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EDITORIAL BOARD - 2013

FORWARD

With well- established ambitious steps on continuing success way, IJST is coming for you all today in its new volume, the eighth volume for year 2013.

Year after year, IJST proves its strength and faithful belief in developing our scientific communities among Arab World, especially in Iraq by giving an opportunity to all researchers to present their fruitful achievements in main vital fields to let all world knows that we are still the first leaders in civilized scientific life, despite all the unfortunate situations or constraints.

This year, IJST had the honor to welcome new Editorial Board Members , Prof. Hazim Al-Daraji from College of Agricuture at Baghdad University, and Dr. Ahmed Abdullah from the Biotechnology Research Centre at Al- Nahrain University, which added another valuable step toward improving the prestigious level and position of the journal.

It is my pleasure to welcome you and present you a new issue of our Journal, Volume 8, No. 1 (2013), the first issue of this year, with diversity of researches and elite experts of the Editorial Board and Advisory Group. The members of Editorial Board, the ICAST and TSTC teamwork and I hope you will find this collection of research articles useful and informative.

IJST has owned a new ISSN registration number, that is: ***2305-9346*** *instead of the previous one, as the first volumes in 2006 issued by Ibn alhaythum, any change in the title needs a new ISSN according to the International Standardization Organization, and this step had been taken for ensuring the high quality and standards of our journal for being internationally recognized.*

The journal is one of the scientific contributions offered by ***the International Centre for Advancement of Sciences and Technology*** *in cooperation with* ***Treasure Est. for Scientific Training and Consultations*** *to the science and technology community (Arab region with specific focus on Iraq and International).*

Finally, on behalf of the International centre, I would like to express my gratitude and appreciation to the efforts of the Editorial Board, Advisory group with their valuable efforts in evaluating papers and the Editorial Board Secretary for 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

Effect of early feeding (*in ovo* injection) amino acids on hatchability, some productive and physiology traits of broiler

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ABSTRACT

Two experiments were conducted to evaluate the effect of early feeding (*in ovo* injection) of lysine and arginine. First experiment was started by setting 360 broiler fertile eggs in the private hatchery from 7th till 28th of February divided into 4 equal treatments (90 eggs per treatment) with three replicates for each treatment. Each egg of the four treatments were injected by the following solutions:
1-T1: Negative control (not injected eggs)
2-T2: Positive control (Sterilized distilled water)
3- T3: Lysine with concentration 2%.
4-T4: Arginine with concentration 2%.
After injection, eggs were returned to the incubation tray to complete the hatching process. Results showed significantly increase ($P<0.05$) hatchability and body weight at hatch.
The second experiment was raising 240 chicks, which were hatched of each treatment and divided into eight equal groups (each replicate involved 30 chicks) to investigate some productive and physiology traits of hatched chicks. Chicks were housed for 42 days (March to April, 2012) at Poultry Experimental Farm of Animal Resources Department, College of Agriculture /University of Kufa. Results showed that *in ovo* injection with lysine and arginine significantly ($P<0.05$) increase weekly live body weight, final body weight and Feed intake compared with control groups. Also *in ovo* ,injection significantly ($P<0.05$) increased in packed cell volume (PCV) and total erythrocytes counts. *In ovo* injection with arginine significantly ($P<0.05$) increased hemoglobin concentration (Hb). Hence, *in ovo* injection with lysine and arginine could be used as a tool for enhancing the hatchability rate and productive performance of chicks hatched from the eggs.

الملخص باللغة العربية

تضمنت الدراسة الحالية تجربتين:
أجريت التجربة الأولى في احد المفاقس الأهلية في محافظة بابل للفترة من 7−28 شباط 2012 لدراسة تأثير التغذية المبكرة (حقن بيض التفقيس) باللايسين والارجنين في نسبة الفقس ومعدل الوزن عند الفقس . إذ تم استخدام 360 بيضة تفقيس والتي قسمت عشوائيا إلى أربع معاملات (60 بيضة لكل معاملة) مع ثلاثة مكررات لكل معاملة وفي اليوم الثامن من الحضن تم حقن البيض في غشاء الكوريوالنتويس حيث حقنت كل بيضة باللايسين والارجنين وكانت المعاملات كما يلي:
المعاملة الأولى: السيطرة السالبة (تركت بدون حقن).
المعاملة الثانية: السيطرة الموجبة (ماء مقطر معقم فقط)
المعاملة الثالثة : حقن البيض بالمحلول الحاوي على اللايسين بتركيز 2%.
المعاملة الرابعة : حقن البيض بالمحلول الحاوي على الارجنين بتركيز 2%.
بعد الحقن تم إرجاع البيض إلى الحاضنات لحين إتمام عملية التفقيس ،وأظهرت النتائج حصول زيادة معنوية ($P<0.05$) في النسبة المئوية للفقس ومعدل الوزن عند الفقس في معاملات الحقن بالأحماض الامينية (اللايسين والارجنين).
أما التجربة الثانية أجريت في حقل الطيور الداجنة التابع لقسم الثروة الحيوانية في كلية الزراعة بجامعة الكوفة للفترة من 1 آذار ولغاية 12 نيسان 2012 إذ تم تربية 240 من الأفراخ الفاقسة والناتجة من البيض المعامل بالتجربة الأولى .إذ تم توزيع الأفراخ على ثمانية معاملات وبواقع 30 فرخ لكل معاملة دراسة بعض الصفات الإنتاجية والدموية للأفراخ الفاقسة وبينت النتائج حصول ارتفاع معنوي ($P<0.05$) في وزن الجسم الأسبوعي ووزن الجسم النهائي في عمر 6 أسابيع والزيادة الوزنية التراكمية عند عمر (2−6 أسابيع) و زيادة في كمية العلف المستهلك وحصول زيادة معنوية ($P<0.05$) في نسبة خلايا الدم المرصوصة وأعداد الخلايا الحمر كمية خضاب الدم وعدم وجود فرق معنوي ($P>0.05$) في أعداد خلايا الدم البيض في المعاملات لمحقونة باللايسين والمثيونين مقارنة بمعاملة السيطرة الموجبة والسالبة.

INTRODUCTION

Amino acids are considered as important elements of feed that are provided to the birds with a desired ratio in order to obtain best production performance . Amino acids are polymerized unit , which can be described as building block of protein (1). Amino acids are the products of protein digestion in the digestive system. Therefore, any deficiency of essential amino acids will lead to produce unremarkable protein inside the body (2).

In ovo technology has been proven to be effective for the commercial vaccination of broilers in the United States. It provides a safer, faster and more uniform means to deliver vaccines for developing embryos (3). This method has not only become a standard procedure for vaccination, but is a potentially effective and pragmatic route to introduce external nutrients to embryos that may have limited nutrient reserves. During late embryogenesis, chick embryos grew rapidly in association with an increased metabolic rate and with increased energy requirements (4). A recent study has also showed that a large number of proteins detected in the pipping muscle are involved in energy producing metabolic pathways and in nucleic acid, carbohydrate, lipid and protein metabolism (5). Previous studies had showed that supplementary carbohydrates and amino acids may improve embryo energy status and growth (6).

Despite the fact that embryos need greater amounts of energy during pipping, not all the nutrients stored in the yolk are mobilized and utilized by embryos at hatch (7). Injected substances can be actively or passively ingested by the embryo via the amnionic fluid and can be subsequently absorbed into various organs prior to hatch (8 ,6). Tullett reported that the broiler embryo is exposed to stress during late term of embryonic development by increasing metabolic heat, thereby affecting the vitality of embryo at hatching, which is negatively reflected on the rate of hatching (9). The hatchability rate of broiler eggs might be low. One reason for this is that the developing embryo inside eggs does not receive the desired amount of critical amino acids. Hence, in order to improve the hatchability it is of great importance for the fertilized eggs to certain all the feed elements (especially amino acids) in a desired amount. Injected broiler eggs with either lysine or methioine acting upon reducing the rate of total mortality and protein degradation (proteolysis) inside body (10). Furthermore, De Olivera *et al*. demonstrated that turkey eggs injected with carbohydrate solution, hatchability rate was increased, while embryonic mortality was decreased (11). Al-Daraji *et al*. indicated that the *in ovo* injection with different levels of 1, 2and 3% arginine into Japanese quail eggs significantly elevated hatchability 86.93,90.24 and 91.45% for levels of arginine respectively compared with control 81.31% (12). Ohta *et al.* found *in ovo* amino acids injection (0 and 7day),chicks from eggs receiving amino acids at day 7 had heavier body weight at hatch than chicks from non injected control eggs(13). Al-Asadi investigated the effect of *in ovo* injection of amino acids (lysine and methionine) at day 18 of incubation; he found both amino acids significantly increased body weight at hatch in comparison to the controls (14). Abdul-Sahib investigated *in ovo* injection of lysine and metionine into the chorio-allantois of broiler eggs at day 10 of incubation he noticed significant ($P<0.01$) increase in body weight in comparison to positive and negative controls (15). Likewise, Japanese quail produce from eggs injected with different levels of arginine (1,2 and 3%) had significant increase on body weight at 7and 42 day-post hatch and body weight gain (12).

Abdul-Sahib also demonstrated that the *in ovo* injection of lysine and methionine in broiler breeder eggs resulted significantly ($P<0.01$) increase in PCV and total erythrocyte counts as compared with those chicks hatched from controls but not significant effect on Hb and total leukocyte count (15). Al-Asadi studied *in ovo* administration of different concentration of lysine (1, 1.5 and 2%) in broiler breeder eggs resulted in significantly ($P<0.05$) increase in Hb, PCV, total erythrocyte and total leukocyte count of chicks as compared with controls (14).

Tako *et al*. revealed that *in ovo* feeding of carbohydrate and /or β-hydroxy-β-methyl butyrate(HMB) significantly enhanced the villus surface area of chicks embryo by approximately 33% over that of controls (16). Moreover, Smith *et al*.suggested that the increase in villus surface area enables faster growing birds to sustain increase demands for nutrition digestion , absorption and assimilation (17) . Likewise, Foye *et al*. reported that *in ovo* feeding of arginine and /or β-hydroxy-β-methyl butyrate (HMB) might increase the hepatic gluconeogenic enzyme to promote hepatic glycogen accretion , thereby enhancing the rapid post –hatch growth (18).

Therefore, the current study aims to realize the points below through two subsequent experiments:

1- The possibility of enhancing hatchability increasing the body weight of hatched chicks and reducing the mortality rate by performed *in ovo* injection of critical amino acids such as lysine and arginine.

2- Enhancement in the productive traits and blood traits for broiler chicks, which produced from injected eggs with lysine and arginine.

MATERIALS AND METHODS

First Experiment:

First experiment was conducted utilizing 360 fertilized commercial eggs of commercial flock. All eggs were obtained from a commercial source from same breeder flock. These eggs were weighted individually to selected homogenously in weight , removed abnormal eggs and randomly allocated to four treatment groups of 90 eggs each. Incubation time from 7th till 28th, February 2012 in private commercial hatchery in Babylon. Through *in ovo* injection, lysine and arginine were administered to the fertile eggs on day 18 of incubation in yolk sac. The *in ovo* injection solutions were as following:

1-T1: Negative control (not injected eggs)

2-T2: Positive control (Sterilized distilled water)

3- T3: Lysine with concentration 2 %(2 g lysine/100 ml sterile distilled water)

4-T4: Arginine with concentration 2%.(2 g arginine/100 ml sterile distilled water)

The injection site of nourishing solution was yolk sac. Then , the used solutions were injected by usin sterilized (1ml) insulin injector. The injection site was disinfected with 70% ethanol before and after injection, sealed with wax. Eggs were incubated at temperature37.7°C under a relative humidity 60 to 65% , and the eggs were turned at each hour for the first to the18th day of the incubation period. Eggs were transferred from incubator trays to hatched trays on the 19th day of incubation and the temperature was decreased to 37°C, the relative Humidity was increased to 80 to 85%. And transferred to incubating baskets in an incubator to complete the hatching process. Hatched chicks were removed from the incubator to determine hatchability rate.

Hatchability was calculated according to the equation below :

(hatched chicks/incubated eggs) x 100 (19)

The average weight of each chick in each replicate was calculate by weight whole chicks in every replicate separately using sensitive electrical balance type Sartorius and divided the total weight by their number.

Second Experiment:

A total sample of 240 chicks were transported from hatchary to Poultry Experimental Farm of Animal Resources Department, College of Agriculture /University of Kufa. Chicks were housed for 42 days (March to April, 2012) to investigate some productive and physiology traits of hatched chicks , which were received solutions previously described at first experiment.

The chicks were fed a diet containing 210 g protein/kg and 12.08 MJ metabolizable energy/kg. The chicks were allowed free access to food and water and housed in wire cages containing 20 chick each. A regimen of 17 h constant lighting and continuous ventilation was provided, and all birds were kept under uniform management conditions throughout the experimental period.

The feeding program consisted of a starter diet used until 21 day of age and a finisher diet until 42 d of age. All diets for each period were prepared with the same batch of ingredients, and all diets within a period had the same composition. Diets were formulated to meet or exceed requirements (20) for broilers of this age. Table (1)

Table (1) composition and calculated analysis of experimental diets

Ingredient (%)	Starter	Finisher
Yellow Corn	41.5	44.8
Soybean meal	27	23.4
Wheat	19	17.3
Protein concentrate *	10	10
Sunflower Oil	1.8	3.5
Di-calcium Phosphate	0.3	0.5
Salt(NaCl)	0.4	0.5
Total	100	100
Calculated chemical analysis		
ME(Kcal/kg)**	3036	3162
Crude protein (%)	22.4	20.9
Me/Crude protein ratio	135.5	151.3
Lysine**	1.19	1.09
Methionine + Cystine**	0.72	0.68

* protein concentration using for experimental produced by Holland company contains .40% crude protein , 2450 Kcal/kg ME,5% fat , 2% fiber ,7% calcium,2.6%phosphor, 3.85% lysine ,3.7% methionine , 4.1% methionine +cystine.
** calculated each one of me, Lysine , methionine+cystine, Ca, AvalP, for each feed materials by using NRC(1994).

Chicks were weighting weekly as a group in replicate throughout the experimental period from the 7th day to the 42nd day.
Live Body Weight (BW): chicks were weighing weekly as a group in each replicates throughout the experimental period (from the 7th day to the 42nd day) and the average body weight were calculated using equation below :

$$\text{Average of live body weight (g)} = \frac{\text{Total life body weight at the end of weeks (g)}}{\text{Number of life chicks at the end of week}} \quad (21)$$

Body weight gain (g) was calculated in replicate weekly by equation below:

Body weight gain=live body weight at the end of week – initial body weight at same week (21)

Feed intake (FI) in each replicated was calculated weekly by following equation :

Feed intake (g)= Total amount of feed provided at the beginning of weeks – amount of feed remaining at the end of week (22)

Blood samples were collected randomly from wing bronchial vein from six birds per treatment on day 42 of age(2 birds per replicate) . Blood samples were collected using lapelled blood collection vials which was contained EDTA as an anticoagulant for hematological studies. A pooled blood sample for each replicate per treatment was analyzed for serum chemistry traits. After overnight clotting at 4 °C, the samples were centrifuged for 15 min at 4000 x *g*. The separated serum was transferred to a laboratory. Hemoglobin concentration (Hb) was determined by using Darbkin's reagent (23). Packed Cell Volume ,PCV (%) has been measured by heparinized capillary tubes were filled to 3/4 with blood, after closing one end of the tubes they were kept in Micro centrifuge (12000 cycle / minutes) for 5 minutes.

The values were recorded with special haematocrit reader (24).Total Erythrocyte count(TRBC)which it referred to Red Blood Cells(RBC) and Total Leukocyte Count (TWBC)which it referred to white Blood cells were estimate by manual standard technique using solution Natt and Herrick according to the standard methods as reported by (25).

Statistical analysis:

Data generated from experiment were carried out in a complete randomized design (CRD) using of SAS software (26). The significant differences among means were determined by using Duncan's multiple range tests. Differences among treatment means were compared at $P<0.05$.

RESULTS AND DISCUSSION

Hatchability and Body weight at hatch

The effect of *in ovo* injection of lysine and arginine during 18th day of incubation period on hatchability, and Body weight at hatch are shown in table(2).

Table (2) Effect of *in ovo* injection of lysine and arginine on hatchability, embryonic mortality, deformed chicks and Average body weight at hatch (Mean ±SE)

Treatments	Hatchability%	Body weight at hatch(g)
Negative control(T1)	$79.59^{c}\pm0.45$	$47.70^{b}\pm0.11$
Positive Control(T2)	$78.41^{c}\pm0.30$	$47.53^{b}\pm0.13$
Lysine 2%(T3)	$87.52^{b}\pm1.45$	$50.20^{a}\pm0.73$
Arginine 2%(T4)	$91.75^{a}\pm1.58$	$51.03^{a}\pm0.60$

a,b, c means with different superscripts within the same column differ significantly(P<0.05)

Hatchability of broiler breeder eggs injected with amino acids (lysine and arginine) were significantly higher ($P<0.05$) than control eggs. Hatchability significantly increased when broiler breeder eggs injected with either lysine or methionine compared to non –injected eggs (14). As such, to complete embryos development, excess amount of proteins or amino acids are required to build the body tissue while as the embryos of poultry are different from those mammals since they do not have feed sources expect eggs (27). Nowadays, the hatching process has been upgraded through utilizing advanced technologies specified for this purpose to achieving the highest hatchability and

guaranteeing the livability of embryos. So, the *in ovo* injection had been performed with amino acids (28). There is interference between the component amino acids of lysine and arginine on hatched chickens through antagonism of lysine over arginine, this act upon raising the level of arginase enzyme which is responsible of break down arginine in liver and kidney of poultry. Results of present study coincides with the finding of several authors who indicated *in ovo* feeding of carbohydrates and proteins (29) have been seen to improve the hepatic glycogen reserves over the control. Furthermore, Foye *et al* . demonstrate that poultry *in ovo* feed arginine have enhanced total hepatic glycogen reserves (30). Consequently, hatchability was positively correlated with liver glycogen content of turkey and chicks embryo before hatching. (29).

Results indicated that chicks produced from eggs that were injected with lysine and arginine were heavier ($P<0.05$) in body weight at hatch than those produced from control eggs. These findings were in confirmation with findings of Al-Murrani(31) and Ohta *et al*.(13) who confirmed that in ovo injection of amino acid have caused an increase chick weights at hatch y increasing amino acid yolk content or increasing amino acid utilization by embryo. The in ovo amino acid injection in broiler eggs led to elevate amino acid level in blood plasma: especially , lysine ad increase amino acid contents of embryo , yolk, albumin , allantois and amnion fluids at day 19 of incubation .Therefore these result might have an important role on the chick weight by formation of muscle protein . These results matched the results obtained by (14).

Productive traits:

Live body weight, body weight gain, and feed intake:

Table(3) shows the results derived from the effect of *in ov*o lysine and arginine injection on live body weight m body weight gain and feed intake of broiler chicks from 2 to 6 weeks of age. In the 2nd and 3 rd weeks of age , all chicks that have received amino acids grew faster and had a higher body weight ($P<0.05$) in comparison with control groups .These results were in consistent with those reported by (10) whom found a significant increase in body weight of chicks at 3 weeks of age , which were hatched from eggs injected with amino acids especially lysine due to increased embryo weights. The present results suggest that amino acids injection into the egg might have stimulate amino acids utilization, which may be explained by an increase in amino acids synthesis and a concomitant decrease in amino acids degradation by embryo, thereby yielding subsequent growth (28). Similar results were reported by (14) , (15) in broiler chicks and (12) in Japanese quail chicks whom found that live body weight significantly increased from injected eggs with amino acids.

Table(4) shows Body weight gain of chicks from amino acids injection group were significantly ($P<0.05$) higher than those of control groups. In the 2nd week , the highest values of body weight gain were obtained in amino acid injection groups. Accumulative body weight gains were calculated during (2-6) weeks. Amino acid injection groups had significantly ($P<0.05$) higher than control groups . Those findings were coincided with the findings of (28) and (29) whom reported that *in ovo* injection of carbohydrates, amino acids and vitamins led to intestinal development through increasing villus size and capacity of digestion y intestinal enlargement, which led to the speed of digestion and absorption of food. Investigation performed by (6) showed that 2 g difference in body weight at hatch due to *in ovo* feeding resulted I 50 to 60 g of increase in body weight gain at 25 day post hatch. These results were in agreement with results reported by (14) and (15) who found that amino acids (Lysine and methionine) injection has significantly increased accumulative body weight gain during (1-6 week) post- hatch.

Table(5) shows Feed intake of chicks from amino acids injection group were significantly ($P<0.05$) higher than those of control groups. In the 3rd week , feed intake of chicks hatched from eggs injected with lysine had significantly increased as compared with other treatments at 3rd week of age . In 5th week, a significant decrease in feed intake was found chicks that received arginine and lysine compared with control groups. These results were in disagreement with results obtained by (14) who found that accumulative feed intake significantly elevated in chicks that were hatched from eggs injected with two levels of lysine (1.5 and 2.%) through 1-8 weeks post-hatch.. In addition these results also were in contrast with those reported by (15) who found *in ovo* injection of lysine and methionine in broiler breeder eggs significantly increased accumulative feed intake as compared to the control during 1-6 weeks post-hatch .

Table (3): Effect of *in ovo* injection of lysine and arginine on average live body weight (Mean ±SE) of broiler

Age / Treatments	Average live Body weight(g)				
	Week 2	Week 3	Week 4	Week 5	Week 6
Negative control(T1)	345.66^{b}±4.56	753.03^{a}±9.76	1315.53^{b}±14.66	2021.30^{b}±9.26	2636.63^{b}±17.15
Positive Control(T2)	348.20^{b}±4.65	760.10^{b}±10.42	1303.03^{b}±8.95	2001.06^{b}±5.83	2615.00^{b}±8.66
Lysine 2%(T3)	367.46^{a}±4.01	805.60^{a}±8.80	1408.23^{a}±11.17	2120.46^{a}±11.52	2738.43^{a}±11.03
Arginine 2%(T4)	368.40^{a}±2.34	807.40^{a}±11.63	1410.30^{a}±6.98	2118.36^{a}±8.77	2748.20^{a}±10.28

a, b, c means with different superscripts within the same column differ significantly(P<0.05)

Table (4): Effect of *in ovo* injection of lysine and arginine on average body weight gain (Mean ±SE) of broiler

Age / Treatments	Average Body weight gain(g)					
	Week 2	Week 3	Week 4	Week 5	Week 6	Week 2-6 Accumulative
Negative control(T1)	184.93^{b}±4.79	407.36^{b}±5.19	562.50^{b}±4.90	705.76^{bcd}±5.40	615.33^{b}±7.88	2475.90^{b}±17.38
Positive Control(T2)	187.20^{b}±4.59	411.90^{b}±5.77	542.93^{c}±1.94	689.03^{d}±3.12	613.93^{bc}±2.83	2454.00^{b}±8.60
Lysine 2%(T3)	206.90^{a}±4.36	438.13^{a}±4.79	602.80^{a}±2.22	712.23^{abc}±0.35	617.96^{b}±0.49	2577.87^{a}±11.37
Arginine 2%(T4)	206.56^{a}±2.34	439.00^{a}±11.63	602.90^{a}±4.65	708.06^{bcd}±1.79	629.83^{a}±1.50	2586.37^{a}±9.61

a, b, c means with different superscripts within the same column differ significantly(P<0.05)

Table (5): Effect of *in ovo* injection of Lysine and Arginine on feed intake (Mean ±SE) of broiler

Age Treatments	Feed intake (g)					
	Week 2	Week 3	Week 4	Week 5	Week 6	Week 2-6 Accumulative
Negative control(T1)	263.40^{d}±1.96	750.66^{b}±1.16	1057.46^{c}±2.85	1333.46^{ab}±7.56	1309.20^{a}±9.24	4714.20^{a}±22.77
Positive Control(T2)	265.80^{cd}±1.84	746.26^{b}±1.53	1040.60^{d}±2.54	1340.10^{b}±5.86	1304.16^{a}±9.62	4696.93^{ab}±21.39
Lysine 2%(T3)	278.20^{a}±1.30	760.36^{a}±1.24	1060.26^{bc}±1.24	1310.06^{cd}±5.80	1278.83^{ab}±10.17	4687.73^{ab}±18.85
Arginine 2%(T4)	277.17^{a}±2.45	747.80^{b}±2.42	1068.36^{a}±2.28	1300.36^{cd}±5.80	1295.33^{a}±8.80	4689.03^{ab}±21.76

a, b, c means with different superscripts within the same column differ significantly(P<0.05)

Blood traits

Table (6) shows the data results of influence of *in ovo* amino acid injection on hemoglobin concentration (Hb), packed cell volume (PCV), total erythrocyte count (TRBC) and total Leukocytes count (TWBC).

Results indicated that there was a significant increase (P<0.05) in hemoglobin concentration of chicks hatched from eggs injected with arginine2% as compared to control groups. This increase could be related to the rule of amino acids in construction of blood proteins (Globulins) which was represented as a hemoglobin-building unit that consists of iron and porphyrin (hem proteins). The significant effect upon Hb content was in agreement with the results obtained by (14) that fund hemoglobin concentration has significantly improved in chicks that were hatched from eggs injected with lysine at concentration 2% as compared with positive and negative controls.

Results showed that hatched from eggs injected with lysine and arginine had significant increase (P<0.05) on PCV in comparison to positive and negative control eggs. Gayton reported an increase in PCV as result to increase in total erythrocyte count (32). Similar result was obtained by (15) who observed that PCV has significantly increase in chicks hatched from eggs that were received either lysine or methonine by *in ovo* injection. Results of current study were in confirmation with those reported by (14) who found that chicks were received

different levels of lysine especially 1, 1.5 and 2% has significantly elevated PCV as compared with controls.

Results indicated that there was a significant increase ($P<0.05$) in TRBC of chicks hatched from eggs injected with lysine and arginine as compared to control groups. This increase could be related to the rule of amino acids in construction of blood protein (Globulins). Results of current study were in confirmation with those reported by (14) and (15).

TWBC of chicks received lysine and arginine by in ovo injection was not affected significantly as compared with control treatment. These results were disagreement with those investigated by (14) who found significant increase on TWBC of chicks hatched from injecting eggs with different levels of lysine (1,1.5 and 2%). These finding were coincided with results demonstrated by (15) who observed that there was no significant different on TWBC between chicks hatched from *in ovo* amino acid injection and non –injected control.

Table (6): Effect of *in ovo* injection of lysine and arginine on hematological Traits (Mean±SE) of broiler

Treatments	Hb (g/100ml blood)	PCV%	TRBC N×10^6/1ml^3)	TWBC N×10^3/1ml^3)
Negative control(T1)	9.22^{bc}±0.19	3.60^{b}±0.31	2.84^{c}±0.01	21.23±0.13
Positive Control(T2)	8.70^{c}±0.36	9.57^{b}±0.30	2.47^{c}±0.01	21.25±0.01
Lysine 2%(T3)	9.62^{bc}±0.28	0.53^{b}±0.09	2.98^{b}±0.01	21.20±0.62
Arginine 2%(T4)	10.01^{ab}±0.12	3.60^{a}±0.41	3.13^{a}±0.06	21.20±0.23

a, b, c means with different superscripts within the same column differ significantly($P<0.05$)

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Response of Different Brassica Vegetables to Manure Source, NO_3_N Accumulation and Nitrate Reductase Activity in Plant

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ABSTRACT

Nutrient mineralization of applied mineral fertilizer or animal manures and the accumulation of NO_3_N in some *Brassica* vegetables in addition to the activity of nitrate reductase enzyme in leaves and edible organs were examined. Fertilizer source included mineral fertilizers as recommended, and cattle or poultry manure at rate of 20% of soil (v/v) were incorporated into 0-30 cm of top soil 20 days before transplanting. Four *Brassica* vegetables were used as test plant for the response to the source of fertilizers. Different vegetables responded to organic manure application in the same trend, but pak choi had the highest and cauliflower had the lowest content of NO_3_N in the edible part (2838 and 539 µg kg dwt^{-1} respectively) when poultry manure was applied. However, elevated NO_3_N in the edible organs of all tested vegetables was below that could cause toxicity to human health. Nitrate reductase enzyme (NR) activity in leaves generally lower than edible organs by 28.9%, and results show that NR activity is not a function of NO_3_N accumulation in plant tissues, where the NO_3_N content of the leaves of kohlrabi, cauliflower, pak choi, and cabbage was greater than the edible organs by 49.8, 30.1, 34.4, or 27.3%, where the NR activity was lower by 33.6, 35.9, 27.6, or 19.6% respectively. These results may suggest that activation of NR in the edible organs is a function of some other biological factors in addition to nitrate level. Crop yield of the tested *Brassica* vegetables increased when cattle or poultry manure was applied as compared to mineral fertilized plants. The percent increases were 31.9 and 52.1% for kohlrabi, 3.9 and 50.7% for cauliflower, 36.6 and 68.9% for cabbage, and 69.5 and 73.4% for pak choi as compared to mineral fertilized plants.

Key words: Brassica vegetables, cattle and poultry manure, Nitrate reductase enzyme activity.

الملخص باللغة العربية

تناولت هذه الدراسة معدنة الاسمدة المعدنية او العضوية المضافة للتربة وتراكم النترات (NO_3_N) في بعض الخضروات الصليبية بالاضافة الى فعالية انزيم مختزل النترات في الاوراق والجزء الذي يؤكل تم اختيارها. مصدر السماد تضمن السماد المعدني الموصى به ومخلفات الابقار او الاغنام بمعدل 20% حجم/حجم مزجت في التربة (0–30 سم) قبل نقل الشتلات بعشرين يوم. اربعة خضروات صليبية استعملت كنباتات اختبار لاستجابتها لمصدر السماد. استجابت الخضروات المستعملة لاضافة الخلفات العضوية بنفس الاتجاه الا ان اللهانة الصينية احتوت على النترات باعلى مستوى بينما احتوى القرنابيط باوطأ مستوى منها في الجزء الذي يؤكل (2838 و539 ميكروغرام.غم–1 وزن جاف) عندما سمدت بمخلفات الدواجن. تراكم النترات في الجزء الذي يؤكل في جميع الخضروات المستعملة هو اوطأ من المستوى الذي يسبب سمية لصحة الانسان. فعالية انزيم مختزل النترات في الاوراق عموما اوطأ من فعاليته في الجزء الذي يؤكل بنسبة 28ز9% واظهرت النتائج ان فعالية هذا الانزيم هي ليست مؤشر لتراكم النترات في انسجة النبات حيث ان محتوى اوراق الكلم والقرنابيط واللهانة الصينية واللهانة من النترات هو اعلى من الجزء الذي يؤكل بنسبة 49.8 و 30.1 و 34.4 و 27.3% بينما فعالية مختزل النترات في الجزء الذي يؤكل كانت اعلى مما هي في الاوراق بنسبة 33.6 و 35.9 و 27.6 و 19.6% بالتتابع. هذه النتائج ربما تشير الى ان تنشيط فعالية هذا الانزيم في الجزء الذي يؤكل هو مؤشر لبعض الفعاليات الحيوية بالاضافة الى تراكم النترات.

حاصل هذه الخضروات ازداد عندما اضيفت مخلفات الابقار او الدواجن للتربة مقارنة بالنباتات المسمدة بالسماد المعدني وبلغت نسب الزيادة في الحاصل 31.9 و 52.1% للكلم و3.9 و 50.7% للقرنابيط و 36.6 و 68.9% للهانة و69.5 و 75.4% للهانة الصينية.

INTRODUCTION

Organic farming differ principally from conventional (mineral fertilised) farming by not using synthetic chemical fertilises and pesticides. Manure (animal or plant residues) is organic matter and contributes to soil fertility by adding organic matter, and its releasing of macronutrients and micronutrients, and improving the physical and chemical properties of the soil (1). In Europe, the conversion from conventional to organic farming has been slowed by the concern about accumulation of nitrate in the soil which might eventually leach into the ground or surface water (2). In plants, nitrates are natural constituents, and often present in the edible organs of many vegetables. The accumulation of nitrates in plant tissues is affected by many factors such as an increasing level of inorganic fertilisers (3), light intensity (4), and species (5). Nitrates accumulation in plant tissues is not uniform, for example, typically they occur in the fruits in only minor amounts. It has been reported that NO_3^- concentrations are highest in petiole and stems, moderate in leaves and roots, and very low in fruits and flowers (6). Leafy vegetables such as spinach, radishes, celery, and lettuce may therefore accumulate nitrates in high concentration (7). Variability's in NO_3^- accumulation among species has been related to the efficiency of reducing NO_3^- in its roots (8). Organic grown vegetables have been reported to have lower NO_3^- contents than mineral fertilized crops (9,10). Nitrate reductase enzyme (NR) (EC 1.6.6.1) is involved in primary metabolism and intensively studied because it catalyzes the rate limiting step in the overall process of nitrate assimilation. Nitrate assimilation often limits plant productivity and the primary product of nitrate reduction is nitrite, which is toxic (11). There are some contradictory reports about the inducible effect of tissue nitrate contents and the activity of NR enzyme. Crawford (12) reported that NO_3^- accumulation is the trigger for NR activity, whereas, Ferrari *et al.* (13) mentioned the importance of nitrate influx to the metabolic pool via the transpiration stream is regulating NR activity. On the other hand, (14) showed that NR activity is independent of nitrate content and ammonium supply in the absence of nitrate stimulated the NR activity more than nitrate. The activity of NR enzyme is not uniform in all plant tissues and is affected by external NO_3^- supply. High NR activity in xylem sap in many cereal plants was found when NO_3^- supplied at low concentration (0.1-1.0 mol NO_3^- m^{-3}), while cereal plants shoot assimilation of NO_3^- increased in importance as applied NO_3^- concentration increased from 1.0 to 20 mol $NO_3^-m^{-3}$ (15).

The primary objective of this study was therefore to compare the accumulation of NO_3_N and NR activity in the leaves and edible organs of four species of *Brassica* vegetables.

MATERIALS AND METHODS

A field experiment was carried out at Horticulture Research Area in Lincoln University, NZ. The soil is classified as Wakanui silt loam. Three fertilizer treatments were implemented: Conventional [mineral fertilization at a rate of 40 kg N ha^{-1} as Urea (46%N), 50 kg P ha^{-1} as triple superphosphate (21% P), and 50 kg K ha^{-1} as potassium sulphate (41.5% K) broadcasted in the first split, and 100 kg N.ha^{-1} as urea a side dressing 3 weeks post planting as recommended by (16)], Cattle manure at a rate of 20% v/v (600 m^3 ha^{-1}), Poultry manure at a rate of 20% v/v (600 m^3 ha^{-1}), and no fertilization as a control. Manures and the first split of the mineral fertilizers were incorporated into 30 cm of the topsoil 20 days before transplanting. The analysis of manures used shown in Table 1.

Seeds of Pak choi (*Brassica campestris* var. *chinensis*) Winter Shanghai F1., Kohlrabi (*Brassica oleracea* var. *gongylodes*) Aka Green Duke, Cauliflower (*Brassica oleracea* var. *botrytis*) Freda F1 Hybrid, and Cabbage (*Brassica oleracea* var. *capitata*) Winter Cross cvs., were sown on 5th May 2008 and at 3-4 true leaves stage were pricked out on 3rd July 2008. Three rows of 6m length were used for each species in each plot with 0.5m apart and 0.25 m between plants. A factorial 4x4 in a Randomized Complete Block Design was implemented in four replicates. At harvest, five random plants from the middle row of each plot of each treatment were taken for the measurements.

Table (1): Analysis of cattle and poultry manures

Properties	Cattle manure	Poultry manure
pH 1:5 KCl extract	7.35	7.79
%C	12.51	22.34
%N	1.23	2.44
C:N ratio	10.98	9.17
%P	0.34	2.32
%S	0.22	0.53
%K	0.35	0.90
%Ca	1.08	8.95
%Mg	0.25	0.43
%Fe	1.18	0.37
%Na	0.04	0.47

Soil analyses

Before transplanting soil samples were analysed for mineral nutrients, pH, ECe and moisture content. Samples of raw material of both manures were also taken for chemical analysis. During plant growth and again at harvest, soil samples from each replicate were taken at 93, 116 (Pak choi harvest), and 151 days (Kohlrabi harvest) after fertilisers were incorporated into the soil.

Fresh soil samples were taken to measure soil moisture content according to (17), the rest of the soil samples were air dried, crushed to pass through a 2 mm mesh sieve, and thoroughly mixed to ensure homogeneity. Soil pH was determined in 1:5 KCl extract and Electrical conductivity of soil extract (ECe $mS.cm^{-1}$) was measured in 1:5 water extract (17). Total N% and C% was also determined in dried soil samples by Macro Elemental Analyser (Varo Max CN Elementor Analyser System GmbH, Germany). Mineral analysis for P, S, K, Ca, Mg, Fe, and Na was performed in acid digest of the soil according to (18) method and read by Optical Emission Spectrometer (Varian 720-ES ICl-OES).

Plant analyses

At harvest five plants were selected and divided into edible organ (The loose head leaves in Pak choi, storage stem in Kohlrabi, curd in Cauliflower, and compacted head in Cabbage), leaves and other plant parts, and the average of fresh weight was recorded. Leaves and edible organs oven (70 ^{o}C) dried for 72 h and the % of dry weight was calculated. Fresh samples of the leaves and the edible organs were kept in a freezer for nitrate reductase (NR) activity assay. Nitrate content in dried leaves and edible organs samples was measured by the salicylic acid method (19). Nitrate reductase (NR) activity was determined using *in vitro* assay following grinding of frozen materials in liquid Nitrogen, extraction and assay procedure used in the *in vitro* assay were as described in (20). The nitrite (NO_2^-) formed were colorimetrically determined at 543 nm and NR activity was expressed as µM NO^-_2 g^{-1} dry weight (DWt.) h^{-1}. Mineral analysis was performed in acid digest according to (21).

Statistical analysis

All data were statistically analysed by analysis of variance (ANOVA) using the 10th ed. of GenStat (22). Least significant differences at probability level of 5% were given to indicate significant variations between treatments.

RESULTS AND DISCUSSION

Nitrogen and carbon content

Soil contents of total N and C was the highest at 20 days after fertilizers were incorporated to the soil, and then declined with time advances (Table 2). Poultry manure treated soil had lost 50% of its contents of N and C, whereas, control and conventional treatments lost only 20% after 116 days of treatment. There was a little change in those elements for cattle manure treated soil for same period of time. At 151 days after treatment as the season got warmer in November mineralization of these elements was enhanced resulted in an increase in %N and %C (Table 2). These increases were expected since the organic wastes decomposition is a temperature dependent process. The high N mineralization of poultry manure which has the lowest C:N ratio (Table 1) is in agreement with the findings of (23) that N mineralized from the manure is inversely correlated to the C:N ratio.

These results demonstrate the value of organic manures as fertilizers because of the potential for manures to mineralize and supply nutrients especially nitrogen to soil. Mineralization of organic material is closely related to its chemical composition including C:N ratio (24). . These results were expected since the rate of the N mineralization is C:N ratio dependent, where the C:N ratio in poultry manure is low (Table 1) and high in other treatments. According to (25) manure with a high N content should result in a high net N mineralization, an increased soil microbial biomass N, high denitrification, or a combination of these outcomes.

NO_3^- accumulation and NR activity in leaves and edible organs

Regardless of fertiliser treatments, NO_3_N accumulated in the leaves of pak choi at higher levels compared to other *Brassica* vegetables tested, and was 93.2%, 126.7%, and 306.6% higher than cabbage, kohlrabi, and cauliflower leaves respectively (Table 3), probably this due to genetic and early maturation effect. Pak choi was harvested one month earlier than kohlrabi, and two months before cauliflower and cabbage. Brown and Smith (1966) reported that early maturing varieties of vegetables tended to accumulate more nitrate than late maturing varieties at a given rate of fertilization. Chinese cabbage accumulated NO_3_N up to 5858.1 mg NO_3_N kg fresh $weight^{-1}$ compared to 1425.5 mg NO_3_N found in the leaves of cabbage (26). Differences of radish (*Raphanus sativus* L.) cultivars in accumulation of NO_3_N in their tissues also has been reported(27).

Poultry manure applied to the soil significantly increased the accumulation of NO_3_N in the leaves compared to cattle manure or other treatments (Table 3). No important NO_3_N differences between cattle manure, conventional or control treatments were noticed. Poultry manure raised NH_4_N and NO_3_N presence as well as the pH in the soil (unpublished data) which may be the reason for the increased NO_3_N in the plant leaves of all *Brassica* vegetables tested (27). Although, all brassica vegetables under investigation have increased NO_3_N in their leaves with poultry manure application, pak choi had the highest and cauliflower had the lowest values.

Accumulation of NO_3_N in the edible organs followed a similar trend as in the leaves where pak choi accumulated the highest while cauliflower curds were the lowest. However, generally the distribution of NO_3_N between the leaves and edible organs was not uniform. The uneven distribution may be due to the fact that in cauliflower the curd (flowers) is the edible organ, whereas, the loose leaves of pak choi is the edible organ, and (28) mentioned that flowers and fruits accumulate very low concentration of NO_3.

Table (2): Effect of fertilizer source on soil content of total N and C at different periods after incorporation.

Fertilizer source	%N	%C	C:N ratio
20 days after soil incorporation			
Control	0.177	1.960	11.097
Conventional	0.162	2.007	12.370
Cattle manure	0.183	1.868	10.226
Poultry manure	0.480	4.025	8.384
93 days after soil incorporation			
Control	0.172	1.96	11.74
Conventional	0.169	1.95	11.59
Cattle manure	0.196	2.16	11.06
Poultry manure	0.282	2.50	8.90
116 days after soil incorporation			
Control	0.136	1.57	12.09
Conventional	0.131	1.54	12.41
Cattle manure	0.164	1.91	11.75
Poultry manure	0.240	1.94	8.63
151 days after soil incorporation			
Control	0.149	1.66	11.30
Conventional	0.163	1.78	10.63
Cattle manure	0.183	2.03	11.09
Poultry manure	0.260	2.14	8.23
Fertilizer source	***	***	***
Interaction $LSD_{0.05}$	Ns	ns	ns

****, significant< 0.001 probability. ns, not significant <0.05 probability.*

The storage stem of kohlrabi acts as a solute translocation channel, therefore, most of the NO_3_N taken up by the plant roots will ascend to the transpiring leaves and not accumulate in it, whereas, in pak choi the transpiring loose leaves accumulate high concentration of the NO_3_N. Poultry manure application resulted in an increase in the content of NO_3_N in edible organs (Table 3).

In pak choi the conventional and cattle manure treatments also increased the accumulation of the NO_3_N in the edible organ relative to the control. In other tested *Brassica* vegetables only the poultry manure treatment resulted in a high accumulation of the NO_3_N in different edible organs. Concentrations of NO_3_N found in the edible organs in this experiment are below values mentioned by (29) where at harvest were 4000-6000 mg kg^{-1} and 5000-8000 mg kg^{-1} on a dry weight basis for cauliflower and cabbage respectively.

The recommendation is the standard concentration of NO_3 in the edible organs of cauliflower, cabbage, and kohlrabi should be less than 500 mg kg^{-1} on a fresh weight basis (30). In this experiment the NO_3 concentration in cauliflower curds and compact heads of cabbage, treated with poultry manure, were well below recommendations (307 and 325 mg NO_3 kg^{-1} on fresh weight basis respectively 'data not shown'). However the loose leaves of pak choi and the storage stem of the kohlrabi had NO_3 concentrations higher than recommended (795 and 1094 mg NO_3 kg^{-1} respectively). The differing NO_3 values in the edible portions may be due to the genotype and the different plant tissues. Toxicity can result if 70-kg adults ingest approximately 0.7 g of NO_3_N (27). Under the conditions of this experiment it would take 880 g of pak choi or 640 g of kohlrabi, on fresh weight basis to give toxic dose. This quantity would have to be ingested at one meal. It is unlikely that a person could eat 880 g of pak choi or 640 g of kohlrabi at one meal. There are no consistent recommendations showing the concentrations of NO_3 in crop edible organs that would be harmful to the human health, probably this due to different cultivars, soil fertilization, and environmental factors. The daily intake of the Chinese person of NO_3 is 422.8 mg (31), while, World Health Organization recommended 3.7 mg NO_3^- kg body weight $^{-1}$ day $^{-1}$ (32) (approximately 260 mg NO_3^- 70 kg body weight^{-1}), whereas, US standard is 7 mg NO_3^- kg body weight^{-1} day^{-1}. Minimizing nitrate content in the edible part of the plants is very important for human health, because of the potential transformation of nitrate into nitrite, which can

interact with haemoglobin and negatively affect blood oxygen transportation (33).

Table (3): Effect of fertilizer source on NO_3_N and nitrate reductase activity in leaves and edible organs of some *Brassica* vegetables

Species	Fertilizer source	NO_3_N leaves (ug N g Dwt^{-1}.)	NO_3_N edible organ (ug N g Dwt^{-1}.)	Nitrate reductase activity in leaves (uM NO_2^- g Dwt^{-1} h^{-1})	Nitrate reductase activity in edible organ (uM NO_2^- g Dwt^{-1} h^{-1})
Pak choi	Control	151	83	1.692	2.458
	Conventional	997	816	3.410	3.749
	Cattle manure	1102	656	2.930	4.918
	Poultry manure	4450	2838	4.048	5.609
Kohlrabi	Control	34	34	0.328	0.505
	Conventional	36	53	0.360	0.451
	Cattle manure	37	38	0.452	0.421
	Poultry manure	2850	2025	0.350	0.481
Cauliflower	Control	191	161	0.394	0.859
	Conventional	169	203	0.404	0.750
	Cattle manure	178	250	0.413	0.791
	Poultry manure	1109	539	0.918	0.920
Cabbage	Control	288	231	0.358	0.626
	Conventional	282	292	0.557	0.607
	Cattle manure	234	341	0.475	0.857
	Poultry manure	2662	877	0.886	1.336
Species	Pak choi	1675	1098	3.029	4.184
	Kohlrabi	739	537	0.373	0.464
	Cauliflower	412	288	0.532	0.830
	Cabbage	867	435	0.569	0.857
Species		***	***	***	***
Fertilizer source		***	***	***	***
Interaction $LSD_{0.05}$		771.4	285.0	Ns	0.9217

****,*, significant< 0.001 probability. ns, not significant <0.05 probability.*

Nitrate reductase activity in the leaves and the edible organs of *Brassica* vegetables is variable. In this experiment pak choi had the highest activity whereas kohlrabi had the lowest NR activity in harvested organs. Poultry manure application increased NR activity in the leaves and edible organs, while other treatments had no significant effects except with pak choi where fertilization regardless of the fertilizer source increased NR activity compared to the control plants. Generally, NR activity in the leaves was lower than that in other edible organs by (28.9%). Nitrate presence in tissues has an inducible effect on NR activity (14) and was seen in this experiment where high content of NO_3_N in both the leaves and edible leaves of pak choi increased NR activity. However, the NR activity increases was not a function of NO_3_N accumulation. For example, the accumulation of NO_3_N in the leaves of kohlrabi, cauliflower, cabbage, or pak choi was higher than in the edible organs (relative to leaves) by 49.8, 30.1, 27.3, 34.4% respectively, whereas, NR activity was lower than the edible organs by 33.6, 35.9, 19,6, or 27.6% respectively. Edible organs usually have higher metabolic activities than other plant organs (strong sinks). Increased NR activity could be one aspect of higher metabolic activity and may be activated by many factors including raised nitrate content of the tissue. Factors that activate NR in plant cells claimed that environmental factors such as light, CO_2, or oxygen availability would rapidly modulate NR activity. Plant development stages could also affect NR activity. The changes in nitrate reduction sites during plant development have

been related to the changes in soluble glutamine in the leaves and roots (34). Ammonium activation to NR enzyme was also suggested (14,35) through the slight acidification in the shoot when ammonium was the sole source of nitrogen supply. Variation in NR activity between shoots and roots of some cereal plants was found (15), where the NR activity in the roots was higher than the shoots when the external supply of NO_3 was low(0.1 to 1.0 mol m^{-3} NO_3^-). Therefore, the higher activity of NR found in leaves and edible organs for the poultry manure treated plants could be due to the high availability of ammonium NH_4^+ in soil solution (unpublished data) which has been taken up by the plants and assimilated either in the roots or in leaves and edible organs, resulting in some changes in the cytosolic pH leading to this high NR activity.

Mineral contents of leaves

Level of P in cauliflower, and S in cabbage treated with poultry manure were the highest among treatments (Table 4). This could be due to the continuous mineralization of decomposed manures during the growing season of the plant. Higher P content in organically fertilized tomato (*Lycopersicon esculentum* Mill.) and potato (*Solanum tuberosum* L.) crops have been reported (36,37).

Among the four fertilizer treatments, poultry and cattle manures showed statistical differences in K, Mg, and Na contents in pak choi and cabbage. Kohlrabi and cauliflower only exhibited elevated level of these nutrients when treated with poultry manure (Table 4). As mentioned above, the organic wastes of either cattle or poultry manure may increases soil nutrient content and rises soil pH towards neutrality (from 6.01 to 6.61), which in turn increase the availability of these nutrients allowing accumulation in plant tissues. Increased soil P and K when organic fertilizer was used has been reported (33), and levels of these nutrients were elevated in chard (*Beta vulgaris* var. *cicla*) and tomato leaves. Warman (2005) and Herencia *et al.* (2007) reported Higher concentration of K in organically produced vegetables also found (33,38). However, others show that application of organic amendment improves soil nutrient content, but does not always increase plant nutrient concentration (38,39). Other studies show that the nutrient content in a plant depends on crop type, nutrient type, climate, and year of study (40,41,42). In this experiment the interaction between *Brassica* vegetables and fertilizer source had no significant effects on the levels of Ca and Fe in the leaves (Table 4).The organically treated plants fresh weight yields were greater than that of mineral fertilized or non-fertilized (control) plants (Table 5). The greatest plant or edible organ weight was found in poultry manure treated *Brassica* vegetables. There were significant yield differences between the organically treated plants, where harvested fresh weight of loose leaves of pak choi, compacted head of cabbage, curds of cauliflower and storage stem of kohlrabi were the greatest when treated with poultry manure or cattle manure as compared to mineral fertilized plants. The percentage increases were 31.9 and 52.1% for kohlrabi, 3.9 and 50.7% for cauliflower, 36.6 and 68.9% for cabbage, and 69.5 and 73.4% for pack choi as compared to mineral fertilizer treatment. Increases in total and marketable yield of potato when plants were grown in organically amended soil was noticed (43).

Increase in chard and tomato yield during the 2-5 yr of manure treatments was reported compared to mineral fertilized plants (33). However, (9) reported that organic treatments lowered vegetable yields in the first 2 yr of treatment, but yields did not differ after the third year. In this study the poultry manure was superior to cattle manure and produced heavier edible organs, probably due to the continuous release of high amount of nutrients and changes in the soil pH which favour the availability and uptake of macro and micronutrients by plant roots improving plant growth and yields of the edible organs.

The nutrient content of cattle manure and its release to the soil, on the other hand, was drastically lower than poultry manure (Table 3).

Dry matter percentage was significantly lower in the leaves of all *Brassica* vegetables when treated with poultry manure and the lowest leaf dry matter percent was found in pak choi. Meanwhile, only pak choi and cabbage shows lower percentage of dry matter in the edible organs relative to cauliflower curd and kohlrabi stem fertilized with poultry manure. This may be related to high levels of nitrogen (NH_4_N and NO_3_N) in plant edible organs when treated with poultry manure (Table 3).

Table (4): Effect of fertilizer source on mineral content (on dry weight basis) of four *Brassica* vegetable

Species	Fertilizer source	P g.kg^{-1}	S g kg^{-1}	K g.kg^{-1}	Ca g.kg^{-1}	Mg g.kg^{-1}	Fe g.kg^{-1}	Na g.kg^{-1}
Pak choi	Control	4.03	3.82	43.14	16.15	1.36	0.093	3.83
	Conventional	4.05	3.60	56.29	17.55	1.48	0.120	3.60
	Cattle manure	4.97	5.56	64.83	18.40	1.61	0.087	5.56
	Poultry manure	4.51	4.29	61.39	24.34	2.83	0.086	4.29
Kohlrabi	Control	2.72	2.47	18.65	13.43	0.98	0.051	2.47
	Conventional	1.88	0.95	19.98	14.63	1.54	0.060	0.95
	Cattle manure	2.14	2.52	20.70	10.79	1.06	0.054	2.52
	Poultry manure	3.00	3.72	34.05	17.44	2.52	0.071	3.73
Cauliflower	Control	2.07	3.43	24.30	10.50	1.25	0.060	3.43
	Conventional	1.76	1.70	17.91	9.55	0.98	0.056	1.70
	Cattle manure	2.68	3.30	21.38	10.24	1.17	0.045	3.30
	Poultry manure	5.10	6.44	30.44	17.16	2.25	0.063	6.44
Cabbage	Control	1.72	3.35	20.15	13.69	1.56	0.042	3.35
	Conventional	1.94	1.91	17.89	12.46	1.47	0.039	1.91
	Cattle manure	2.60	4.04	23.34	13.23	1.84	0.047	4.04
	Poultry manure	3.83	9.71	32.76	23.04	2.90	0.057	9.71
Species	Pak choi	4.39	4.32	56.41	19.11	1.82	0.097	4.32
	Kohlrabi	2.43	2.41	23.35	14.04	1.52	0.059	2.41
	Cauliflower	2.90	3.72	23.51	11.86	1.41	0.056	3.72
	Cabbage	2.53	4.75	23.54	15.61	1.94	0.046	4.75
Species		***	***	***	***	***	***	***
Fertilizer source		***	***	***	***	***	***	***
Interaction $LSD_{0.05}$		0.437	1.591	5.200	Ns	0.359	Ns	1.591

****, significant< 0.001 probability. ns, not significant <0.05 probability.*

Table (5): Effect of fertilizer source on vegetative growth and edible organs of *Brassica* vegetables.

Species	Fertilizer source	Plant fresh weight g plant^{-1}	Edible organs fresh weight g plant^{-1}	% Plant dry weight	% Edible organs dry weight
Pak choi	Control	92	37	10.33	10.23
	Conventional	181	72	7.97	7.97
	Cattle manure	590	236	5.92	5.92
	Poultry manure	678	271	6.33	6.33
Kohlrabi	Control	138	50	18.14	12.69
	Conventional	495	162	16.66	11.79
	Cattle manure	670	238	16.29	13.12
	Poultry manure	1132	338	15.10	12.26
Cauliflower	Control	218	92	19.75	10.66
	Conventional	570	367	22.28	12.02
	Cattle manure	800	382	19.93	12.06
	Poultry manure	1462	745	14.86	12.87
Cabbage	Control	477	130	16.82	10.52
	Conventional	930	374	17.29	9.83
	Cattle manure	1350	590	17.67	9.40
	Poultry manure	2395	1202	14.23	8.38
Species		***	***	***	***
Fertilizer source		***	***	***	ns
Interaction $LSD_{0.05}$		375.2	261.3	2.700	2.015

**** significant< 0.001 probability, ns, not significant <0.05 probability*

CONCLUSIONS

The high nitrogen content and mineralization of poultry manure could serve as the sole fertilizer source of nitrogen and possibly other nutrients for the *Brassica* vegetable tested under the conditions of this experiment. Cattle manure released insufficient amounts of nitrogen and other nutrients to meet the demand of *Brassica* plant. The amount added could be increased and further work is necessary to establish the quantities of cattle manure required so that might be used as the sole source of nitrogen and other nutrients for the growth of these vegetables. Although, poultry manure resulted in greater weight of edible organs for all the tested *Brassica* vegetables, NO_3_N was also increased to high levels particularly in pak choi
and kohlrabi. However, NO_3_N should pose no harmful threat to humans unless more than 640 g of these vegetables were consumed in one meal. Further studies will be required to test other sources of manure, in particular sheep manure at different levels to assess the possibility of using them as the sole nutrients source for annual crops.

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An investigated study of soil contamination of some heavy metals in two regions at north (Al-Tarmiya) and south (Hor-Rejab) of Baghdad

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ABSTRACT

The pollution of soil is a source of danger to the health of people, even to those living in cities. Municipal solid waste, in developing cities projects a strong threat to soil environment. (Pb, Zn, Cu, Cd, Co, Cr and Ni) are considered environmentally important heavy metals in soil. An investigation is conducted on the concentration of these heavy metals in six sites divided into two regions at north (Al-Tarmiya TZ: given the symbols T1, T2, T3 zones) and south(Hor-Rejab RZ: given the symbols R1, R2, R3 zones) of Baghdad. The highest concentration of heavy metals Pb and Zn are presented in the following sites:
TZ1, RZ1 > TZ2, RZ2 > TZ3, RZ3 and
Metals Cu, Co, Cr and Niare presented in the following sites:
TZ2, RZ2 > TZ3, RZ3 > TZ1, RZ1
While metal Cd is ordered as:
TZ2, RZ2 > TZ1, RZ1 > TZ3, RZ3
pH is regarded as the most important factor affecting metal solubility in soil Organic matters, soluble salts, sulfates and chlorides in addition to carbonates were also measured to depict complexity and mobility of heavy metals.

Key words: soil contamination, heavy metals(Pb, Zn, Cu, Cd, Co, Cr and Ni), soil factors (pH,organic matter, total soluble salts, sulfates, chlorides and carbonates).

الملخص باللغة العربية

أصبح تلوث التربة في العراق حالياً مصدراً خطراعلى صحة الانسان وتمثل المخلفات الصلبة في المدن النامية الجديدة تهديداً حقيقياً لبيئتها. أجري التحليل الكيميائي لنما ذج من التربة في ستة مواقع موزعة على منطقتين رئيستين أحدهما في شمال بغداد (الطارمية وقد أعطيت الرمزTZ) والثانية في جنوبها (هور رجب وقد أعطيت الرمز RZ) اذ قدرت تراكيز الرصاص، الخارصين، النحاس، الكادميوم،الكوبلت، الكروم والنيكل فيها.
نتائج التحليل أوضحت أعلى تركيز للملوثين الرصاص والخارصين قد توزع بالشكل التالي:
TZ1, RZ1 > TZ2, RZ2 > TZ3, RZ3
فيما أعلى تركيز للملوثات النحاس، الكوبلت، الكروم والنيكل قد توزعت على المواقع بالترتيب الاتي:
TZ2, RZ2 > TZ3, RZ3 > TZ1, RZ1
أما أعلى تركيز للملوث الكادميوم قد وجد كما في أدناه:
TZ2, RZ2 > TZ1, RZ1 > TZ3, RZ3
أجري قياس قيم الأس الهيدروجيني للنماذج كونه من العوامل المؤثرة على ذوبانية العناصر الثقيلة في التربة، كما أجريت فحوص المواد العضوية، الاملاح الذائبة الكلية، الكبريتات والكلوريدات وكذلك الكاربونات للحصول على تصور واضح للمعقدات التي تكونها العناصر الثقيلة وبالتالي امكانية معرفة حركة هذه الملوثات.

INTRODUCTION

Soil health is defined as the continued capacity of soil to sustain its biological productivity, maintain the quality of the surrounding air and water environments, and promote plant, animal, and human health. Soil health is threatened by various materials from human activities, which include industrial pollutants, pesticides, live stock waste water, and petroleum contamination (1).

The boundaries between toxicity, sufficiency and deficiency are vague. The amounts of elements vary with species of plant and animal, vary within the specie's growth cycle and vary with the organism's general health and the supply of the other essential metals.

Contamination of soil with heavy metals is becoming one of the most severe environmental and human health hazards. Elevated levels of heavy metals not only decrease soil microbial activity and crop production, but also threaten human health through the food chain (2).

A.K. Krishna and P.K. Govil determined extent and distribution of heavy metals (Ba, Cu, Cr, Co, Ni, Sr, V and Zn) to find out the large scale variability of those metals in soils taken from an industrial area from top 10cm layer of the soil (3).

Jose Luis Moreno *et. al.* studied the contamination of an agricultural soil with the heavy metals (Cd, Zn, Ni, Cu, Fe and Mn) contained in different sewage sludge composts and their effect on a crop grown in the soil and to evaluate the transfer of these heavy metals to the food chain (4).

E. Tipping *et.al.* analyzed total heavy metal concentrations in soil solution and predicted free metal ion concentration from solution-only speciation calculations (5).

Open dumps unfortunately still mostly observed in developing cities, where the waste is dumped in uncontrolled manner, can be detrimental to the urban environment. The solid waste is dumped in the most unscientific manner is heterogeneous in nature. The municipal solid waste in addition to biodegradable waste such as cellulose, lignin, protein, lipids and motor oils, also possess a variety of chemicals like detergent, inorganic chemicals and complex organic chemicals and metals. These compounds are themselves very much toxic for the environment and additionally uncontrolled microbial action may result in release of more toxic elements which were not present in a free or reactive form in the waste (6).

Other different source of heavy metals to the urbanized areas is vehicle emission.

It has been noted that locations close to roads are severally polluted by heavy metals from traffic. Generally, the distribution of these metals is influenced by the nature of parent materials, climate and their relative mobility (3,7).

The aim of the study:

1. Determine the heavy metals (Pb, Zn, Cu, Cd, Co, Cr and Ni) concentrations in soils in two development cities at north and south of Baghdad.
2. Determine some important soil factors that influence on the migration of heavy metals concentrations in the soils under study.

MATERIALS AND METHODS

Materials:

HCl (37%), HNO_3(65%), barium chloride, potassium chromate and dichromate, silver nitrate and ammonia were used.

All of these reagents and materials were provided from BDH with high purity.

Procedure:

(Al-Tarmiya city) at north and (Hor-rejab city) at south of Baghdad are two suburb population regions with a little tend to be rural life style that symbolize new small slum development cities with many sprawling activities inside each one. The two cities were chosen as they were to some extent a stage of military operations after 2003 with huge lack of infrastructure services most important is the open dumped of waste in uncontrolled manner between and inside the neighbors.

The concentrations of soil heavy metals were determined in each city and for the purpose of research; each region is divided into three zones as below:

Region TZ:

1- Al-Tarmiya zone (TZ1): 5 meters away from outside main road that headed to the city.

2- Al-Tarmiya zone (TZ2): 2 meters away from drive road inside and through the center of the slum city with many different sprawling a ctivities (local domestic in dustrial and agricultural purposes, cars and generators repairs, household, painting, etc.) distributed at random sites.

3- Al-Tarmiya zone (TZ3):5 meters away and around an open dump solid waste abandonment area inside the city.

Region RZ:

1. Hor-Rejab zone (RZ1):5 meters away from outside main road that headed to the city.

2. Hor-Rejab zone (RZ2):2 meters away from drive road inside and through the center of the slum city with many different sprawling activities (industrial and agricultural purposes, cars and generators repairs, household, painting,etc.) distributed at random sites.

3. Hor-Rejab zone (RZ3):5 meters away and around an open dump solid waste abandonment area inside the city.

Sampling of soil before analyzing is of a great importance and precautions steps were taken as follow:

Step 1: Three different holes with an area of 80× 80 cm^2 each were chosen to be dug for each region and marked as mentioned above.

Step 2: A layer of about 1cm was scraped off the surface of the holes.

Step 3: 2Kg net weight from three different spots of each hole with a deep through 15 cm are taken for analysis, mixed well and homogenized to be one specimen and kept in a tied plastic pack.

Step 4: The homogenized specimen was oven dried, powder crushed then sieved through a 2 mm sieve and finally dissolved and extracted in hydrochloric acid and/or nitric acid.

Step 5: The extracts were collected by filtration through whatman no.42 filter paper and analyzed for (Pb, Zn, Cu, Cd, Co, Cr and Ni) using Atomic Absorption Spectrophotometer model 500 Perkin Elmer – USA.

Step 6: The soil pH values, organic matter, carbonate, sulfate and chlorides were determined according to BS 1377(8).

Step 7: Total soluble salts was carried on according to Earth Manual (9).

The values of soil factors and heavy metals represent the average , and all the samples for the purpose of analysis were taken in April/2012.

RESULTS AND DISCUSSION

Table (1) shows some factors of soil samples under study with their RSD%.

The pH values of all samples at different zones are ranged between 6 and less than 7 as the solubility of these heavy metal ions are vary widely. In general heavy metal cations are most mobile under acid conditions (10,11).

Organic matter content (O.M.) is ranged between (0.77% – 1.72%) in region A and between (0.32%–2.28%) in region B. Generally, the organic fraction is the most chemically active portion of the soil. Soil organic matter is mostly an accumulation of dead plant matter and partially decayed are synthesized plant and animal residues. Arid and semiarid soil thought to have low organic matter because the soils lack the dark color that characterizes organic matter in temperate soils. Organic matter forms stable complexes with Cu^{+2}, Zn^{+2} and other polyvalent cations, the insolubility of organic matter results partially from association with clay; salts of divalent cations with organic matter are also insoluble while isolated organic matter is partly soluble in water.Organic matter buffers soil pH in the slightly acid and neutral ranges. Decomposition of organic matter yields CO_2, NH_4^+, NO_3^-, PO_4^{-3} and SO_4^{-2}(12).

Total soluble salts (T.S.S.) are ranged between (0.20% – 0.29%) in region A and between (0.20% - 0.25%) in region B. The concentration of the soluble salts in the soil solution is influenced by the moisture content (13).

Sulfate content is less than 1% for the two regions and this also applies to the chloride content. Sulfate is readily soluble and widely involved in soil equilibrium processes, while chlorides as being considered soluble salts in arid soils, they easily form soluble complexes with Cd cation (14).

Carbonate content is ranged between (44.0%-49.5%) in region A and between (42.2%-49.0%) in region B. Carbonates affect soil texture. The heavy metals (Pb, Zn, Cu, Cd, Co, Cr and Ni) may co-precipitate with greatest affinity with carbonates or sorbed by oxides that were precipitated onto the carbonates (14).

Table (2) demonstrates the concentrations of the heavy metals (Pb, Zn, Cu, Cd, Co, Cr and Ni) in ppm with their RSD% in the two regions (Al-Tarmiya) and (Hor-rejab) cities.

Table (1): Factors of soil samples with their RSD% in the two regions A and B at north and south of Baghdad

location		pH	O.M(RSD%)	T.S.S(RSD%)	%SO_3(RSD%)	%Cl(RSD%)	%CO_3(RSD%)
Region (A)	TZ1	6.0	0.77(0.041)	0.29(0.054)	0.08(0.004)	0.080(0.002)	44.0(1.385)
	TZ2	6.7	1.70(0.149)	0.25(0.029)	0.07(0.006)	0.078(0.001)	49.5(1.563)
	TZ3	6.6	1.72(0.152)	0.20(0.045)	0.05(0.002)	0.047(0.002)	46.0(1.571)
Region (B)	RZ1	6.1	0.32(0.091)	0.25(0.002)	0.10(0.009)	0.109(0.091)	42.2(1.364)
	RZ2	6.9	0.73(0.150)	0.22(0.007)	0.09(0.003)	0.075(0.005)	49.0(1.518)
	RZ3	6.6	2.28(0.190)	0.20(0.006)	0.05(0.004)	0.071(0.006)	44.0(1.781)

Table (2): Concentrations of the heavy metals (Pb, Zn, Cu, Cd, Co, Cr and Ni) in ppm with their RSD% in the two regions A and B

Location		Pb(RSD%)	Zn(RSD%)	Cu(RSD%)	Cd(RSD%)	Co(RSD%)	Cr(RSD%)	Ni(RSD%)
Region (A)	TZ1	70(1.25)	61.7(0.21)	7.6(0.25)	8.24(0.12)	13.3(0.55)	45(1.24)	97.0(0.81)
	TZ2	56.7(0.49)	61.0(0.21)	9.5(0.20)	8.82(0.16)	14.0(0.25)	50(0.94)	111.8(0.77)
	TZ3	36.7(0.45)	54.3(0.40)	9.4(0.20)	8.20(0.09)	14.7(0.24)	52(0.47)	102.9(0.75)
Region (B)	RZ1	41.7(0.25)	74.3(1.08)	9.8(0.31)	5.00(0.21)	14.7(0.49)	50(1.04)	91.2(0.47)
	RZ2	41.6(0.26)	62.2(0.75)	11.7(0.58)	8.83(0.24)	15.1(0.12)	51(1.00)	105.9(0.53)
	RZ3	38.3(0.40)	59.3(0.94)	11.0(0.36)	3.83(0.08)	15.3(0.26)	52(1.41)	100.0(1.02)

Lead concentrations are between (36.7-70) ppm in region A and (38.3-41.7) ppm in region B. Gasoline combustion being the main Pb source most of the lead, will be deposited as the soluble and insoluble compounds. Lead is also used in the production of batteries. Because of the formation of these solids and the adsorption of Pb when present as divalent cation, lead displacement in soils is mostly small (15).

Zinc concentrations are between (54.3-61.7) ppm in region A and (59.3-74.3)ppm in region B. One of the major factors controlling Zn availability is the pH. The possible accumulation of zinc in soils after disposal of waste materials and its consequences deserve special attention. Total Zn levels in soil are up to 300ppm, with 30-50ppm as a rough average value (15).

Copper concentrations are between (7.6-9.5) ppm in region A and (9.8-11.7) ppm in region B. Prime use of Cu is as wire and brass, and as alloys with a number of different other metals. Most water supply systems consist of copper tubing. Normal Cu contents of soil are around 20ppm with variations over the range 2-100ppm. Mobility and displacement of Cu in soils is slow, as a large number of Cu complexes are known to occur in soils (15).

Both zinc and copper ions increase with increasing soil acidity and are complexed strongly by soil organic matters.

Cadmium concentrations are between (8.2-8.82) ppm in region A and (3.83-8.83) ppm in region B. Cadmium is widely used as a coating material, applied in batteries, in motor oil and car tires which explain to some extent the relative accumulation in roadside soils. Cd contents of soil in non-polluted areas are usually below 1 ppm.

Cadmium is relatively soluble and its retention in soil is relatively in dependent of pH. Its complexion might contribute to the mobilization of Cd in the environment (15, 16).

Cobalt concentrations are between (13.3-14.7) ppm in region A and (14.7-15.3) ppm in region B. It is used in the production of alloys and in paints, varnishes, enamels and inks. Co contents of soil usually do not exceed about 10ppm.

Co is one of the heavy metals that are known to be subjected to chelation in soils. Due to previously expectation, Co concentrations is

low in waste products;so there has been little concern so far on hazardous effects of this heavy metal in soils (15).
Chromium concentrations are between (45-52) ppm in region A and (50-52) ppm in region B. Many metals, used in household, traffic and industry are chrome plated to extend their durability. Use of chromium in smaller quantities is made in the production of varnishes, inks and dyes. The Cr content of most soils is usually limited to traces only (15).
Nickel concentrations are between (97-111.8) ppm in region A and (91.2-105.9) ppm in region B. nickel is used in the production of steels and alloys. It is applied in paint pigments, cosmetics and in the production of machinery parts, batteries and electrical contacts. Total Ni-content of soil may vary from 5-500ppm, with 100ppm as a rough mean value (15).
Nickel ion is expected to be adsorbed on the soil complex.
Figures (1& 2) illustrate the concentrations of the heavy metals (Pb, Zn, Cu, Cd, Co, Cr and Ni) under study in both the two major regions at north (Al-Tarmiya) and south (Hor-Rejab) of Baghdad.

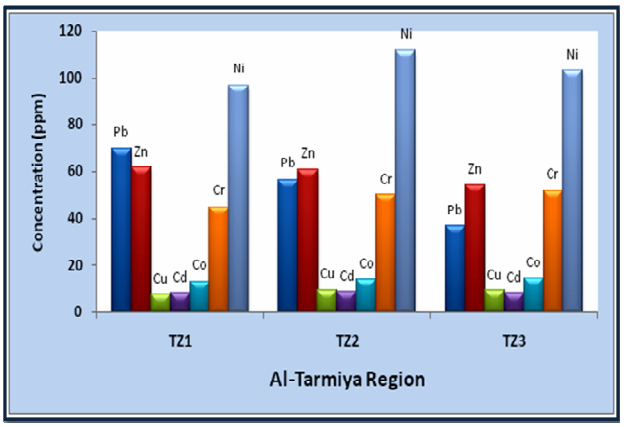

Fig (1): illustrates the concentrations of the heavy metals (Pb, Zn, Cu, Cd, Co, Cr and Ni) in (Al-Tarmiya) soil at north of Baghdad

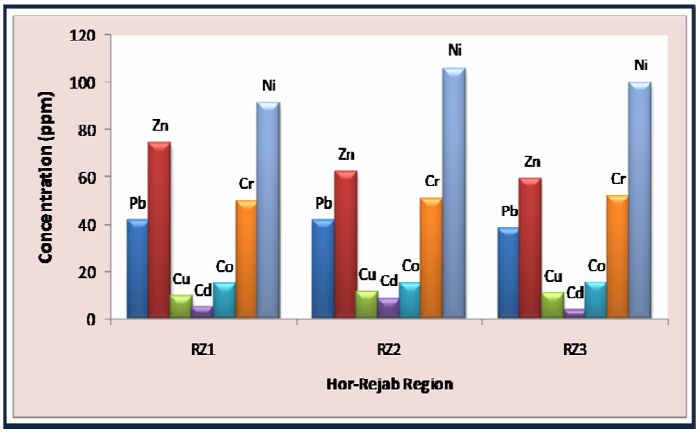

Fig (2): illustrates the concentrations of the heavy metals (Pb, Zn, Cu, Cd, Co, Cr and Ni) in (Hor-Rejab) soil at south of Baghdad

CONCLUSION

The concentrations of the heavy metals (Pb, Zn, Cu, Cd, Co, Cr and Ni) were measured in six sites in two major regions located at north (Al-Tarmiya: TZ) and south (Hor-Rejab: RZ) of Baghdad as recently being considered as new slum development cities. The research concluded that the highest concentrations of the heavy metals measured in soils of the two suburb cities were arranged as follows:
1.For metals Pb and Zn respectively TZ1(70ppm,61.7ppm),RZ1(41.7ppm,74.3pp > TZ2(56.7ppm,61.0ppm),RZ2(41.6ppm, 62.2ppm)>TZ3(36.7ppm,54.3ppm), RZ3(38.3ppm, 59.3ppm).
2. For metals Cu, Co, Cr and Ni respectively TZ2(9.5ppm, 14.0ppm, 50.0ppm and 111.8ppm), RZ2(11.7ppm, 15.1ppm, 51.0ppm and 105.9ppm)> TZ3(9.4ppm, 14.7ppm 52.0ppm and 102.9ppm), RZ3(11.0ppm, 15.3ppm, 52.0ppm and 100.0ppm)> TZ1(7.6ppm, 13.3ppm, 45.0ppm and 97.0ppm), RZ1(9.8ppm, 14.7ppm, 50.0ppm and 91.2ppm).
3.For metal Cd: TZ2(8.82ppm), RZ2 (8.83ppm)>TZ1 (8.24ppm), RZ1 (5.0ppm)>TZ3 (8.2ppm), RZ3 (3.83ppm)

The study points out that the concentration of metal ions is increasing with respect to the vicinity to the dumping site, and continued practice of waste dumping in the similar way may result in further increments of metal ions aggregation and pollution of environment, further more, the contribution of deposition of particles from urban sources (such as electrical stations or refineries, etc) as industrial

emission. It also has been noted that locations close to roads are severally polluted by heavy metals such as Pb, Zn and Cd from traffic waste disposal.

One aid to developing a perspective of soil contamination is to arrange the elements according to their behavior in soils viewing in mind that all substances are toxic above an ill-defined threshold concentration that varies with each substance.

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General multi-component rate model for adsorption of Cd(II) and Pb(II) ions from aqueous Solution by Sea Shell powder

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ABSTRACT

Many of industrial wastewater effluents contain a wide range of heavy metals. The adsorption of Cd^{2+} and Hg^{2+} metal ions from aqueous solutions by sea shell powder was studied. The results showed that maximum adsorption capacity occurred at 471.5×10^{-3} mg/kg for Pb^{2+} ion and 531.6×10^{-3} mg/kg for Cd^{2+} ion. The adsorption in a mixture of the metal ions had an equilibrium effect on the adsorption capacity of the sea shell powder. The adsorption capacity of each metal ion was influenced by the presence of other metal ions rather than its presence individually. The General Multi-Component Rate Model were used to describe the adsorption kinetics mathematically, The study showed the adsorption process obeys the Freundlich isotherm for both ions.

Key words: Adsorption, heavy metal ions, multi-component rate model, sea shell powder

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الملخص باللغة العربية

تحتوي الكثير من النفاياتا السائلة المتخلفة من مياه الصرف الصناعي على مجموعة واسعة من أيونات المعادن الثقيلة، وقد تمت دراسة خاصية الامتزاز لكل من أيونات الكادميوم Cd^{2+} وأيونات الزئبق Hg^{2+} من المحاليل المائية باستخدام مسحوق صدف البحر.

أظهرت نتائج الدراسة بأن أقصى سعة امتزاز وقعت عند 471.5×10^{-3} mg/kg لأيون الرصاص Pb^{2+}، وعند 531.6×10^{-3} mg/kg لأيون الكادميوم Cd^{2+} .

كما تبين أن امتزاز خليط من أيونات المعادن الثقيلة كان له تأثير إحداث التوازن في سعة الامتزاز بواسطة مسحوق صدف البحر، حيث كانت سعة الامتزاز لكل أيون معدني متأثرة بوجود أيونات المعادن الأخرى بكشل أكبر مما لو كان الامتزاز فرديا.

في الدراسة الحالية، تم استخدام نموذج النسب العام للمركبات المتعددة لوصف حركية الامتزا بتعبير رياضي، حيث أوضحت الدراسة أن هناك امتثالا من عملية الامتزاز للمركبات ذات التشابه التركيبي الكيميائي للأيونات المعدنية.

INTRODUCTION

A great interest in the research for the removal of heavy metals from industrial effluents had concentrated on using materials such as sea shell powder that is easy handle, economical and durable materials. Adsorption process considered as technique for the removing of heavy-metal from industrial, mineral and petroleum industry, (1). This process is mainly less cost and can be executed on site, hence, reducing the hazards of transport the toxic materials to the de-pollution sites. The adsorption process is a good alternative for the recovery of metals contained in other media (2).

The use of sea shell powder as filters were introduced in the 1947's for the ultra pure of water in the food industry. Industry has also taken advantages of the unique ability to adsorb a variety of organic and non-organic compounds by utilizing the crushed sea shell in industrial wastewater treatment (3).

The Freundlich equation has been widely used for many years. This equation which originally proposed as an empirical equation is used to describe the data for the heterogeneity in the adsorbent surfaces, in which the energy term (b) in the Langmuir equation varies as a function of surface coverage (q_e), that due to variations in the heat of adsorption (***4***).

Freundlich studied the adsorption phenomenon and showed that adsorption from solution could be expressed empirically by:

$$q_e = K C_e^{1/n} \quad \ldots (1)$$

When, (K and n) are constants, and n > 1 (***5***).

As Freundlich equation is an empirical equation then it is useful as a means for data description. Data are usually fitted to the logarithmic equations as follows:

$$\text{Log}\, q_e = \text{Log}\, K + \frac{1}{n} \text{Log}\, C_e \quad \ldots (2)$$

This equation gives a straight line with a slope of (1/n) and an intercept equal to the value (Log K), for C = 1. The intercept is roughly indicated of adsorption capacity.

Freundlich equation generally agrees quite well for the experimental data of a wide range of concentration (6).

Zenedy and Murphy found that the equilibrium data for adsorption of mercury onto activated carbon were correlated well with Langmuir and Freundlich equations.

Prasert and Parasaut found that the pistachios shell can be examined for removal of copper and lead from aqueous solution and the Freundlich isotherm models well fitted the data (7).

Badmus and Anyate showed that the adsorption isotherm of zinc onto reed bed seemed generally to approach Freundlich models (8).

Diarati and Taliki showed that the Freundlich isotherm fits well the data of adsorption of chromium by powdered activated carbon at 24^{o}C (9).

Qader and Akhtars found that the adsorption kinetic study of lead and cadmium individually, the resulted data fits well Freundlich isotherm (10).

The mechanism of adsorption may be particle diffusion controlled (11) or a film diffusion controlled depends on many parameters that will dominate which mechanism of the above of favorable depends on the slowest step of the adsorption process that will consider the rate step. Here the result of the amount adsorbed against time for a mixture of the metal ions.

Since adsorption is a particle diffusion controlled (12) and this could be affected by the following processes:

(i) diffusion of the solute from the solution to the film surrounding the particle;
(ii) diffusion from the film to the particle surface (external diffusion);
(iii) diffusion from the surface to the surface to the internal sites (surface diffusion or pore diffusion), and
(iv) Uptake which can involve several mechanisms: physicochemical sorption, ion exchange, precipitation or complexion (13). The first process is bulk diffusion, the second is the external mass transfer resistance and the third is inter-particle mass transfer resistance.

When the adsorption is particle diffusion controlled, it means that inter-particle mass transfer resistance is rate limiting. Therefore, in the presence of a mixture of the metal ions, the metal ions (i.e. compete) for the adsorption sites on the adsorbent. This competition affects the diffusion properties of the metal ions, hence decreases the adsorption capacity of the metal ions. Thus, the metal ion that successfully reaches the adsorption site faster depends on the above factors and also on the ionic radii of the metal ions. Competition among the metal ions for adsorption sites deviously affected the adsorption capacity (11).

It seems that surface attachment might also be taking place on the functional groups on the surface of the adsorbent. More of what happens is volumetric filling of the micro-pores found in the adsorbents. Since adsorption takes place in these micro-pores (14), these results decrease in the amount of metal adsorbed with time, by inspection of the plots, the application of the lagergen equation (equation 3) shows a zero order reaction. This is true since amount adsorbed remain fairly

constant with increased time. The lagergen equation is given by:

$$\text{Log } (q_e-q) = \log q_e - K_{ad}\, t/2.303. \quad \ldots (3)$$

Where q is amount adsorbed (mg/g) at time t, q_e is amount adsorbed (mg/g) at equilibrium time and K_{ad} is the rate constant of adsorption (min^{-1}) (11).

General Multi-Component Rate Model (GMRM)

It is most used and detailed model available to describe the adsorption process (11). This model takes into consideration all the phenomena affecting the adsorption process. Such model is widely used in many cases to describe the adsorption and mass transfer process in multi-component adsorption (8). Zhank used a binary system consists of (Zn, Cr) from electroplating industry and from the simulation results, agreement between the experimental data and calculated value were good (15).

Wright investigated the use of (GMRM) to simulate the binary adsorption system consists of (Cr, Mn) and the simulation gave a good matching between the experimental and calculated data(16).

Khanna conducted the (GMRM) to simulate the adsorption of (Fe, Mn) from iron industry and the model fitted well for experimental and theoretical data (17).

The general multi-component rate model indicates a model that has a rate expression, which describes the mass transfer between the mobile phase and the stationary phase .

Generally, such model is widely used in many cases to describe the adsorption and mass transfer processes in a multi-component systems.

The general multi-component rate model which takes into consideration (18) axial dispersion, external mass transfer, inter-particle diffusion and nonlinear isotherm.

For the modeling of multi-component adsorption, the column is divided into two phases. The bulk-fluid phase and the particle phase. This approach considers three phases (19): the mobile phase flowing in the space between particles, the stagnant film of mobile phase immobilized in the macro-pores and the stationary phase where adsorption occurs.

Model Assumptions

The following basic assumptions are considered in order to set-up a general rate model (19):

- The compressibility of the mobile phase is negligible.
- The adsorption process is isothermal. There is no temperature change during a run.
- The adsorbent particles in the column are spherical and uniform in diameter.
- The concentration gradients in the radial direction are negligible.
- The fluid inside particle (macro-pores) is stagnant, i.e., there is no convective flow inside macro-pores.
- An instantaneous local equilibrium exists between the macro-pore surface and the stagnant fluid inside macro-pores of the particles.
- The film mass transfer mechanism can be used to describe the interfacial mass transfer between the bulk-fluid and particle phases.

All the mechanisms which contribute to axial mixing are gathered into a single axial dispersion coefficient (18).

Model Equations

Depending on the above assumptions, A set of equations can be formulated from differential mass balances of the bulk-fluid phase and the particles phase, respectively, for component i. The following equations can be derived from equations of continuity (11).

1. Continuity Equation in the Bulk-Fluid Phase

$$-D_{bi}\frac{\partial^2 C_{bi}}{\partial Z^2}+\nu\frac{\partial C_{bi}}{\partial Z}+\frac{\partial C_{bi}}{\partial t}+\frac{1-\varepsilon_b}{\varepsilon_b}\frac{\partial q_i}{\partial t}=0 \quad (4)$$

Transport by axial dispersion in the bulk-fluid phase; Convective transport in the bulk-fluid phase; Accumulation in the bulk-fluid phase; Accumulation in the particle phase

Using C_{pi}, the concentration in the stagnant fluid-phase (in the macro-pores), and writing the expression of flux leads to:

$$\frac{\partial q_i}{\partial t}=\frac{3k_{fi}}{R_p}\left(C_{bi}-C_{pi,R=R_p}\right) \quad (5)$$

Substitution of equation (5) into equation (4) gives:

$$-D_{bi}\frac{\partial^2 C_{bi}}{\partial Z^2}+\nu\frac{\partial C_{bi}}{\partial Z}+\frac{\partial C_{bi}}{\partial t}+\frac{3k_{fi}(1-\varepsilon_b)}{\varepsilon_b R_p}\left[C_{bi}-C_{pi,R=R_p}\right]=0 \quad (6)$$

2. Continuity Equation Inside the Macro-pores

The particle phase continuity equation in spherical coordinates is:

$$(1-\varepsilon_p)\frac{\partial C^{*}_{pi}}{\partial t}+\varepsilon_p\frac{\partial C_{pi}}{\partial t}-\varepsilon_p D_{pi}\left[\frac{1}{R_p^2}\frac{\partial}{\partial R_p}\left(R_p^2\frac{\partial C_{pi}}{\partial R_p}\right)\right]=0 \quad (7)$$

Accumulation in the micro-pores stationary phase

Accumulation in the macro-pores

Radial diffusion inside the porous particle

$$C_{bi}=C_{bi}(0,Z)=0$$

$$C_{pi}=C_{pi}(0,R,Z)=0$$

$$Z=0: \quad \frac{\partial C_{bi}}{\partial Z}=\frac{\nu}{D_{bi}}\left(C_{bi}-C_{oi}\right)$$

$$Z=L: \quad \frac{\partial C_{bi}}{\partial Z}=0$$

$$R=0: \quad \frac{\partial C_{pi}}{\partial R}=0$$

$$R=R_p: \quad \frac{\partial C_{pi}}{\partial R}=\frac{k_{fi}}{\varepsilon_p D_{pi}}\left(C_{bi}-C_{pi,R=R_p}\right)$$

Model Solution

The model becomes nonlinear whenever a nonlinear isotherm, such as the Langmuir isotherm is used. A true multi-component case is almost certainly nonlinear, since no linear isotherm can be used to describe true multi-component adsorption. For such a nonlinear multi-component model, there is no analytical solution. The model equations must be solved numerically.

The numerical method of lines is used in order to obtain, through space discretization of the PDE system, an ODE system which can be solved with a common ODE solver. The bulk-fluid phase and the particle equations are first discretized using the finite element (FE) and the orthogonal collocation (OC) methods, respectively (19). The resulting ODE system is solved using an existing ODE solver provided by MATLAB.

The scope of the study

In the current study, sea shell powder was used as adsorbent for the removal of Cd(II) and Pb(II) ions and the mechanisms of sorption were investigated respectively. The effect of having two metal ions in the wastewater had been studied. Since the pollution of the environment with heavy metals is important and as result of many human industrial, agricultural and petroleum activities, it means that these effluents would carry heavy metal ions in solution.

MATERIALS AND METHODS

All materials used are from the Iraqi market, a solution of $Cd(NO_3)_2.H_2O$ and $Pb(NO_3)_2.2H_2O$ were prepared with initial concentration of 1000 mg/l by the dissolving 1.35 gm of $Pb(NO_3)_2.2H_2O$ in 4.3 l of distilled water and 1.21 gm of $Cd(NO_3)_2.H_2O$ in 4.6 l distilled water. The sea shell was first collected from Al-Razzazza artificial lake (107 km south west of Baghdad), the sea shell then washed with distilled water and dried in the oven at 85 C for 24 hour, then the sea shell were crushed and grinded by a ball mill (Clarkson) with an average particle size used is 600 μm, the process of screening was held in the petroleum research and development center lab / Ministry of Oil using screen shaker of type (BAUS). 40 ml of each of two metal ions were mixed and put in a conical flask containing 50 g of sea shell powder. The flask was uniformly agitated at a temperature of 25^{o}C and optimum pH of 7 using a rotary shaker. Figure (1.a) shows the agitation process using rotary shaker at constant agitation speed.

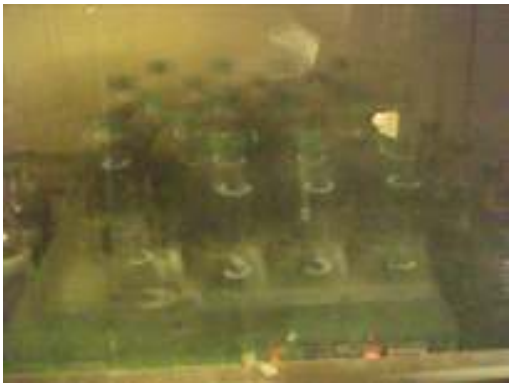

Fig. (1.a): Agitation process for different flasks

The experimental set-up was repeated for time intervals of 20, 30, 40, 50, and 60 min after the end of every (10min) a sample was taken from the flask and the concentration of (Pb, Cd) in the filtrate water was measured using atomic absorption device in the Baghdad environmental directory / Ministry of Environment. Figure (1.b) shows the samples after treatment with sea shell powder.

Fig. (1.b): The samples after treatment with sea shell powder

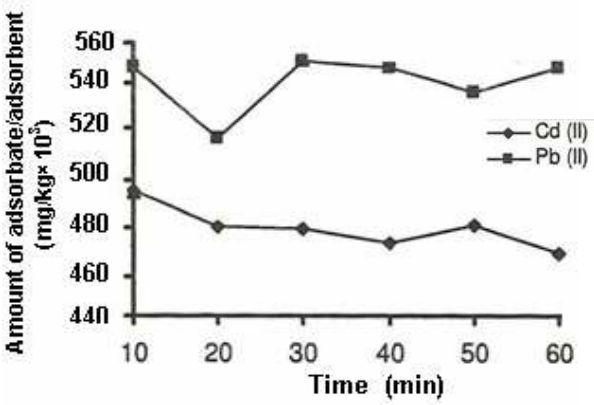

Fig. (2): Amount adsorbed vs. time for Cd(II), Pb(II) ions on sea shell powder of 600µm average particle size at 1000 mg/l initial mixed metal ion concentration

RESULTS AND DISCUSSION

The amount of the adsorbate adsorbed at different time interval for both Cd, Pb ions were measured and table (1) refers the values of these amounts.

Table (1): Amount of adsorbate adsorbed by sea shell powder

Time (min)	Amount Pb adsorbed (mg/kg)×10^3	Amount Cd adsorbed (mg/kg)×10^3
10	531.60	471.50
20	515.20	470.11
30	547.31	473.20
40	542.01	471.75
50	530.57	475.54
60	545.71	436.71

Figure (2) shows the amount of metal adsorbed against time for sea shell powder, 1000 mg/l initial metal ions concentration and 600 μm average particle size.

From Figure (2), its clear that the adsorption was unsteady for Pb(II) but varied a little for Cd(II) on sea shell powder. The adsorption will expected to become less varied in the curve behaviour as preceding the time interval. By applying (equation 1) the equilibrium concentration for each ions C_e at different q_e values were calculated and tabulated in Table (2).

Table (2): Equilibrium concentration C_e (mg/l) for Cd and Pb

Cd	Pb
862.60	878.20
871.20	880.70
863.17	881.70
864.49	882.06
866.07	881.11
863.57	890.89

By applying the linearized form of Freundlich isotherm model, Equation (2) to the data in Tables (1) and (2) and plotting the equation, a straight line will be resulted according to the model isotherm as in Figure (3) which is refer to a good fitting of the data with Freundlich isotherm for both Cd and Pb.

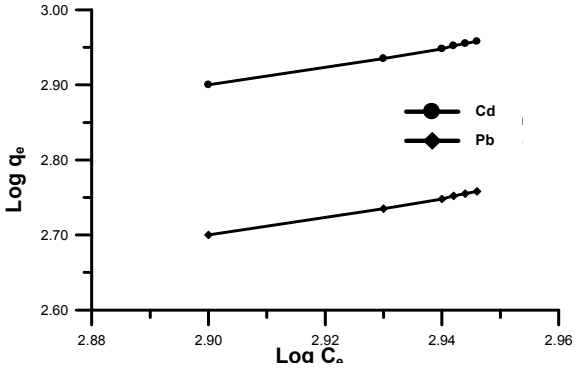

Fig. (3): The linearized form of the equilibrium concentration for both Cd and Pb

CONCLUSION

The amount of Cd(II), Pb(II) metal ions in aqueous solutions adsorbed did not increase as time increased as expected. Rather, the amount adsorbed remained fairly constant with time during the competitive sorption. This was attributed to the fact that all the metal ions, will have to be struggling for the same number of adsorption sites at the same time. Therefore, this study significantly reveals that the presence of other heavy metals and chemicals are influential factors and should be design parameters in the treatment and management of heavy metal pollutants using sea shell powder. The multi-component rate model gave a good explanation for the adsorption of the two ions onto sea shell powder , as a results from the MATLAB , applications referred to that as results from the computer program.

Furthermore, the fluctuation in the amount of Cd adsorbed with respect to time rather than Pb may attribute to the high affinity of the Pb rather than Cd for adsorption onto sea shell powder due to the variation in the electro-charges properties of both ions. As Pb is more attracted (attached) than Cd on the vacant sites of the sea shell powder due to is less positivistic than Cd. Both Cd and Pb obeys well Freundlich isotherm.

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Assessment of some serological tests for the diagnosis of Acute human *Brucellosis*

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ABSTRACT

Brucellosis is a public health problem in many developing countries including Iraq. The most reliable diagnosis of an infectious disease is confirmed by isolation of its pathogen by culture ,but It is well known that culture for *Brucella* is relatively difficult, time consuming and very hazardous for laboratory workers therefore it is reasonable to use serological tests for routine diagnosis of this important disease in human patients . In order to choose and evaluate the most applicable test in term of speed of performance, and accuracy , three serological tests were assayed:
Rose Bengal plate test (RBPT) , Modified Rose Bengal with 2ME ,and Standard tube agglutination test (STAT) for Brucellosis. The fourth test assayed was *anti brucella- IgM* by Enzyme-linked immunossorbant assay (ELISA) which was used as a referent test to compare the results of the above tests with it for its high specificity and sensitivity.
Blood samples were obtained from 210 patients who were highly suspected of having Brucellosis . The above 4 serological tests were performed on all serum samples . Out of the total samples , 118(56.2%) gave positive results by Roes Bengal plate test (RBPT), 58(27.6%) by 2ME , 130 (61.9%) by STAT ,and 70(33.3%) by Elisa. All negative cases by RBPT gave negative results by 2ME. Twelve samples of negative results by RBPT and 2ME were found to be positive by STAT . The sensitivity of RBPT ,2ME ,and STAT were found to be 100%,82%,and 100% respectively ,while the specificity of these three serological tests were found to be 74%,82%,and 97% respectively as compared with the results of *anti Brucella –IgM* by ELISA .

Key words: Brucellosis , Serodiagnosis , Rose Bengal , ELISA

الملخص باللغة العربية

تعد حمى مالطا من المشاكل الصحية المهمة في الدول النامية بضمنها العراق. إن من أفضل الطرق لتشخيص المسببات المرضية البكتيرية بشكل عام هو الزرع البكتيري ولكن من المعروف ان استنبات الجرثومة المسببه لحمى مالطا يتميز بصعوبة نسبية إضافة إلى انه يستغرق وقتا طويلا كما أن فيه خطورة عالية على العاملين في المختبر وعليه فقد صممت الدراسة الحالية بهدف تقييم فاعلية ثلاث طرق مصلية لتشخيص المرض هي الفحص المباشر للمصل باستعمال طريقة Rose Bengal والطريقة المحورة بإضافة 2MEوتخفيف الأنابيب القياسي STAT وإجراء فحص الأجسام المضادة النوعية *anti Brucella –IgM* بطريقة الاليزا لغرض مقارنة النتائج به وتقييم مدى الاستفادة من هذه الفحوص لتشخيص المرض دون الرجوع إلى الزرع . تم جمع نماذج دم من 210 مريض يشتبه بإصابتهم بحمى مالطا وأجريت الفحوص المصلية الأربعة على كافة نماذج مصل الدم . بينت الدراسة الحالية أن من بين المجموع الكلي للعينات , 118 عينة أعطت نتائج ايجابية باستخدام Rose Bengal و58 بطريقة 2ME و62 عينة بطريقة تقنية الاليزا،اما بالنسبة الى فحص STAT فقد اعطت 12 حالة نتيجة موجبة رغم انها كانت سالبة في الفحصين السابقين مما يعطي هذا الفحص أهميته . تبين ان حساسية الفحوص الثلاثة RBPT و 2ME و STAT 100% و82% و 100% على التوالي ونوعيتها 74% و 82% و 97% على التوالي بالمقارنة مع نتائج الاليزا .

INTRODUCTION

Brucellosis is a chronic infection in animals mainly localized in reproductive organs (male and female),and are shed in in milk and urine. Transmission to humans usually occurs either through direct contact with infected animal tissues or ingestion of unpasteurized milk or milk products. Human to human transmission is rare (1,2).

Human brucellosis is an infectious disease of worldwide importance .Due to the wide spectrum of manifestations of this disease, its diagnosis cannot be made solely on clinical grounds and it always essential to perform bacteriological and serological tests (3).

This disease is characterized by acute ,chronic , sub acute , localized and relapsing arthralagia and the main clinical features are undulating fever ,headache ,night sweats ,fatigue and anorexia (4).

The diagnosis of brucellosis is based on clinical features and the results of laboratory tests (5).The common serological test used for the diagnosis to brucellosis is Rose Bengal Plate test (RBPT) based on agglutination of colored particulate antigen (Killed Brucella organisms) by the antibodies present in the patient's serum .Although it is a simple ,cheap and effective test , the RBPT is generally considered to be less sensitive than other tests like standard tube agglutination test (STAT),complement fixation test (CFT)and enzyme linked immunosorbant assay (Elisa).Elisa has been claimed to be a good screening test whether used alone or in combination with the (RBPT) (6). the standard tube *Brucella* agglutination test (STAT) except for the addition of 2ME to a final concentration of (0.05 M) in each agglutination tube .The 2ME disrupt disulfide bonds ,making immunoglobulin (IgM) inactive and permitting only *Brucella* agglutination by immunoglobulin IgM agglutinating antibodies that are resistant to 2ME (7).

The aim of the present study was to assess the validity of 3 serological methods (RBPT ,2ME , and STAT) in the diagnosis of acute human brucellosis in comparison to the results of *anti-Brucella – IgM* by ELISA .

MATERIALS AND METHODS

Blood samples were obtained from 210 patients who were highly suspected of having Brucellosis .Both genders were included .Ages of patients ranged from 10 to 65 years .All blood samples were centrifuged to get serum , and all serum samples were subjected to 4 serological diagnostic methods including Rose Bengal Plate Test (RBPT),2-Mercaptoethanol test (2ME) ,Standard Tube agglutination Test (STAT),and *Anti-Brucella-IgM* by Elisa . Tests were done as described by (8-10).

Standard Tube Agglutination was performed as described by (11) as fellows :

A series of 10 test tubes were placed in a rack, and then 0.9ml saline was delivered in the first test tube and 0.5ml in each of the remaining test tube. After that, 0.1ml of the tested serum was added to the first test tube .After mixing ,0.5ml of the diluted serum was transferred to the second test tube . Then 0.5ml diluted serum was transferred from the second test tube to the third test tube, and so on until the contents of tube 10 were mixed, from which 0.5ml diluted serum was discarded. The resulting dilutions in the 10 test tubes ranged from 1:10 in tube no. 1 to 1:5120 in tube no 10 .As an antigen control another tube was added to the series containing 0.5ml saline .Then 0.5ml *B.abortus* antigen diluted 1:50 in saline , was added to each tube to make a final dilution varying from 1:20 to 1:10240 . After shaking the rack well , it was placed in a 37˚C water bath for 48 hours .The same procedure was repeated at the same time for positive and negative controls. Sensitivity , specificity , and accuracy of each test were calculated as follows :

sensitivity = no. of True positive/(no. of true positive + no. of false negative)

specificity = no. of true negative /(no. of false positive +no. of true negative).

RESULTS

The total number of patients enrolled in the study was 210 . The ratio of males to females was 58/60. Ages of patient ranged from 10 – 65 years .The ratio of patients from urban to rural area was 44/74.Of the total serum samples ,118 samples gave positive results by RBPT as shown in Table 1.

Out of 118 positive cases with different titers of RBPT , only 58(49.1%) were found to be positive by 2Me and the remaining 50.9% gave negative results (Table 2).

Table (1) :Distribution of positive results by RBPT according to gender , residency and age groups

Age groups in years	No. of patients	Gender Male/female	Residency Urban/rural	RBPT titer		
				1/320 No(%)	1/160 No(%)	1/80 No(%)
10-20	27	12/15	8/19	12(44.5)	5(18.5)	10(37)
21-30	26	13/13	10/16	8(30.8)	6(23)	12(46.2)
31-40	33	17/16	14/19	11(33.4)	8(24.2)	14(42.4)
41-50	19	7/12	6/13	3(15.8)	4(21)	12(63.2)
>50	13	9/4	6/7	3(23)	1(7.7)	9(69.2)
Total	118	58/60	44/74	37(31.4)	24(20.3)	57(48.3)

Table (2) : Results of 2 ME in comparison to RBPT titers

RBPT titer	Total	Positive by 2 ME No(%)	Negative by 2 ME No(%)
1/320	37	27(73)	10(27)
1/160	24	18(75)	6(25)
1/80	57	13(22.8)	44(77.2)
Total positive	118	58(49.1)	60(50.9)
Negative	92	0(0)	92(100)

Tube agglutination tests were standardized into 9 dilutions starting in 1/20 to 1/5120 as shown in Table (3) .All positive cases by RBPT gave positive results by STAT in different titers in addition to 12 cases that were negative by RBPT were found to be positive by STAT.

Among the total positive cases by RBPT 62(52.5%) gave positive results by Elisa while all negative cases by RBPT were found to be also negative by Elisa(Table 4).

All positive cases by 2ME gave positive results by ELISA while 3 of negative cases gave positive results by ELISA . Among 130 positive cases by STAT , only 70(53.8%) were found to be positive by ELISA while all negative cases by STAT were found to be also negative by ELISA (Table 5).

The validity of the above three serological tests in comparison with Anti Brucella – IgM was calculated as %sensitivity, %specificity , and %accuracy as shown in table 6 .

DISCUSSION

Brucellosis has a worldwide distribution and remains a major problem in humans and animals in Middle Eastern and Mediterranean countries , where the prevalence is high (12).

Isolation and identification of the causative agents remains the gold standard in the diagnosis of infectious diseases , however , the isolation of *Brucella* bacteria from blood is difficult and time consuming and the rate of success range from 47- 94% depending on the method used for cultivation and period of incubation (13,14). Furthermore , the clinical diagnosis of Brucellosis is difficult because the disease affect many organs and the symptoms may be non- specific (15).Particular problems for the final diagnosis are inadequate data on case history, course of the disease, chronic period, infections caused by microorganisms which are alike in terms of antigens, and also eventual treatment with antibiotics. Due to a number of subjective and objective problems which are the result of pathogen isolation, which is not even possible in the most of the cases, it takes a lot of time to isolate the pathogens even when some of the modern microbiologic methods are applied. If all of this is taken into consideration, immunological methods in brucellosis diagnosis are obligatory with a good reason (16).

IgM antibodies are present in acute brucellosis and it potentially aid in the diagnosis of the disease (17) .In the present study , we evaluated the diagnostic value of three serological tests to be compared to the results obtained by *anti-Brucella – IgM* by ELISA for its high sensitivity , specificity , and accuracy in the diagnosis of acute Brucellosis. Many investigators proved that ELISA is the most sensitive and specific test serological test in the diagnosis of acute brucellosis and it can be used as a referent test (18-20).

Table (3) : Results of STAT titers in comparison to RBPT titers

RBPT	Total	STST titer								
		1/20 No(%)	1/40 No(%)	1/80 No(%)	1/160 No(%)	1/320 No(%)	1/640 No(%)	1/1280 No(%)	1/2650 No(%)	1/5120 No(%)
1/320	37	21(56.4)	13(35.1)	1(2.7)	2(5.4)	0(0)	0(0)	0(0)	0(0)	0(0)
1/160	24	8(33.3)	7(29.2)	5(20.8)	2(8.3)	0(0)	2(8.3)	0(0)	0(0)	0(0)
1/80	57	5(8.8)	13(22.8)	10(17.5)	11(19.3)	10(17.5)	8(14)	0(0)	0(0)	0(0)
Total positive	118	34(28.8)	33(27.9)	16(13.5)	15(12.7)	10(8.5)	10(8.5)	0(0)	0(0)	0(0)
negative	92	0(0)	1(1)	2(2.2)	2(2.2)	1(1)	2(2.2)	4(4.3)	0(0)	0(0)

Table (4) : Results of Anti-Brucella-IgM by ELISA according to RBPT titers

RBPT titer	Total	Positive by ELISA No(%)	Negative by ELISA No(%)
1/320	37	31(87.8)	6(12.2)
1/160	24	19(79.2)	5(20.8)
1/80	57	12(21)	45(79)
Total positive	118	62(52.5)	56(47.5)
Negative	92	0(0)	92(100)

Table (5) : Results of 2ME and STAT as compared to Anti-Brucella-IgM by ELISA

Test	Total	Positive by ELISA No(%)	Negative by ELISA No(%)
Positive by 2ME	58	58(100)	0(0)
Negative by 2ME	152	3(2)	149(98)
Positive by STST	130	70(53.8)	60(46.2)
Negative by STAT	80	0(0)	80(100)

Table (6) : validity of RBPT , 2ME , and STAT as compared to Anti-Brucella – IgM by ELISA results

Test	% sensitivity	%specificity
RBPT	100	74
2ME	82	82
STAT	100	97

Comparing these results with classical methods of serological diagnosis, (21), who described the competitive immunoenzyme test (cELISA) as a more selective test for detection of and differentiation between infected and uninfected animals in comparison to all other serological tests, including iELISA. On the basis of the analysis of the results on sensitivity and specificity of the tests applied, it was concluded by the above researchers that the most sensitive were iELISA (100 %) and it can be used as a referent test in the diagnosis of acute cases of brucellosis . Out of the total number of cases assayed ,118 cases gave positive result by RBPT , only 62 cases were found to be positive by ELISA while the remaining 56 cases were found to be negative. Both genders were affected and patient rural area were affected more than those from urban , these findings are supported by many investigators (22,23).

Comparing the results of RBPT and 2ME , 27(69%) of high titer RBPT results gave positive results by 2ME while 10 (27%) were found to be negative , and in low titer RBPT (1⁄160 - 1⁄80), only 18(70 %) and 1(20%) gave positive results by 2ME respectably But despite the differences in percentage positivity , negative results were found to be almost equal in RBPT and 2ME. 2-Mercaptans (2ME) cause the cleavage of disulphide bonds of IgM and loss of agglutinin activity , Thus , comparisons of results obtained in the absence or presence of theses agent is often used to distinguish IgM from IgG activity and differentiate between early and persistent infection (24, 25), which may explain the differences between the percentage positivity in these two methods .

The present study has shown that there are differences in the ability of the three serological methods in detection of the specific anti *Brucella* antibodies in patient′s sera .The majority of positive cases by RBPT gave positive result by 2ME and all negative cases by RBPT gave negative results by 2ME(Table 2),however , it was reported that 2ME can detect the disease at 18 months after the onset of the disease because it detects IgG only (26). In the present study as shown in table 3 and 4, out of 92 negative cases by RBPT were 12 cases were found to be positive by STAT however in Elisa All negative cases by RBPT were found to be also negative by Elisa . Similar results obtained by (27), while (22) stated that diagnosis of Brucellosis cannot be achieved sometimes by STAT because of the low titer antibodies , and the presence of blocking antibodies . Kostoula *et. al* reported that Elisa is more sensitive than STAT because Elisa detects the specific IgM or IgG (23). On the other hand, (24) reported that in patients with brucellosis the sensitivity of either Elisa –IgM or IgG is higher than of STAT .

It is considered that a sensitive test will determine the most true – positive patients and a specific test will determine the most true – negative patients[28].In the present study , the three serological tests were found to be sensitive but with different specificity ,however the whole 3 serological tests are simple , inexpensive , and rapid when compared by culture , ELISA ,PCR ,and other diagnostic methods (23).

Based on the analysis of results obtained in the present study , STAT was recommended as the best serological test in the diagnosis of Brucellosis because it detect false negative cases and its sensitivity and specificity was the highest .Each of serological tests has its advantages and limitations and requires careful interpretation and advanced techniques e.g. PCR and other molecular methods are still required to confirm diagnosis because of the complicity of the disease .

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Biofilm formation by intestinal spore- forming Bacilli

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ABSTRACT

Bacillus spore-formers has been identified in human feces . It has been shown that spores of a laboratory strain of *Bacillus subtilis*, are able to germinate in the jejunum and ileum of mice dosed orally with spores .25 intestinal isolates of *Bacillus* spp.formerly characterized , used in this study Our work revealed that most of the GIT *Bacillus* isolates could form biofilms, and these might enable *Bacillus.* spp. to survive within the GIT, either attached to the mucosal wall or food particles, or in mixed biofilms. Of particular interest is the finding that the majority of these *Bacillus* isolates from adhering biofilms on inert surfaces.These results suggest a new aspect of *Bacillus.* spp*,* interactions GIT surface. If rapid dendritic or surface biofilm growth occurs in the GIT zone, this could allow these bacteria to increase access to nutrients, or find favorable colonization sites.
In this study, we recovered *B. subtilis* isolates from human gastrointestinal tract and revealed biofilm traits that could be beneficial to an intestinal existence.

Key words: *Bacillus subtilis* ,dendritic growth ,colony morphology ,biofilms

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الملخص باللغة العربية

عزلت بكتريا الباسلس المكونة للسبورات من براز الانسان. وقد لوحظ ان سبورات العزلة المختبرية لبكتريا *Bacillus subtilis* قادرة على النمو في الصائم والفائفي للفئران المجرعة فمويا بالسبورات . 25عزلة من بكتريا الباسلس المعزولة من الامعاء والمشخصة مسبقا استخدمت في هذه الدراسة. النتائج تشير الدراسات الى ان معظم بكتريا الباسلس المعزولة من الامعاء قادرة على تكوين الفلم الحيوي, وهذا قد يمكن بكتريا الباسلس من البقاء في المعدة والامعاء, سواء ملتصقة الى جدار الامعاء او جزيئات الغذاء, او في مزيج من الفلم الحيوي. . ومن الدراسات المهمة وجد بان اغلبية بكتريا الباسلس عزلت من الفلم الحيوي على السطوح الخاملة ، هذه النتائج تشير الى وجود تداخل بين انواع الباسلس وسطح القناة الهضمية. في حالة ضهور النمو الشجيري السريع او الفلم الحيوي السطحي في المعدة والامعاء, فانه يساعد البكتريا من الوصول الى المغذيات, او ايجاد المكان المفضل للتوطن.في هذه الدراسة عزلنا انواع من الباسلس في القناة المعوية المعدية واظهرت قابليتها على تكوين افلم الحيوي والذي يعتبر ميزة لتواجدها في الامعاء.

INTRODUCTION

Although normally considered soil organisms, members of the spore- forming genus *Bacillus* can inhabit the gastrointestinal tract (GIT) of insects and animals (1) . In the case of pathogens such as *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus sphaericus*, entry into the GIT is an essential part of their virulent life cycle (2,3) . It is probable, though, that *Bacillus* spores present in the soil enter the GIT associated with ingested organic matter and this could explain the abundance of spore- formers in soil-dwelling animals, e.g.,earthworms (5). However, a number of studies have also recovered *Bacillus* species in mammals (1), for example, members of *Bacillus* were readily recovered in the feces of broiler chickens (4), deer (7,8) as well as from the mouse GIT (9). A recent study has identified *Bacillus* spore-formers in human feces (10). It has been shown recently that spores of a laboratory strain o f *Bacillus subtilis*, strain PY79, are able to germinate in the jejunum and ileum of mice dosed orally with spores (6,11) . Surprisingly, germinated spores could outgrow and then, as they progressed into the upper colon, re-sporulate. This phenomenon was also observed with other, natural isolates of *B. subtilis* that had been recovered from human feces, suggesting that *B.subtilis* could use the GIT for both growth and sporulation. This raises the intriguing question of what is the real habitat for spore-formers? Is it possible that the presence of spores in soil is a byproduct of having been shed into the environment in feces? If so, then over time the soil would accumulate large quantities of spores that might mistakenly be considered soil organisms due to their 'apparent' presence there in high numbers.

With the presence of *B. subtilis* in feces samples, we observed an interesting phenotype: biofilm or rapid dendritic growth on semi-solid media. Dendritic or fractal growth of *Bacillus* and *Paenibacillus* strains has been the subject of numerous investigations (12, 15), but notably until now this behavior was usually associated with very slow growth on hard agar media over the period of many days. This growth habit has been modeled and proposed to result from a cooperative response of groups of cells to nutrient gradients and formation of a lubricant fluid that aids surface motility (14,15). Biofilm formation in bacteria associated with biological surfaces in animals is a very active field of investigation (14, 16, 17), and adhering biofilm formation in laboratory cultures of *B.subtilis* recently been demonstrated (18).

If indeed *Bacillus* species are intestinal residents or commensals, then not only should it be possible to isolate them readily from the GIT, but it should also be possible to identify strains that carry attributes enabling their survival within the gut.

In the current study, we recovered *B. subtilis* isolates from human gastrointestinal tract and revealed biofilm traits that could be beneficial to an intestinal existence.

MATERIALS AND METHODS

Bacterial strains

Bacillus isolates were obtained from culture maintained in the microbial culture collection of Department of Structural and Functional Biol. University of Naples, Federico II, Italy.

Reference *B. subtilis* strain used was PY79, a prototrophic derivative of the 168-type strain (19). Was obtained from laboratory of molecular bacteriology, intercollegiate faculty of biotechnology, poland.The vegetative cells were harvested from an overnight culture of cells in Luria–Bertani broth at 37 °C and 100 rpm. The harvested cells were washed twice in phosphate buffer until used.

Biofilm formation

For analysis of dendritic growth, biofilms and surface colonization phenotype strains were grown on a pellicle medium MSgg described by Branda et al (3) was used with some modifications: 1) the 5 mM KH2PO4 component was replaced by 5 mM NaH2PO4 and K+ ion was provided by addition of KCl as described below; 2) the metals solution, prepared as a separate 100× filter sterilized stock and stored at 4°C, was supplemented with 5 mM ascorbic acid to keep the iron component (5 mM FeSO4) from oxidizing and precipitating; and 3) thiamine, tryptophan and phenylalanine were added. For sliding motility the MSgg medium, prepared with varying amounts of KCl, was solidified with 0.3% w/v agarose. The 60 mm plates were allowed to air dry on a leveling table in a laminar flow hood overnight, and then routinely inoculated in the center with a sharpened toothpick from overnight cultures of bacteria on Luria-Bertani (LB broth) , the inoculum (2.5 µl) contained 5 × 10^3, 5 × 10^4, or 5 × 10^5 viable cells. Following growth at 37°C for 16–24 h the MSgg plates were photographed. To observe biofilm formation on and adherence to a surface , the general method described by (3) with some modifications. In short, polystyrene microtitere plates were filled with 200 µl MSgg medium, and for each strain, three wells were inoculated with 1.5 % (vol/vol) overnight

cultures, grown in MSgg broth. The plates were incubated 48 h. at 37°C without shaking. After incubation for 48 h, wells were gently washed three time with 200 µl of phosphate buffered saline, and subsequently biofilm cell were stained with 200 µl of 1% (wt/vol) crystal violet for 30 min. Then the wells were washed twice with 200 µl sterile deionized water to remove unbound crystal violet. The remaining crystal violet was dissolved in 200µl 96% ethanol, and the absorbance was measured at 595nm with spectrophotometer.

RESULTS

Isolation of spore-forming bacteria from the GIT

Twenty five of ethanol-resistant, aerobic spore forming colony were used in this work(Table 1), were *Bacillus subtilis* (thirteen isolates) *Bacillus pumilus* (four isolates), *Bacillus licheniformis* (two isolates), *Bacillus clausii* (two isolates), *Bacillus megaterium* (one isolate), *Bacillus thurengensis* (one isolate), *Paenibacillus chibensis* (one isolate), and *Bacillus* spp. (one isolate).We focus only on isolates of *B. subtilis* that comprised 52% of total *Bacillus* isolates in GIT.

Surface-spreading film formation

Fig. 1 shows a typical result, where appearance of numerous spreading films emerging from intestinal isolates were point inoculated to the center of MSgg agarose plates and incubated overnight at 37 °C. The majority of GIT isolates were able to form surface film, as judged by their appearance on MSgg agarose, with the exception of SF151, SF153, SF154, SF168, SF106, SFB2, SF170, SF173 and SF174 strains. For those strains that produced biofilms, they fell into two groups as described previously (**3**), being either a colony type or a pellicle-like biofilm. Growth on MSgg plates containing 2% agar restricted surface growth somewhat and raised the colony profile. As noted before, the laboratory strain PY79 was unable to form biofilms (**3**) . For almost all of the strains that produced biofilm on MSgg agarose, these biofilms were also shown to be adherent to plastic using a simple liquid assay. Measured strains formed dendritic growth on MSgg agarose, we identified four types of dendritic growth, as shown in Fig.1 Group A consisted of the most extensive dendritic growth, producing a finger like project pattern on plates (Fig. 1A), while group B exhibited recognisable 'spoked-wheel dendritic g rowth (Fig. 1 B), group C showed a unique sunflower pattern rhizoid pattern on agarose (Fig. 1 C). As shown in Fig. 2A the domesticated type *B. subtilis* PY79 and 2B the intestinal isolate *B. subtilis* SF155, this medium yielded single colonies with distinct large, irregular, undulate morphology.

Table (1): list of intestinal strains isolated

Strain	Species	Source/ reference
SF119	*Bacillus pumilus*	Human feces (Fakhry *et al*.2008)
SF120	*Bacillus licheniformis*	Human feces (Fakhry *et al*.2008)
SF147	*Bacillus pumilus*	Human feces (Fakhry *et al*.2008)
SF148	*Bacillus subtilis*	Human feces (Fakhry *et al*.2008)
SF149	*Bacillus subtilis*	Human feces (Fakhry *et al*.2008)
SF150	*Bacillus clausii*	Human feces (Fakhry *et al*.2008)
SF151	*Bacillus subtilis*	Human feces (Fakhry *et al*.2008)
SF152	*Bacillus subtilis*	Human feces (Fakhry *et al*.2008)
SF153	*Bacillus subtilis*	Human feces (Fakhry *et al*.2008)
SF154	*Bacillus subtilis*	Human feces (Fakhry *et al*.2008)
SF155	*Bacillus subtilis*	Human feces (Fakhry *et al*.2008)
SF168	*Bacillus thuringensis*	Human feces (Fakhry *et al*.2008)
SF85	*Bacillus pumilus*	Human ileum (Fakhry *et al*.2008)
SF106	*Bacillus subtilis*	Human ileum (Fakhry *et al*.2008)
SFB2	*Bacillus subtilis*	Human ileum (Fakhry *et al*.2008)
SFB3	*Bacillus subtilis*	Human ileum (Fakhry *et al*.2008)
SF128	*Bacillus subtilis*	Human ileum (Fakhry *et al*.2008)
SF169	*Bacillus licheniformis*	Human ileum (Fakhry *et al*.2008)
SF170	*Bacillus sp.*	Human ileum (Fakhry *et al*.2008)
SF173	*Bacillus megaterium*	Human ileum (Fakhry *et al*.2008)
SF174	*Bacillus clausii*	Human ileum (Fakhry *et al*.2008)
SF185	*Bacillus subtilis*	Human ileum (Fakhry *et al*.2008)
SF186	*Paenibacillus chibensis*	Human ileum (Fakhry *et al*.2008)
SF188	*Bacillus pumilus*	Human ileum (Fakhry *et al*.2008)
SF195	*Bacillus subtilis*	Human ileum (Fakhry *et al*.2008)

A

B

C

Fig. (1): (A) Dendritic growth. Representative plate cultures showing the appearance of *B. subtilis* isolates growing on MSgg agarose. Plates were inoculated with a single drop of culture and grown overnight at 37° C. Panel A, finger like project appearance of type A growth; panel B, dentritic appearance type B; panel C, sunflower pattern typical of type C.

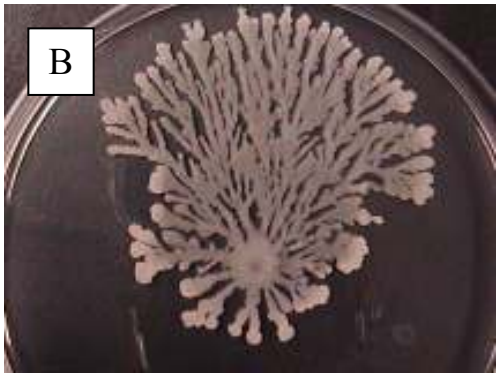

Fig. (2): A, Single colony isolation on Msgg agar shows distinctive PY79 *B. subtilis* colonies. overnight at 37 °C. B, the intestinal isolate *B. subtilis* SF155.

Adhering biofilms

The question of whether the appearance of surface films on hard media correlate with adhering biofilms. The standard method for visualization (and quantification) of such biofilms is to assess a ring of bacterial growth on plastic or glass surfaces. We used methods in widespread use for *Pseudomonas* and *Staphylococcus* biofilms (6) and most recently for *B. subtilis* biofilms (10). In preliminary tests with *B. subtilis* grown overnight in MSgg broth in glass or polystyrene plate, we detected pronounced adhering biofilms in glass and polystyrene with MSgg broth. use of MSgg broth was very satisfactory for demonstration of biofilm formation, as shown in Fig. 3. This figure illustrates the formation of adhering biofilm by *B. subtilis* (SF 155), but lack of biofilm formation strain of *B. subtilis* (PY79). Two *B. subtilis* (SF148, SF149) and one *B. licheniformis* (SF120) produced dense biofilm rings in this test. Three other isolates produced much weaker biofilm rings, including *B. thuringiensis* (SF168) , *Bacillus* sp.(SF170), and *B. megaterium* (SF173).

Results of this type for each of the GIT *Bacillus* isolates are summarized in Table 2. In general, isolates that showed dendritic growth on MSgg agarose also produced adhering biofilms in MSgg broth.

Table (2): Biofilm formation

Strain	Surface film formation	Biofilm liquid adherence
SF119	+	+
SF120	+	++
SF147	+	+
SF148	+	++
SF149	+	++
SF150	-	-
SF151	-	-
SF152	+	+
SF153	-	-
SF154	-	-
SF155	+	+
SF168	-	±
SF85	+	+
SF106	-	-
SFB2	-	-
SFB3	+	+
SF128	+	+
SF169	+	+
SF170	-	±
SF173	-	±
SF174	-	-
SF185	+	+
SF186	+	+
SF188	+	+
SF195	+	+

A B C D

Fig. (3): Visualization of adhering bioflm formation by Bacillus isolates. Photograph of polystyrene plate from cultures of Bacillus strains stained with crystal violet to visualize the formation of biofilm rings. The strains shown include: (A) isolated strains of biofilm-forming *B. su*btilis SF 148 (Table 1), (B) non-biofilm-forming *B. subtilis* PY 79, (C) strain of Bacillus isolated from feces sample *B. licheniformi*s SF 169 (Table 1), (D) *B. pumilus* SF 147.

DISCUSSION

B. subtilis might be closely associated with human intestine. Here we show that *B. subtilis* isolates and close relatives can be detected on a MSgg medium by formation of very distinct dendritic surface growth. This surface film formation and colony morphology seen with pure cultures of B. *subtilis* and its close relatives.

It is notable that we have recently shown that both dendritic growth surface film growth is eliminated by a deletion of an essential gene (srfA-A) of surfactin synthesis and restored by addition of authentic surfactin (20); in addition, this type of surface motility is independent of flagella.

The surface films of *Bacillus* observed here are very similar in appearance to the highly structured surface pellicles that have been seen in wild type *B. subtilis* strains; these multicellular structures have been described as laboratory versions of naturally-occurring *B. subtilis* biofilms (3). Adhering biofilm formation in *B.subtilis*, as measured by cell adherence to the wells of microtiter plates is highly dependent on the nutrients used in the assay (18). Some authers speculate that *B. subtilis*, as a soil bacterium, might use biofilm formation to avoid desiccation in dry soil.

Our work also revealed that most of the GIT isolates could form biofilms, and these might enable *B. subtilis* to survive within the GIT, either attached to the mucosal wall or food particles, or in mixed biofilms. Biofilm formation by undomesticated strains of *B. subtilis* is well recognised and it has been shown that biofilms facilitate the formation of fruiting bodies whose apical tips support sporulation-specific gene expression and differentiate into spores (3). In the GIT, the biofilm would also serve to protect the colony from antimicrobials produced by competing bacteria and possibly shield cells from gastric and bile juices present within the gut lumen.

Here we have noted that growth of many wild type *B. subtilis* isolates and related species in MSgg liquid media also promotes film-adherence to polypropylene in assays like those used by Hamon and Lazazzera (18), and have recently shown that the wild type *B. subtilis* Marburg strain forms profuse, viable biofilms. It is possible that another role for *B. subtilis* biofilms might be attachment to GIT surface. These results suggest a new aspect of *B. subtilis* interactions GIT surface. If rapid dendritic or surface film growth occurs in the GIT zone, this could allow these bacteria to increase access to nutrients, or find favorable colonization sites. It is known in other microbial systems that motility is essential to

the development of biofilms on various environmental and biological surfaces (21). Perhaps our finding of *B. subtilis* is indicative of its presence in tightly-adhering biofilms.

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Characterization of marine pigmented *Bacillus pumilus* SF214

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ABSTRACT

Numerous studies have reported the existence of pigmented Bacillus species . to identify and characterize the diversity of pigmented aerobic spore formers bacilli frome the environment. *B. pumilus* SF214 isolated from sea water, this isolate was identified and characterized to species level using API 50CHB kit (Biomerieux).

In this study the production of pigment take place on L.B (favouring vegetative broth on LB(favoring vegetative growth) and on DSM medium (which induces the formation of spores , pigment was very intense at 25cº progressively less intense with increasing temperature , SF214sporulation efficiency was determined after 24h , purification spores from SF214 was observed at a pure culture,the quantification of the biofilm was performed after 3 days of incubation at 37 cº *B. pumilus SF*214 isolate produce biofilm at all temperatures.

Our work has shown that pigmentation can vary dependent upon growth conditions (nutrition and temperature)as well as cell density.

Key words : pigmented Bacillus, biofilm, sporulation

الملخص باللغة العربية

افادت الدراسات الى وجود انواع الباسلس الملونة في البيئة المائية.و لغرض تشخيص وتوصيف انواع الباسلس الهوائية الملونة المكونة للاسبورات من البيئة استخدمت العزلة 214 *B. pumilus SF* التي حصل عليها من مختبرات جامعة نابولي الثانية بعد اجراء فحوصات الكيموحيوية عليها باستخدام API50 CHB. في هذه الدراسة انتجت الصبغة على وسطي L.Bالذي يحفز النمو الخضري وكذلك وسط DSM الذي يحفز تكوين السبورات ,الصبغة كانت واضحة جدا عند درجة حرارة نمو 25 ° م وقلت كثافة اللون بالتدريج بزيادة درجة الحرارة . كفاءة تكوين السبور حددت بعد مرور 24 ساعة لحظة دخول البكتريا في طور التوقف .العزلة 214 *B.pumilus SF* انتجت الفيلم الحيوي في جميع درجات الحرارة .نتائج الدراسة اظهرت ان الصبغة تعتمد على ظروف النمو (غذاء ,درجة الحرارة) اضافة الى كثافة الخلية.

INTRODUCTION

Pigments are widespread in nature and are found in both eukaryotes and prokaryotes. In photosynthetic organisms, pigments mainly function in light harvesting, but they can also serve as photoprotective agents (1,2). For others, pigmentation can help protect the cell from predation, for example prodigiosin, the red pyrrole-containing pigment commonly found in *Serratia*, *Streptomyces* and *Vibrio* species has well-defined antibacterial properties (3). Similarly, the purple violacein of *Chromobacterium* species is able to provide resistance to being consumed by predatory protozoa together with inherent antibiotic properties (4). Photoprotective pigments include the melanins and carotenoids (6). Interestingly, the latter have also been shown to act as virulence factors in a number of pathogens, including *Staphylococcus aureus* (6) and *Mycobacterium* spp. (7,8). Here, the carotenoid helps protect bacteria attempting to survive within an intracellular environment, that is, following phagocytosis where they provide resistance to oxidation and neutrophil attack (6). Presumably, carotenoids, that originally evolved to protect the cell from UV damage, have assumed an additional and more sophisticated role as part of a pathogenic life cycle. Numerous studies have reported the existence of pigmented *Bacillus* species. The best known example is *B. subtilis* var *niger* (also known as *B. atrophaeus*), which produces a soluble pigment black (9). The endospores of *Bacillus* species that are more resistant to UV light and this is mainly due to the presence of a pigment found (probably) a carotenoid (5).

Further studies have reported the existence of other *Bacillus* species that produce one or more pigments, such as *B. marisflavi* and *B. acquimaris*, species found in the Yellow Sea in Korea, respectively, which produce yellow and orange-yellow pigment (10), *B. indicus*, species found in the waters of Bengal, which produces a yellow-orange pigment (11), *B. vedder* characterized by a yellow-white pigment, *B. okuhidensis* which has a yellow pigment, *B. jeotgali* isolated homonymous Korean food, which produces creamy-yellow pigment (12).

The role of carotenoids in photosynthetic bacteria of the genus *Bacillus*, appears to be related to different protective function both vegetative and spore cells, although in many cases, a function has not been precisely defined. In bacteria of the genus *Bacillus* of marine origin, the role of these carotenoids in the vegetative cells, may be to protect them from photo-oxidation damage to which aquatic organisms are more susceptible. The carotenoid pigments, as mentioned above, however, are also present in dormant spores which are already resistant. TLC (Thin Layer Chromatography) has been shown, in fact, that the carotenoids found in spores of *B. megaterium deiliposomi* increase the anisotropy of the membrane as dimiristoilfosfatidilcolina. This feature may explain why different species produce different molecules to modulate the stability of the membrane (13).

The current study seeks to isolate new species of *Bacillus* to characterize both the pigmented and the pigment-producing bacteria product. For this purpose samples were used river water to isolate spore-forming bacteria and verify the presence of pigmentation.

MATERIALS AND METHODS

Bacterial strains:

B.pumilus SF214, reference *B. subtilis* strainPY79, *B. firmus, B. amyloliquefaciens* were obtained from culture maintained in the microbial culture collection of Departement of Structure and Functional Biol. University of Naples, Federico II.

General methods:

Vegetative cell growth was made on Luria–Bertani (LB) solid or liquid medium unless otherwise indicated in the text. Sporulation was made in Difco sporulation medium (DSM) agar or liquid medium (14). For analysis of sporulation efficiencies, spores recovered from plate cultures (30°C, 3days old) were examined microscopically using a haemocytometer counting chamber or by determination of heat resistance (65°C, 45min).

Biochemical test:

Kit API 50CH (BioMerieux): A single colony of each bacterium was inoculated into 5ml of LB and grown at 37°C for 24h. The next day was made a dilution in 5ml of LB to 0.1 OD was read every hour and the absorbance at 600nm in 1ml of each culture. When all the

crops they were in exponential phase were centrifuged at 7000 *g* for 10 min, the pellet was washed twice with PBS 1X. After washing the pellets were resuspended in 10ml of medium and 50CHB rate of each culture was added, using a pasteur, in the 49 wells of the kit. The diagnosis was carried out as described in the instruction manual.

Determination of sporulation efficiency:

Sporulation of PY79 and SF214 (used as control) were measured in parallel using DSM medium for induction of sporulation (15). A colony of both strains were inoculated in 20ml of LB and incubated at 37°C for 24h, then dilution in 200ml of DSM (0.4OD) was made and bacterial growth was checking after entry the stationary phase (T0) and reading by spectrophotometric at 600nm every hour. After 3, 8 and 24 hours, 200µl of (T0) were taken from both cultures, 100µl were subjected to heat treatment (80°C for 20min) and 100µl were not subjected to heat treatment. Treated and untreated samples were plated on DSM agar by serial dilution. The efficiency of sporulation was determined by comparing the number of cells resistant to the heat in relation to untreated cells.

Biofilm production:

Biofilm production were made on liquid MSgg medium (100 mM l^{-1}MOPS pH 7.0, 0.5% glycerol, 0.5% glutamine, 5 mM potassium phosphate pH 7.0, 50 µgm l^{-1}tryptophan, 50 mg ml^{-1} phenyl alanine , 2 mM l^{-1} $MgCl_2$, 0.7 mM l^{-1} $CaCl_2$, 50 µM l^{-1} $FeCl_3$,50 µM l^{-1} $MnCl_2$, 2 µM l^{-1} thiamine, 1 µM l^{-1} $ZnCl_2$) (16) and cells grown at 37°C in static conditions up to 48h. Cells forming solid layer at the liquid-air interface were considered as biofilm producers. To quantify biofilm formation, bacteria were grown in MSgg medium at 37°C for 3 days in 6 well polystyrene microtiter plates, then culture medium was removed and wells washed with phosphate-buffered.

RESULTS

Pigment production

The production of pigment takes place both on, LB (favoring vegetative growth) and on DSM medium (which induces the formation of spores), (Fig 1).

DSMAGAR **LB AGAR**

Fig. (1): *Bacillus pumilus* SF214 on DSM agar (left) and LB agar (right), PY79 (*Bacillus subtilis*) is a non-pigmented *Bacillus* as control

Growth temperature:

The optimal temperature for growth of strain SF214 was determined in LB liquid medium at various temperatures, such as (25, 30, 37, 42 and 50°C) (Fig 2). Plates of solid medium LB and DSM incubated at various temperatures were observed that the pigment was very intense at 25°C and progressively less intense with increasing temperature (Fig 3). for example, isolate SF214 would develop an orange pigment at 25°C but at 42°C white colonies were found.

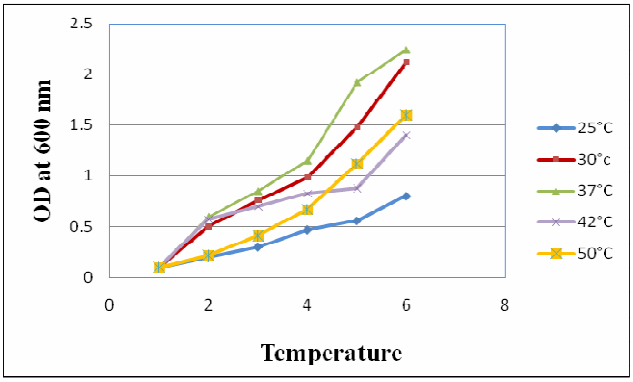

Fig. (2): Curve of strain SF214 growth at various temperatures in LB medium

Fig.(3): Spore pellet of SF214 strain

Sporulation efficiency:

To further characterize SF214 determine sporulation efficiency, expressed as a percentage of cells resistant to heat (80°C for 20min) present in a culture of DSM, after 3, 8 and 24 hours, after entry into stationary phase (T0), compared to total cells. The results showed that after 24h from (T0) of SF214 strain, sporulation efficiency was 10.1%. The low efficiency of sporulation observed for SF214 strain compared to control strain *B. subtilis* PY79 was at least partly due to the fact that the experimental conditions used (culture medium, pH and temperature) are ideal for those *B. subtilis* (Table 1)**.**

Table (1): Sporulation efficiency

Strain	One hour after (T0)	Cell unmber (ml^{-1})	Spore number (ml^{-1})	Sporulation effieciency(%)
SF214	3	$3.5x10^7$	0	0
	8	$8x10^7$	$0.12x10^7$	1.5
	24	$128x10^7$	$13x10^7$	10.1
PY79	3	$13x10^7$	0	0
	8	$35x10^7$	$2.3x10^7$	6.5
	24	$40x10^7$	$18x10^7$	76.6

The production of pigment in SF214:

In experiments for purification spores from SF214 strain was observed at a pure culture, pigmented, always get a mixed pellets formed in part by white spores and partly pigmented spores (Fig. 3). White phase was fixed in the bottom of the tube after centrifugation, when pigment appeared viscous, repeated washing was possible to separate the two phases.

Biofilm formation:

A similar rule for the formation of carotenoid and biofilm was assumed that processes could be coordinated. Production of biofilm was tested at 25, 37 and 42°C by MSgg medium. The experiment was conducted in the presence of a positive control *Bacillus amyloliquefaciens*, and a negative control *Bacillus firmus*. The quantification of the biofilm was performed after 3days of incubation at 37°C and calculated at spectrophotometer at 570 nm (Fig. 4**).**
As can be seen in figure 4, SF214 strain produce biofilm at all temperatures degree, while producing the carotenoid at 25°C and less at 37°C but it produces at 42°C . This observation allows us to conclude that although the two processes are regulated in a similar manner, but are independent of one another.

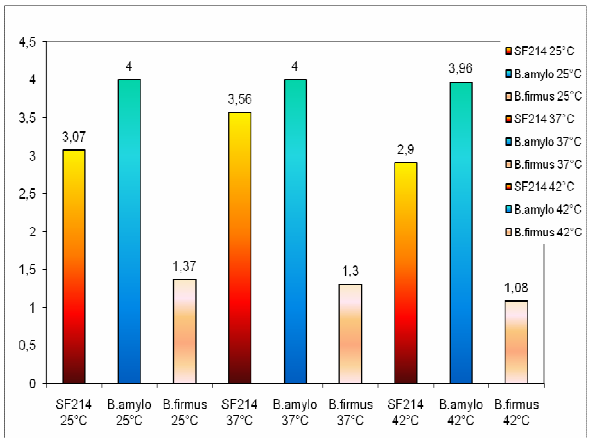

Fig. (4): Production of biofilm in *Bacillus pumilus* SF214 at 25, 37 and 42°C. *B. amyloliquefaciens* was used as positive control; B. firmus as a negative control.

DISCUSSION AND CONCLUSION

As mentioned in the introduction to this article, a relatively small number of publications have reported the identification and characterization of pigmented bacilli(9) provide the first details about pigmented marine *Bacillus* species. we emphasize that this study showed the diversity of spore pigmentation. Our study has revealed that for the most part, the abundance of colored bacilli has probably gone unnoticed. We attribute this to the technicalities of identifying colored colonies, where plating out bacteria at low dilution masks the true abundance of pigmented species. Only at high dilutions do the pigments become apparent, and in some cases one in ten colonies were found to be pigmented. Another contributing factor is the medium and temperature used to culture bacilli where we have found that significant variation in the colony coloration can result. The variation in colour suggests that environmental or nutritional factors could be important. The pigmented spore-forming bacteria are still poorly known. With this work we have proposed to identify new species of pigmented *Bacillus*. We got different pigmented strains from samples of river water and decided to focus our attention about SF214 isolate, belong to species *Bacillus pumilus*. The physiological characterization of this microorganism allowed to understand the production of the pigment and its stress response in poor conditions, temperature and nutrients. Our work has shown that pigmentation can vary dependent upon growth conditions (nutrition and temperature) as well as cell density, while other showed that *B. indicus* HU36, the yellow coloration of vegetative cells changes to orange pigment, which belong to spore formation (9). This suggests that developmental signals may affect to the biosynthetic pathways. Carotenoid biosynthetic pathways will therefore prove a complex task, yet there are a number of incentives for attempting this, first and foremost is the ability to metabolically engineer bacteria to synthesize high levels of endogenous isoprenoids, if this can be achieved, these bacteria could be included amongst the cohort of metabolically engineered bacteria, second was the development and third was indicated to generation biofuels (17). population of spores of SF214, the same part of the population form biofilm (observed viscosity) and that the same part of the population is pigmented so we suggest that the formation of carotenoids, such as biofilm formation, is a regulated process in bistable mode. From experiments conducted subsequently aimed at understanding a common pathway of regulation of carotenoid production and biofilm, we realized that the two processes are independent, but this did not rule out that both can still be adjusted, although independently, in a bistable.

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Immunological effect of a crude soluble extract (CSE) from *Listeria monocytogenes* on the Listerial infection in mice

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ABSTRACT

In order to determine the effect of a crude saline soluble extract(CSE) from *L.mnonocytogenes* on stimulate immune response and protect immunzed mice against the course of listeric infection,sixteen mice were immunized twice with CSE with two weeks interval at a dose of 0.5ml subcutaneously ,both humoral and cellular response were monitored using radial immunodiffusion plate kit(IgG RID) and skin test.

At twenty seven days post first immunized the mice were challenged with 0.5/ml subcutaneously of CFU 1×10^8 / ml of virulent *L.monocytogenes* ,while six mice was inoculated with 0.5 ml of sterile phosphate buffered saline as control. Then all immunized and control mice were sacrificed at fifteen days post challenge. The results showed, immunization has increased humoral and cellular immune responses in mice as judged by increas the IgG titer and hyper sensitivity reaction. No *L. monocytogenes* was recovered from internal organs; two mice were died in fifth day after immunization and challenged with total 80% protection.Mild histopathological changes were observed which characterized by lymphoid hyperplasia in spleen and lung, lymphocytes infilitration, small granuloma formation in liver. Control mice died within 3-10days postchallenge. High Bacterial isolation levels were obtained from internal organs. The pathological changes revealed multiple extensive lesions characterized of necrosis of hepatocytes ,multiple granulomatous lesion,and congestion in internal organs.the data investigat that the CSE induced humoral and cellular response and protect the mice from infection .

الملخص باللغة العربية

من اجل معرفه التاثيرالمناعي للمستخلص الذائب من جرثومه *Listeria* monocytogenes على التحفيز المناعي وحمايه الفئران ضد الاصابه بهذه الجرثومه ,تم تمنيع سته عشر فارا بمستخلص الذائب بجرعه مقدارها 0.5مل تحت الجلد ولمرتين متتاليتين بينهما اسبوعين. وقد قيست الاستجابه المناعه الخلطيه والخلويه باستخدام اطباق الانتشار المناعي الاشعاعي والفحص الجلدي . وبعد 27 يوما من التمنيع الاول اجري فحص التحدي وذلك بحقن الفئران الممنعه تحت الجلد ب0.5مل من عالق جرثومه *Listeria* monocytogenes الضاريه الحاوي على 1×10^8 CFU/مل ,بينما حقنت سته فئران ب0.5مل من المحلول دارئ الفوسفات الملحي. قتلت الفئران بعدمرور خمسه عشر يوما من حقنها بجرعه التحدي , اوضحت النتائج بان الفئران الممنعه اظهرت استجابه خلطيه وخلويه بارتفاع مستوى معيار IgG وزياده فرط الحساسيه وهلاك اثنين فقط من الفئران الممنعه وبنسبه حمايه 80%. كذلك عدم عزل الجرثومه من الاعضاءالداخليه للفئران الممنعه مع تغيرات مرضيه طفيفه تمثلت بفرط تنسج اللمفي في الطحال والكبد والرئه , ووجود اورام حبيبيه صغيره في الكبد وارتشاح الخلايا اللمفيه . اما حيوانات السيطرة هلكت جميعها خلال 3-10ايام وتم عزل الجرثومه من الاعضاءالداخليه مع تغيرات مرضيه شديده تميزت تنخرات الخلايا الكبديه , احتقان ونزوفات , واورام حبيبيه متعدده في الاعضاء الداخليه .نستنتج من الدراسه ان المستخلص حفزاستجابه مناعيه خلويه وخلطيه واعطى حمايه ضد الاصابه بجرثومه*Listeria monocytogenes* .

INTRODUCTION

Listeria monocytogenes a Gram-positive pathogenic bacterium that has adapted to various environments, from soils and food products to the intestinal tract and intracellular compartments of diverse animal species and humans (1). In order to infect mammalian host and to cause the most severe pathological changes, *L.monocytogenes* is able to cross the intestinal, blood-brain and maternal fetal barriers(2).

As several bacteria possess an array of components which participate in the host immune system .Like wise , Listeric infections in several animals species and man are characterized by the presence of large circulating mononuclear cells. Although this phenomenon is not unusual in infections, the large numbers of such cells produced in response to *L.monocytogenes* infection is unique(3). (4,5) demonstrated that a chloroform-soluble extract of Listeria contained the agent which caused this response and named the extract monocytosis-producing agent (MPA), this agent can be found in saline extract (SE) of heat-killed ,dilapidated cells of *L.monocytogenes* (6,7). The SE is no-toxic, water soluble contain of protein, carbohydrates and phosphorus. CSE promotes a significant elevation in the level of circulating monocytes ,this characteristic is present only in both live and killed virulent strain of *L.monocytogenes* but not in another Listeria Spp. Monocytes is essential for the development of cellular and humoral incompetence and there is an intimate inter play between the mononuclear phagocytes and T-lympthocytes and the mononuclear phagocytes play critical roles in the defense against Listeric infection(8).

In relation to the role of MPA in immune responses studies were undertaken to survey the immunological properties of crude SE and their fractions partially purified preparation from *L.monocytogenes* which is enriched in MPA and the immunostimulation of saline extract have been reported (9,5).

MATERIALS AND METHODS

The crude SE was obtained from delipidated of heat killed *L.monocytogenes according* to (7,10) Briefly SE was prepared as follows: the residue from the delipidated cells was mixed with 1M NaCl in flask glass beads and agitation for 18hr. at 4c,then centrifugation at 20,000xg for 20 min. and the supernatant present the saline extract. Total protein was measured by Biuret method .

Sixteen mice was immunized with a twice dose of CSE (0.5 ml/ S.C) with two weeks intervals, to detect humoral immune response after ten days from second dose, six mice and three mice as control (not immunized) were sacrificed and blood collected from heart in sterile test tubes and allowed to clot for two hours at 4c, tubes were centrifuged for 10 minutes at 4000 xg , and the serum was separated, the titer of IgG were determined by radial immune-diffusion plate kit (IgG RID/ Bussero-MilanITALY) .

Delayed type hypersensitivity (DTH) test was performed by inject the remaining ten immunized mice with 0.05ml of CSE on right foot pad, and inject 0.05ml of sterile phosphate buffered saline on the left foot pad as control. The thickness of foot pads were measured after 24 & 48 hours. after that immunized mice (four of them become pregnant in late stage) during experiment and six mice used as control were challenged with 0.5 ml S.c virulent *L.monocytogenes* (CFU 10^8 / ml). At fifteen days post challenge immunized and control mice sacrificed to isolate *L.monocytogenes* from internal organs ,and pieces from internal organs were taken in 10% formalin for fixation ,then after ,processing routinely in histokinette,cut at 5µm thickness and stained with hematoxyline and eosin and examined under light microscope(11) and study the histopathological changes.

RESULTS

The saline extract was obtained from delipidated of heat killed *L monocytogenes*. Total protein was measured by Biuret's method. The protein in CSE was 90mg/ml.

Effect of CSE on Humeral and Cellular response:

The result showed increased level of IgG titer (1235.2) as compared with control mice (158.1). On other hand the CSE stimulate of delayed hypersensitivity reactions in immunized mice by increase the thickness of foot pad after skin injection with mean 1.9±0.09, 1.4±0.2 after 24, 48 hr. respectively while no changes in thickning of foot pad in control mice. (Table 1).

Table(1): Cellular and and humoral response in mice immunized with CSE

Cellular response			**Humoral response**	
Thickness of food pad				
No.of mice (10mice)	**After24 h**	**After48 h**	**No.of mice (6mice)**	**IgG titer**
1	2.1	1	1	1509.5
2	1.5	0.9	2	860.9
3	2.3	1.6	3	1249.4
4	1.6	0.8	4	1333.8
5	2.2	1.5	5	1208.0
6	2.1	1.7	6	1249.4
7	1.8	1	-	-
8	1.6	0.9	-	-
9	1.7	1.2	-	-
10	1.9	1.3	-	-
mean±SE	**1.9±0.09**	**1.4±0.2**	**mean**	**1235.2**
Control(6mice) mean	**0**	**0**	**Control(3mice) mean**	**158.1**

Effect of CSE on the course of Listerial infection:

The results showed dies only tow mice from immunized which were pregnants through fife days post challenge , the protection percentage was 80%, and isolated *L.monocytogenes* from liver,spleen ,Lung and heart from these mice while not isolated from other immunized mice that sacrified at fifteen days post challange . all control mice were dies within 3-10 days post challenge and *L.monocytogenes* was isolated from liver,spleen Lung and heart .(Table2).

Histopathological changes:
Immunized mice:

The histopathological changes in Liver characterized by proliferation of kuffer cells ,small granulomatous lesion of the liver paranchyma, lymphocytic aggregation area in bile duct and central vein In another section theres focus aggregation of mononunclear cells specially(macrophages) in liver paranchyma with cuffing cells from lympthocyetes and monocytes arround the blood vesseles of portal area and central vein. (Fig 1).

Table (2):Bacterial isolation from internal organs of immunized and control mice

Immunized mice sacrified at fifteen day	No. of mice	Bacterial isolation			
		Liver	Spleen	Lung	Heart
	*1	+	+	+	+
	*2	+	+	+	+
	3	+	-	-	-
	4	-	-	-	-
	5	-	-	-	-
	6	-	-	-	-
	7	-	-	-	-
	8	-	-	-	-
	9	-	-	-	-
	10	-	-	-	-
Control died within 3-10	1	+	+	+	+
	2	+	+	+	+
	3	+	+	+	+
	4	+	+	+	+
	5	+	+	+	-
	6	+	+	+	-

**pregnant died at 5th day of Challenge*

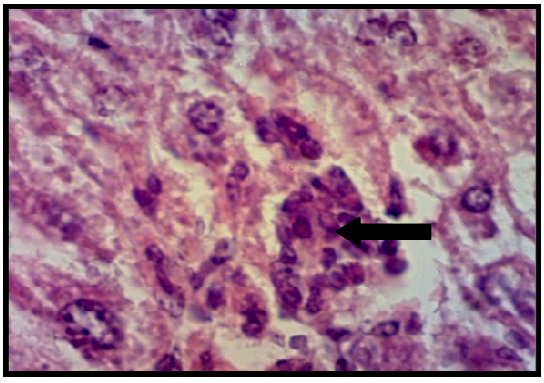

Fig (1) : liver of immunized mice show granulomatous consist of aggregation of macrophagesin the paranchyma of liver with profilartion of kuffer cells (H&E ×40)

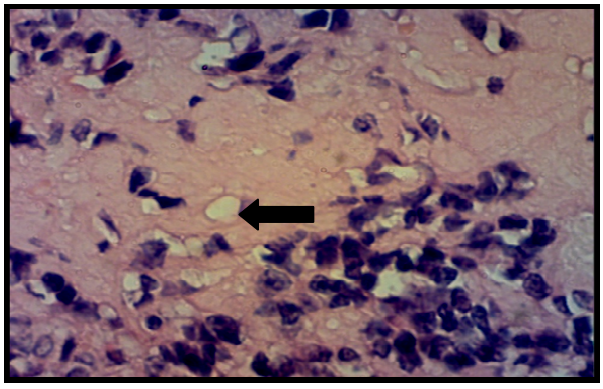

Fig (2): spleen of immunized mice show amyloid like substances deposition around atrophic white pulp (H&E ×40)

In spleen there is hyperplasia arround central vein and mononuclear cell infiltration ,also amyloid depostion(fig 2). Also theres infiltration of mononuclear cells including macrophages and plasma cells in red pulb. While in Lung theres lymphocytes aggregation arround the bronchules and lung pranchyma and hyperplacia ,a nother section theres lymphoid hyperplacia of lymphoid tissues arround peribronchial lymphoid tissue (fig 3).

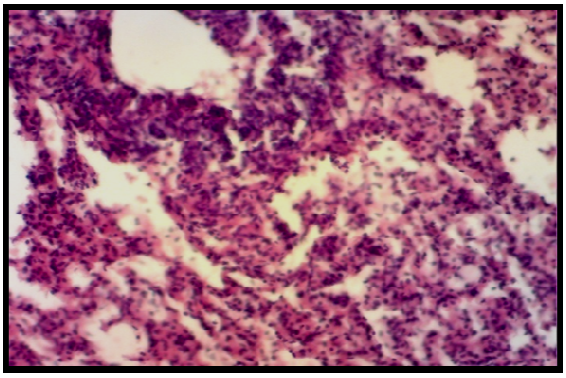

Fig (3):lung of immunized mice show hyperplasia and lymphoid tissue around bronchules and in the lung parenchyma (H&E ×40)

Control mice:

The main lesion in liver characterized by multiple extensive lesion characterized by necrosis of hepatocytes with neutrophils, in other section macrophages aggregation together and congested of blood vessels and sinusoid and proliferation of kuffer cells and aptosis of hepatocytes(fig 4). Lesions in spleen there is sever congestion and Hemorrhage of red pulp, capsular area with neutrophiles infiltration and depletion of white pulp, multiple granulomatous lesion and proliferation of megakaryocyte (fig5). the lung show thickening intera alveolular septum due to mesnchymal proliferation and congestion of capillaries blood vessels and mononuclear infiltration, also Hemorrhage in other sector were reported and emphysemia and collapse well seen, other section there is hyperplacia of tunica media of consephector blood vessels ,also red blood cells was seen in the lumina of alveoli.

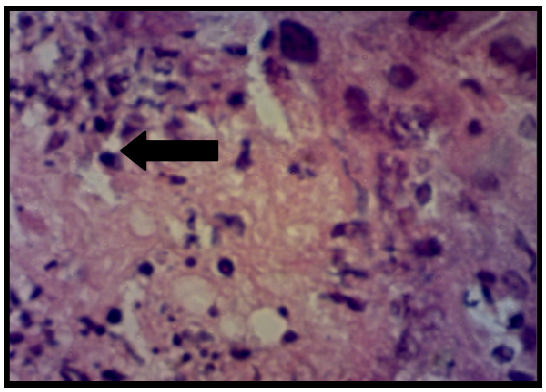

Fig (4): liver of control mice show diffuse necrotic area and infiltration with inflammatory cells (H&E x40)

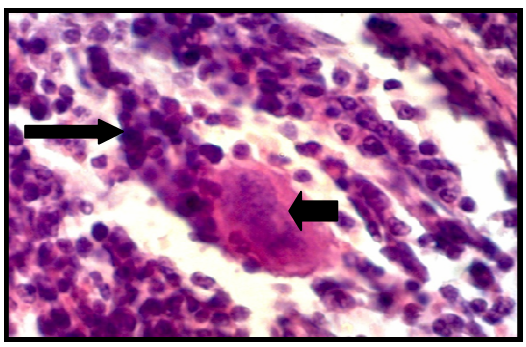

Fig (5): spleen of control mice show congestion of red pulp with neutrophils infiltration and proliferation of megakaryocyte (H & E ×40)

DISCUSSION

In the present study the CSE have been found to stimulate both the humoral and cell-mediated immune response in the experimental animals and give 80% protection even the two pregnant mice. The augmentation of the humoral response by CSE ,as evident by increase in the number of respective antibody titers in mice Indicated that the CSE have the ability to stimulated the immune response, involved in the antibody synthesis (12).in view of the pivotal role played by macrophages in coordinating the processing and presentation of antigen to T-and B- cells, the elicitation of the humoral response to the antigen reveals that SE may enhance the effect by facilitating these processes.

Increase in the delayed type hypersensitivity reaction in mice response to SE revealed the stimulatory effect of on T-lymphocytes and accessory cell types required for the expression of the reaction. The roles of thymus-derived lymphocytes (13,14)and of activated macrophages (15-18) in resistance of infection of *L.monocytogenes* has been convincingly demonstrated.

In some experiments, SE showed presence of *in vivo*, adjuvant activity which was presumably masked by the immunosuppressive activity as reported previously by (10).SE may lack the components which cause the *in vivo* suppressive activity. The findings outlined above have demonstrated that the saline extract possesses imunostimulant activity .(Kim, *et al* al) showed that the crud SE of delipidiated cells suppressed the antibody response of mice to sheep erythrocytes, provided that the extract is administered before antigen. Similarly prepared extract in our experiment showed immunostimulating activity which is in accordance with the work of(5,7,20). In the present work immunostimulating activity of SE was observed when the extract was administrated a twice dose.

The Results of cellular and humoral response corresponded with histopathological changes as well as with the results of bacterial isolation, the immunized animals show enlargement of the spleen and infiltration of lymphocytes in organs which can be attributed to hyperplasia in lymph tissue. That hyperplasia indicated infiltration of lymphocytes type T in huge numbers as a result of persist stimulation lymphocyte cells type Th0 which sensitized by histocompatibility complex and expression of antigens and then divides and differentiation

into CD4 $^{+}$ cells which produces INF Kama in addition to present of cytotoxic CD4 $^{+}$ cells for the same purpose and that is explain the development of hyperplasia which indicated to cellular response after infected with the challenged dose ,These findings are agreement with previous studies indicated development hyperplasia in lymphoid tissues of immunized animals after challenge dose (21-23).

The presence of granulomas, which can explained by transmission a small number of L.*monocytogenes* to the liver, and because of severe immune response led to the restriction and eliminate them by granulomas. (24) investigated that the growth of granulomas corsponding with severe immune response as delayed hypersensitivity accelerates the growth of granulomas.

The inclusion of histopathological changes in control animal which injected with infected dose during 3-7 days can be indicated the transition the bacteria across the blood to organs and causing typical physical response to bacterial infection and causing congestion of blood vessels ,heamorrhges ,odema and other lesions resulting isolation of bacteria from the organs and this results agreement with(25-28).

It is generlly accepted that the development of cell-mediated immunity is crucial for resistance against *L.monocytogenes* (29).in contrast,the role of circulating antibodies in the protection from Listeriosis has been neglected after it was shown by (30) that anti-listerial antisera transferred no resistance to recipients. However,in this experiment antilisterial antibodies protect mice from the lethal effect of *L.monocytogenes* which agreement with (5) which found the role of antibodies protect mice from infected dose of *L.monocytogenes*.

CONCLUSION

The data reported in the present study have resulted in very useful biological activity of a water –soluble extract of *L.monocytogenes* which markedly increased mice resistance toward the challenged dose of *L.monocytogenes*,resulting in decreased mortality of the mice and also protection pregnant mice. this immunostimulating activity ,which can be useful in the development of vaccine against this important food pathogen.

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Prevalence of *Neisseria meningitidis* in Iraqi children presented with meningitis

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ABSTRACT

Neisseria meningitidis is a leading cause of bacterial meningitidis and septicemia in children and young adults. Rapid and consistent identification of *N. meningitidis* serogroups is critical for thoughtful and convenient response to cases of meningococcal infections. *N. meningeitidis* isolates collected in Iraq through the Children Welfare Teaching Hospital in Baghdad and confirmed by the Central Public Health Laboratory (CPHL), Baghdad. Out of fifty suspected specimens of cerebrospinal fluid (CSF) two isolates of *N. meningitidis* were detected using culture and Real Time-PCR techniques (RT-PCR). Api NH confirmed the results of culture. Slide Agglutination Serogrouping (SASG) revealed that both isolates were belonging to the serogroup W135. This study compared the conventional methods with the RT-PCR for detecting *N. meningitidis* with specific primers targeting *ctrA* in short time with high specificity and quality.

الملخص باللغة العربية

تعتبر الناسيريه المننجايتس من الاسباب الرئيسية التى تؤدى الى التهاب السحايا البكترى وتجرثم الدم عند الاطفال والبالغين،و عليه فان ايجاد طريقة سريعة وفعالة لتشخيص هذه الجرثومة يعتبر بالغ الاهمية للاستجابة مدروسة وذات مردود فعال. جمعت خمسون عينة من السائل النخاعى من الاطفال فى منطقة بغداد وجرى توصيف وتشخيص البكتريا المسببة فى مختبر الصحة المركزى/بغداد . تم توصيف عزاتين على انهما بكتريا الناسيرية المننجايتس باستخدام تفاعل البلمرة فى الوقت الحقيقى وكذلك بالمقارنة مع فحص الايبى والتلازن على الشريحة. تم تحديد النمط المصلى والذى يعود الى النوع المصلى دبليو 135 . قارنت هذه الدراسة بين الطرق التقليدية لتشخيص هذه البكتريا مع فحص تفاعل البلمرة فى الوقت الحقيقى باستخدام بادى خاص يعطى نتائج سريعة ودقيقة.

INTRODUCTION

Neisseria meningitidis is a Gram-negative diplococcal bacterium which considered as an important cause of morbidity and mortality worldwide. Additionally, it is the leading cause of bacterial meningitis and septicemia (1). There are 13 clinically significant serogroups in this species, which are classified according to the antigenic structure of their polysaccharide capsule. Five serogroups (A, B, C, Y and W135) are responsible for nearly all cases of the disease. However, serogroup B is the predominant one (2,3). Changes in the epidemiology of meningococcal disease have imposed important implications for vaccination and on the prevention strategies (4). Up to 36 hr is needed to identify *N. meningitidis* by conventional culture methods. What's more, starting antimicrobial treatment prior to specimen collection will decrease the capacity to confirm detection of the causative agent of bacterial meningitis and septicemia (5).

Unlike biochemical tests and slide agglutination serogrouping, polymerase chain reaction (PCR) does not require viable bacteria and can be used to identify and characterized even non groupable *N. meningitidis* meningococci (6,7). Additionally, it is necessary to detect small numbers of *N. meningitidis* in clinical specimens; bacterial loads in cerebrospinal fluid (CSF) of patients range from 3×10^1 to 4×10^3 CFU/ml (8,9). TagMan RT-PCR has been shown to detect as few as 8 meningococcal genomes per reaction (10,11).

The capsular transport protein (*ctrA*) gene is unique to *N. meningitidis* and can be found in all meningococcal serogroups (12). Therefore it may be the most frequently targeted gene to detect *N. meningitidis* using PCR (13).

This study aimed to detect of *N. meningitidis* isolated from CSF by RT-PCR assay in Iraqi children.

MATERIALS AND METHODS

Isolation and identification

Fifty CSF specimens from young children (1-14 year) presented with meningitis were cultured as soon as possible after collection on blood agar and chocolate agar and incubated for 24-48h at 37ºC with 10% CO_2.

Identification was achieved by api NH (bioMérieux -France).

Serogrouping

The sero- grouping was done with 50µl of each biological sample by using commercial kit (BD UK) according to the manufacturer's instructions.

Antibiotic susceptibility test (AST)

Neisseria meningitidis isolates were test for AST using Amoxicillin (20µg), sulfamethoxazole-trimethoprim (10µg), ceftriaxone (30µg), cefotaxime (30µg), and gentamicin (10µg) on chocolate agar and incubated at 37ºC with 10%CO_2.

Standardization of bacterial culture

Clinical isolates of pure culture was emulsified in 2ml of sterile injectable water in a microbiological class 2 safety cabinet. Using a spectrophotometer (Pharmacia, St. Albans, England) set at 650 nm, the bacterial suspension was standardized to an optical density of 0.1 and adjusted to a concentration of approximately 2×10^4 cell/ml, which represents 40 cells per 2µl of inoculum.

DNA extraction

peqGOLD bacterial DNA kit was used for the isolation of up to30µg genomic DNA from CSF specimens and bacterial cultures following the manufacturer's instructions. The extracted DNA was stored at −20°C.

RT-PCR

A partial region of *ctrA* (110bp) was amplified using 300nM (each) specific primers. The bacterial isolates and CSF samples were analyzed by real-time PCR (RT -PCR) as reported by Corless *et al*. (14). Briefly, based on a 25-ml reaction volume, the master mixture was prepared from the (Taqman, Applied Biosystem, USA), this comprises a 300 nM concentration of each oligonucleotide primer (*ctrA* Forward ^{617}-GCTGCGGTAGGTGGTTCAA-^{635} and *ctrA* Reverse ^{727}-TTGTCGCGGATTTGCAACTA-^{708}); 25 nM 6-carboxyfluorescein-labeled probe (6-FAM–680-CATTGCCACGTGTCAGCTGCACAT-657); 5.5 mM $MgCl_2$; 200 mM (each) deoxynucleoside triphosphates dATP, dCTP,

dGTP, and dUTP; and 0.125 U of *Taq* DNA polymerase. A negative (no-template) control and control DNA preparations (2 ml) for each of the bacterial pathogens were included in every run.

DNA was amplified with the Applied Biosystem 7500 system (Applied Biosystem Inc.,USA) using the following cycling parameters: heating at 95°C for 10 min followed by 40 cycles of a two-stage temperature profile of 95°C for 15 s and 60°C for 1 min. RT-PCR results were based on the fluorescence readings, which are used to calculate a baseline reading for each reaction. The cycle threshold (C_T) value is the PCR cycle number (out of 40) at which the measured fluorescent signal exceeds a calculated background threshold identifying amplification of the target sequence. If no increase in fluorescent signal is observed after 40 cycles, the sample is assumed to be negative.

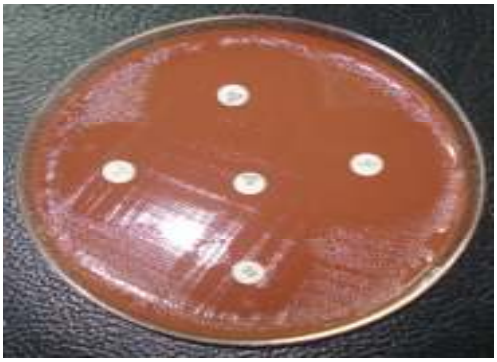
RESULTS

Isolation and identification

Fifty CSF specimens were collected and cultured on blood agar and chocolate agar incubated for 24-48 hours at 37ºC with 10% CO_2. On a blood agar, colonies of *N. meningitidis* were grey, unpigmented round, smooth, moist, glistening, and convex, with a clearly defined edge. On chocolate agar they appeared large, colorless and opaque. The results revealed that two bacterial isolates (N6 and N23) were identified as *N. meningitidis* by api NH. Serogrouping were employed to identify the type of *N. meningitidis* by using *N. meningitidis* antisera, the results showed that both of them were belonging to the serogroup W135.

Antibiotic susceptibility

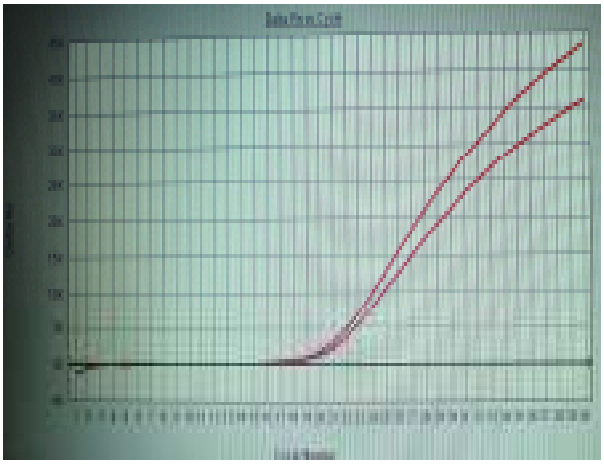
AST illustrated that the two isolates were sensitive to amoxicillin, cefotaxime, ceftriaxone, chloramphenicol, and resistant to sulfamethoxazole trimethoprime (fig.1).



Fig(1): Antibiotic susceptibility of isolated *N. meningitides.*

RT-PCR

Results from real-time PCR assays using nucleic acids extracted from CSF specimens and bacterial cultures are given in Fig.(2). Real-time PCR using primers and probes targeting the capsule transport gene, *ctrA,* of *N meningitidis* confirmed the presence of *N meningitidis* in all positive samples. Serogroup-specific, real-time PCR assays were able to determine the *N meningitidis* serogroup W135. Of the 50 cases of *N meningitidis* serogroupW135, were confirmed using Aggultination test and RT-PCR.



Fig(2): RT-PCR analysis revealing the presence of *ctrA* of *N. meningitidis*

DISCUSSION

The prevalence of *N. meningitides* was relatively high (4%) in Iraqi children in comparison to global incidence of meningococcal carriage which is shown to be <3 % in children younger than 4 years and increased to 24–37 % in the age-group 15–24 years (15,16) a alongside with the probable emergence of new serogroups and potential increase in incidence of this bacteria which necessitates a need for more sensitive techniques.

Serological typing methods have been useful for rapid public health decisions and vaccine development, but they have a number of weaknesses, which make them inappropriate for epidemiological studies. These methods are based on variation in cell surface antigens, which are likely to be under selective pressure (17). Especially, strains recovered from carriers often lack expression of the capsular antigen and the serotype and/or serosubtype cannot be identified either because of low level of expression of the genes (18). In order to reduce such pitfalls, the present work adopted CSF specimens as the source of isolation.

Although detection of *N. meningitides* by conventional culture technique is highly specific and reliable, it is limited by time consuming and low sensitivity (19). On contrary the relative rapidity, sensitivity, and specificity of RT-PCR are emphasize the consistent of this technique. Upon such fact many laboratories use *ctrA* as a gene target for RT-PCR detection of *N. meningitidis* (20).

In this study, we demonstrated the presence of *N meningitidis* in two samples Out of fifty suspected specimens of cerebrospinal fluid (CSF), the two isolates of *N. meningitidis* were detected using culture and Real Time-PCR techniques (RT-PCR). Api NH confirmed the results of culture. Slide Agglutination Serogrouping (SASG) revealed that both isolates were belonging to the serogroup W135.

Routine methods for the detection of bacteria in CSF specimens' by cultural and morphological methods, such as Gram and silver impregnation stains, can be difficult to interpret and are nonspecific; thus, detection of *N meningitidis* by RT-PCR and slide agglutination test for determining the serogroup is very important. Our study was in agreement with other studies round the world (21-23), whose demonstrated that the sensitivity of throat swab culture was higher than a PCR assay based on *ctrA*. They explain such variation by failure to detect some non-capsulated strains and probable presence of inhibitory factors in the samples. On the other hand, a study conducted by de los Monteros (23), *N. meningitidis* was isolated from 14 out of 736 (1.9%) children; while the total prevalence of *N. meningitidis* was 1.6%. The most frequent serogroups were Y (29.7%), C (24.3%) and B (10.8%).

The prevalence of *N. meningitidis* is conditioned by inherent factors in the bacterium, the host, the environment, the sample-taking technique, the number of samples taken, and the sensitivity of the methodology that is used in the laboratory (24). Other factors that impact the carrier state are age, gender, socioeconomic level, exposure to tobacco, pollution, alcohol consumption in entertainment centers, immunological status, and viral and/or bacterial infections (25,26).

Upon present study findings, certain recommendations concerning vaccination could be made to prevent *N. meningitidis* from spreading between Iraqi children as well as its prevalence among various Iraqi populations. Moreover, special attention should be paid to the serogroup W135.

We concluded that this study demonstrated the ability of RT-PCR for detecting *N. meningitidis* with specific primers in short time with high specificity and quality between the CSF samples and the bacterial isolates.

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Probiotic marine pigmented *Bacillus pumilus* SF 214

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ABSTRACT

The genus *Bacillus* is a member within the group of probiotic microbes, This study investigated the isolate *B. pumilus* SF 214 for some properties (adhesion to cell lines,resistance to GIT conditions and bacteriocin assays),results provide some interesting into a potential probiotic mechanisms, and they also raise further concerns over the potential using of this pigmented Bacilli as probiotic.
Cells or spores suspended in simulated gastric fluid and incubated at 37 °C for 1h. Samples were plated to determine the number of CFU per ml on Luria-Bertani (LB) agar plates, the results indicate that *B. pumilus* SF214 is not adversely affected by strongly acidic conditions.
Caco-2 cells cultured in Dulbecco's modified Eagle's minimal essential medium (DMEM) to investigated the adherence of *B. pumilus* SF214, The human Caco-2 cell line is one of the best model systems for evaluating the interactions between bacterial and intestinal epithelial cells, In this study, *B. pumilus* SF214 were adherent to approximately 65 % of Caco-2 cells, respectively. *B. pumilus* SF214 was used as a probiotic in the normal intestinal microflora to counteract invasion by pathogenic bacteria.
Germination of the spore could allow production of antimicrobial agents, such as bacteriocin-like inhibitory substances thereby contributing to the competitive exclusion of pathogens
We conclude that, *B. pumilus* SF214, remained stable in artificial gastric juice and bile salt, adhered to Caco-2 intestinal epithelial cells, and produce bacteriocin- like inhibitory substance, these characteristics indicate that *B. pumlius* SF214 is suitable for industrial use as a probiotic.

Keywords: *Bacillus pumilus*, Probiotic , Caco-2 cells, Bacteriocin like substance

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الملخص باللغة العربية

ان بكتريا الباسلس تعتبر ضمن مجموعة المعززات الحيوية الميكروبية ، في هذه الدراسة تم اختبار العزلة *B. pumilus* SF214 لخصائصها الاخرى ، واعطت النتائج بعض الاهتمام في امكانية آلية المعزز الحيوي وكذلك تشير ايضا الى امكانية استعمال هذه العصيات الملونة كمعززات حيوية.
علقت الخلايا البكتيرية او السبورات لهذه البكتريا بالسائل المعوي المقلد وحضنت في درجة حرارة 37°م لمدة ساعة واحدة ثم تصب النماذج لتحديد عدد المستعمرات CFU لمل مليلتر باستخدام وسط LB agar ، اشارة النتائج الى عدم تاثر بكتريا *B. pumilus* SF214 بالظروف الحامضية القوية.
ان خلايا *B. pumilus* SF214 للتحري عن امكانية التصاق بكتريا (DMEM) زرعت في وسط Caco-2 cells خلايا Caco-2 cells البشرية هي احد افضل الانظمة النموذجية لتقييم التداخلات (التفاعلات) بين البكتريا والخلايا الطلائية المعوية ، في هذه الدراسة التصقت بكتريا *B. pumilus* SF214 بنسبة 65% مع خلايا Caco-2 cells وعليه فان هذه البكتريا تستخدم كمعززات حيوية في الفلورا الطبيعية في الامعاء لصد غزو البكتريا المرضية.
عند استنبات السبورات يمكن ان ينتج عوامل مضادة للميكوبات مثل مواد مثبطة تشبه البكتريوسين وبذلك تساهم في منع التنافس مع الممرضات ، ومن خلال هذه الدراسة تبين ان بكتريا *B. pumilus* SF214 تبقى مستقرة في العصارة المعوية الصناعية والاملاح الصفراء وكذلك التصاقها بخلايا Caco-2 cells المعوية الطلائية وانتاجها مواد مثبطة تشبه البكتريوسين، ان جميع هذه الخصائص تشير بان *B. pumlius* SF214 مناسبة للاستخدامات الصناعية كمعززات حيوية.

INTRODUCTION

Within the group of probiotic microbes are the spore-forming bacteria, normally members of the genus *Bacillus*. Here, the product is used in the spore form and thus can be stored for along time . The use of spore- based products raises a number of questions though . Since the bacterial species being used are not considered resident members of the gastrointestinal microflora (1). The natural life cycle of spore formers involves germination of the spore, proliferation and then re-sporulation when nutrients are exhausted, the logical question is whether it is the germinated spore (that is the vegetative cell) that produces the probiotic effect or is it the spore itself(2,3) If the former model is correct then it would suggest that probiotics show a unified mode of action involving the action of a live bacterium within the GIT (4). Spore- forming bacteria can survive and, indeed, proliferate within the GIT of animals, Although it is unlikely that they are true commensals, a case will be made that many spore formers exhibit a unique dual life cycle of growth and survival in both the environment and within the gut of animals and it is this bimodal life cycle that could provide the basis of their probiotic effect (5).

Few *Bacillus* species had been obtained from marine environments (6,7). Among the numerous *Bacillus* species, only species of *B. badius, B. subtilis , B. cereus , B. lichenifirmis, B. firmus , B. pumilus , B. mycoides* , and *B. lentus* have been detected from marine environments, including marine-derived species, such as *B. marinus, B. dipsosauri* (6). Furthermore, marine-originated species have been reported to produce unusual metabolites, different from the species of terrestrial origin (7).

B. pumilus SF214 isolated from sea water, this isolate was identified and characterized to species level using API 50CHB kit (Biomerieux) The entirely *rrnE* (16S rRNA) gene was sequenced, together with phylogenetic analysis revealed that this isolate were identical to known *B. pumilus* , and tested for sporulation efficiency, starch lysis, haemolysis, motility, resistance to arsenate and arsenite, surfactin production, tolerance to NaCl, anaerobic growth, UV resistance, hydrogen peroxide resistance, and pigment profile, the pigment identified as a caratenoid and was particularly interesting because carried high levels of carotenoids and was fully resistant to UV-C, the pigment was water soluble and produced in a temperature-regulated fashion leading to rise the question to understand their physiological role Khaneja *et al* (8).

In this study we tested the isolate *B. pumilus* SF 214 for other properties (adhesion to cell lines,resistance to GIT conditions and bacteriocin assays), our results provide some interesting insights into potential probiotic mechanisms, and they also raise further concerns over the potential using of these pigmented Bacilli as probiotic.

MATERIALS AND METHODS

Bacterial strain:

B. pumilus SF214 was obtained from culture maintained in the microbial culture collection of Department of Structural and Functional Biol. University of Naples, Federico II, Italy. Reference *B. subtilis* strain used was PY79, a prototrophic derivative of the 168-type strain (9).

Resistance to GIT conditions:

Resistance was assessed as previously reported (10). cells or spores were suspended in simulated gastric fluid [sgf: 1mg of pepsin(porcine stomach; Sigma) per ml; ph 2.0] or small intestinal fluid [sif: 1 mg of pancreatin(porcine pancreas; sigma) per ml and 0.3% bile salts(50% sodium cholate-50% sodium deoxycholate; sigma); PH 7.4] and incubated at 37 °C for 1h. Samples were serially diluted and plated to determine the number of CFU per ml on LB agar plates.

Adhesion to Caco-2 cells:

Caco-2 cells were cultured in Dulbecco's modified Eagle's minimal essential medium (DMEM) containing 25 mM glucose, 1.0 mM sodium pyruvate, 10% heat inactivated fetal bovine serum, 1% nonessential amino acids solutions, and antibiotics (100 IU of penicillin G per ml and 100 μg of streptomycin sulfate per ml). The Caco-2 cells were grown under standard conditions (37°C, 5% CO2) and the medium was replaced every two days. Monolayers of Caco-2 cells, which were used in the adherence assay, were prepared by seeding 6-well tissue culture dishes with 9.5×10^4 cells per 4 ml of culture medium. Next, 3.1×10^6 CFU/ml of *B. pumilus* SF214 were added to the Caco-2 cells in the culture dishes. The samples were then incubated for 2 h and then washed 3 times with sterile PBS (pH 7.4), after which the number of adhered B.

pumilus114 was determined by plating the diluted *B. pumilus* SF214 suspensions on TSA (11).

Bacteriocin assays:

A colony overlay assay was used to screen for bacteriocin activity (12). The tested bacterium grows on Luria-Bertani (LB) medium. For this reason, culture of the bacterium was incubated overnight in LB medium at 37ºC for 24 h. Then 5µl portions of the overnight cultures were incubated as spots on LB medium plates, which were incubated at 37°C for 24h. the plates were overlaid with LB medium soft agar that had been inoculated with an overnight culture of an indicator strains which were (*B. subtilis, B. pumilus, B. licheniformis, B. megaterium, B. clausii, Staphylococcus aureus, E.coli , Salmonella enteric , Pseudomonas aeruginosa* and *Enterobacter aeroginosa*) and reincubated. The presence of zones of growth inhibition around the spots at any of the times examined (5, 8, 24, and 48h postinoculation) was considered a positive response.

Preparation of spores:

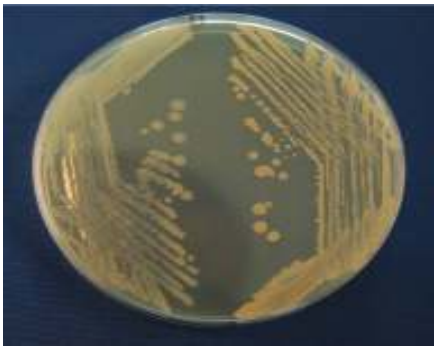
Sporulation was induced in Difco sporulation medium (DSM) by using the exhaustion method as described elsewhere (13). Sporulation cultures were harvested 24h after start of sporulation. Purified suspensions of spore were prepared as described by Nicholson and Setlow (13), followed by washing in 1 M NaCl, 1 M KCl, and distilled water (twice). Spore suspension was titrated immediately to determine of CFU per milliliter before used it.

RESULTS AND DISCUSSION

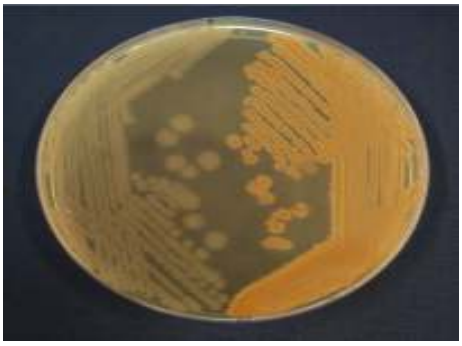
B.pumilus SF214 was isolated from sea water. Table (1) shows some properties for this isolate, and this isolate form pigmented colony both on LB and DSM as shown in (Fig 1). Cellular stress begins in the stomach, which has a pH as low as 2.0. To determine the effect of the acidic pH of the stomach on the survival of *B. pumilus* SF 214, an in vitro system with a pH of 2.0 was utilized (Table.2). When *B. pumilus* SF214 was exposed to pH 2.0, it survived for 2h. These results indicate that *B. pumilus* SF214 is not adversely affected by strongly acidic conditions. After the microorganisms pass through the stomach, they enter the upper intestinal tract, where bile salt is secreted into the duodenal (14). Therefore, it is necessary for probiotic bacteria to be resistant to bile. Accordingly, we evaluated the sensitivity of *B. pumilus* SF214 to bile by culturing it in LB containing 0.3% bile (Table. 2). The results revealed that the level of *B. pumilus* SF214 was maintained in the presence of bile,which indicates that it is relatively resistant to bile. The viability of domesticated strain PY79 was identical for treated and untreated spores to the condition used (data not shown)**.**

Table(1): Some growth and morphological characters of *B. pumilus* SF214

Characteristics	Description
Growth temp	37°C (24 h)
Culture medium	LB for normal growth and DSM for sporulation
Pigmentation	on TY agar orange to red pigment on DSM agar yellow to orange
Colony morphology	Small to medium white edge, flat colony, orange to red color on LB agar.
Spore formation	2-3 days at 37°C



B. subtilis ***B. pumilus***
On LB agar



B. subtilis ***B. pumilus***
On DSM agar
Fig (1):Overnight growth on LB and DSM agar

We investigated the adherence of *B. pumilus* SF214 to Caco-2 cells. The human Caco-2 cell line is one of the best model systems for evaluating the interactions between bacterial and intestinal epithelial cells (15). In this study, *B. pumilus* SF214 were adherent to approximately 65 % of Caco-2 cells, respectively. *B. pumilus* SF214 is used as a probiotic in the normal intestinal microflora to counteract invasion by pathogenic bacteria. Therefore, the ability of *B. pumilus* SF214 to inhibit the adhesion of pathogenic bacteria is expected to be highly specific and depends on both probiotic and pathogenic bacteria (16).

We used a colony overlay assay for antimicrobial activity in *B. pumilus* SF214. Ten indicator strains, including both gram positive and gram negative organisms, were used. The strains used included strains of *B. subtilis, B. pumilus, B. licheniformis, B. megaterium, B. clausii, Staphylococcus aureus*, *E.coli, Salmonella enterica, Pseudomonas aeruginosa and Enterobacter aeroginosa*. Under our assay conditions *B. pumilus* SF214 exhibited measurable activity (Table 3). Germination of the spore could allow production of antimicrobial agents, such as bacteriocin-like inhibitory substances thereby contributing to the competitive exclusion of pathogens, and it is one factor that could support the probiotic effect. A number of *Bacillus* species produce antimicrobial agents, and more than 80 different types have been reported (17). These antimicrobial agents are active mostly against gram-positive bacteria, but some are active against gram negative bacteria. Recently, an antibiotic compound isolated from a strain of *B. subtilis* found in probiotic Biosporin activity against *Helicobacter pylori* (18). We show here that *B. pumilus* SF214 produce antimicrobial agent (or bacteriocin-like inhibitory substances) are active also against other *Bacillus* species that have been isolated from GIT. In any case, production of the antimicrobial agents could be an element in the probiotic effect.

Two types of spores came from the same vegetative cells and spores as the various viscosity is presumably attributable to differences in surface of the spores (Fig. 2).

In conclusion, *B. pumilus* SF214, remained stable in artificial gastric juice and bile salt, adhered to Caco-2 intestinal epithelial cells, and pro produce bacteriocin- like inhibitory substance. Taken together, these characteristics indicate that *B. pumlius* SF214 is suitable for industrial use as a probiotic.

Table(2): Simulated GIT conditions with *Bacillus pumilus* SF214

Time	Control	PH 2.0	PH 6.5
Zero time	$20X10^8$ cell/ml	$26X10^8$ cell/ml	$28X10^8$cell/ml
1h	$9.8X10^8$ cell/ml	$1.75X10^8$ cell/ml	$2.8X10^8$ cell/ml
2h	$14X10^8$ cell/ml	$1.65X10^8$ cell/ml	$2.0X10^8$ cell/ml

Table (3): Bacteriocin production by B.pumilus SF214

Indicator organism	Reaction
B. subtilis	++
B. pumilus	++
B. licheniformis	++
B. megaterium	+/-
B. clausii	+
Staphylococcus aureus	+++
E.coli	+/-
Salmonella enteric	+/-
Pseudomonas aeruginosa	+
Enterobacter aeroginosa	+

+, clear halo of growth inhibition at least one times (results were determined after 5, 8, 24, and 48h of incubation); +++, ++, different degrees of inhibitory activity, as assessed by decreased inhibition zone. +/-, clear reduction in growth but not complete inhibition.

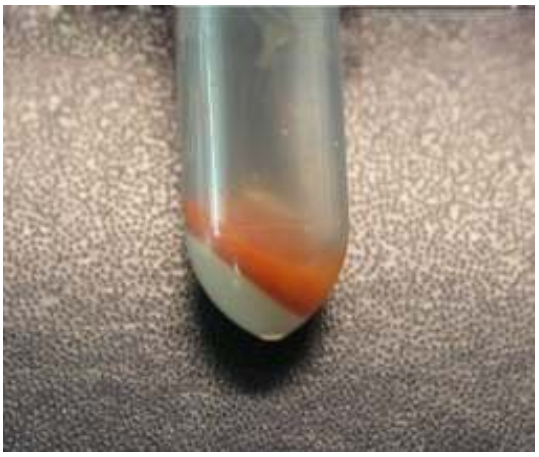


Fig. (2): Spore pellet of *B. pumilus* SF214 strain

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Concentrations of Some Serum Elements in Clinically Healthy and Anemic Iraqi Awassi Sheep

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ABSTRACT

The study was conducted on 181 normal healthy and 66 diagnosed iron deficiency anemia (IDA) Awassi sheep to evaluate serum calcium (Ca), magnesium (Mg), and phosphorus (P) concentrations. The normal sheep were divided into 73 males; 36 ram lambs and 37 rams,108 females; 35 ewe lambs, 37 pregnant and 36 lactating ewes, while the 66 anemic divided into 19 males (12 ram lambs and 7 rams) and 47 females (5 ewe lambs, 21 pregnant and 21 lactating ewes), all aged 7 months to 4 years in Baghdad governorate / Iraq.

The samples were collected from October 2011 to March 2012, and the sera were investigated in Clinical Pathology Laboratory / College of Veterinary Medicine-Baghdad University.

The results showed that the range and mean ± standard error (SE) of serum Ca, Mg, and P in clinically normal and anemic Awassi sheep were as follows : serum Ca 0.82 – 2.56 mmol/L and 1.97 ± 0.02 mmol/L, 0.78 – 2.26 mmol/L and1.76 ± 0.04 mmol/L , serum Mg 0.57– 3.29 mmol/L and 1.35 ± 0.05 mmol/L, 0.53 – 2.79 mmol/L and 1.02 ± 0.06 mmol/L and serum P 0.82 – 3.87 mmol/L and 2.31 ± 0.04 mmol/L, 0.8 – 3.00 mmol/L and 1.89 ±0.05 mmol/L respectively. However significant differences ($P<0.05$) were recorded between males and females in serum Mg and P, as well as in different subgroups of normal Awassi sheep. On the other hand, the results revealed significant differences ($P<0.05$) between normal and anemic values. In conclusion, the present data was recorded the reference range and Mean ± SE of serum Ca, Mg, and P in normal Awassi sheep and compared their values with that of anemic sheep.

Key Words: serum calcium, magnesium, phosphorus , and Iraqi Awassi sheep

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اجريت هذه الدراسة على 181 رأساً من الاغنام العواسية العراقية السوية سريرياً و 66 مشخصة بفقر دم نقص الحديد (IDA) لتقييم مستوى الكالسيوم والمغنيسيوم والفسفور في المصل. قسمت الاغنام السوية سريرياً الى 73 ذكراً (36 حملان ذكرية و 37 كباش) و 108 اناث (35 حملان انثوية و 37 حوامل و 36 مرضعات) في حين تم تقسيم المصابة بفقر الدم الى 19 ذكراً (12 حملان ذكرية و 7 كباش) و 47 من الاناث (5 حملان انثوية و 21 حوامل و 21 مرضعات) وقد تراوحت اعمارها ما بين 7 شهور الى 4 سنوات في محافظة بغداد \ العراق.

جمعت العينات من تشرين الأول 2011 لغاية آذار 2012 وفحصت الامصال في مختبر التشخيصات المرضية في كلية الطب البيطري \ جامعة بغداد.

اظهرت نتائج المديات والمعدلات ± الخطأ القياسي لكل من الكالسيوم والمغنيسيوم والفسفور في الحيوانات السوية سريرياً والمصابة بفقر الدم كما يلي : كالسيوم المصل 0.82 – 2.56 mmol/L و 1.97 ± 0.02 mmol/L ، 0.78 – 2.26 mmol/L و 1.76 ± 0.04 mmol/L ومغنيسيوم المصل 0.57 – 3.29 mmol/L و 1.35 ± 0.05 mmol/L ، 0.53 – 2.79 mmol/L و 1.02 ± 0.06 mmol/L و فسفور المصل 0.82 – 3.87 mmol/L و 2.31 ± 0.04 mmol/L ، 0.8 – 3.00 mmol/L و 1.89 ± 0.05 mmol/L على التوالي. على أية حال، سجلت فروقات معنوية بمستوى معنوية ($P<0.05$) بين الذكور والاناث لأيوني الفسفور والمغنيسيوم في المصل، كذلك وجدت اختلافات معنوية في المجاميع الفرعية للاغنام العواسية. ومن ناحية اخرى، كشفت النتائج عن وجود فروقات معنوية بين الحيوانات السوية والمصابة بفقر الدم. ثبتت هذه الدراسة المديات المرجعية والمعدلات مع الخطأ القياسي للكالسيوم والمغنيسيوم والفسفور في امصال الاغنام العواسية الطبيعية ومقارنة هذه القيم مع المصابة بفقر الدم.

INTRODUCTION

Awassi sheep was a breed of multipurpose uses (milk, meat, and wool), it was the most domestic breed of sheep in Iraq due to its ability for adaptation to cruel environmental conditions and poor nutrition (1, 2). Many researchers had take care this breed via studying their blood criteria including constituents like minerals in different ages in both males and females (3-6). Also, in Iraq was studied status of some serum electrolytes in clinically normal Iraqi Awassi sheep (7).

The scientists (8) were denoted to an important thing that blood is a good medium by which general health evaluation could be estimated, and when variation noticed in blood it might be refers to many factors like age, sex, altitude, breed, seasonality, food, climate, or physiologic status like pregnancy or lactation (9).

Calcium (Ca) and Phosphorus (P) were important minerals that plays a vital role in building of strong skeleton, muscle contraction and neuronal excitability; in adult sheep, there was a stable ratio 2:1 Ca to P, with moderate importance of Mg with an important role of Vitamin D which increases Ca absorption, while excess Mg decreased Ca absorption, while excess Ca or Mg decreases P absorption (10-12).

There were little researches performed on sheep flocks connecting to minerals in Iraq, especially in Awassi sheep, and because of Ca, Mg, and P are most likely deficient minerals in sheep rations and / or in the grazing pasture (13). For those reasons, this study was conducted to evaluate their levels in normal healthy and diagnosed iron deficient anemic sheep in sex, different age groups and physiologic status in Iraqi Awassi sheep.

MATERIALS AND METHODS

Blood samples were collected into plain tubes from jugular vein from October 2011 to March 2012 of 181 clinically normal Awassi sheep (73 males which subdivided into 36 ram lambs aged 7 – 12 months and 37 rams aged 1.5 – 4 years, and 108 females subdivided into 35 ewe lambs aged 7 – 12 months, 37 pregnant ewes aged 1 – 4 years, and 36 lactating ewes aged 1.5 – 4 years. On the other hand, a total of 66 iron deficient anemic sheep were subdivided into 19 males (12 ram lambs aged 7 – 12 months, and 7 rams aged more than 2 years) and 47 anemic females (5 ewe lambs aged between 7 – 12 months, 21 pregnant ewes aged 1 – 4 years, and 21 lactating ewes aged 1.5 – 4 years) from Baghdad / Iraq.

The sera were obtained by centrifugation of blood samples at 3000 rpm for 5 minutes (14) and used for measurement of serum Calcium, Magnesium and Phosphorus in the Clinical Pathology Laboratory / College of Veterinary Medicine – Baghdad University / Iraq. The serum Ca, Mg, and P concentrations were estimated according to the colorimetric method (15, 16, 17).

SAS program was used for statistical analysis. Data were subjected to analysis of variance (ANOVA) and significant means were compared by T-test at a level ($P<0.05$).

RESULTS

The serum values of Ca, Mg, and P for Awassi sheep independent of any subdivisions are presented in (table 1), according to sex (table 2), and the physiologic status (table 3).

Serum Ca in normal Awassi sheep has been recorded mean values of 1.97 ± 0.02 mmol/L ranged 0.82 – 2.56 mmol/L, and 2.04 ± 0.03 mmol/L in males, while in females 1.93 ± 0.03 mmol/L with a significant differences ($P<0.05$) between them (table 2). Moreover, serum Ca concentration was significantly ($P<0.05$) higher in normal compared to that of iron deficient anemic sheep (table 1). However, in normal sheep, the serum Ca levels were significantly higher in rams and lactating ewes compared to pregnant ewes, as well as rams and pregnant ewes showed significant increase compared to the respective anemic subgroups (table 3). Serum Ca level in IDA was 1.76 ± 0.04 mmol/L ranged 0.78 – 2.26 mmol/L, it was 1.84 ± 0.07 mmol/L and 1.73 ± 0.05 mmol/L in males and females respectively.

On the other hand, Serum Mg in normal Awassi sheep was found to be 1.35 ± 0.05 mmol/L ranged 0.57 – 3.29 mmol/L (table 1). It was 1.91 ± 0.11 mmol/L in males and 0.98 ± 0.01 mmol/L in females with significant differences ($P<0.05$) between them (table 2). In general, serum Mg levels were significantly higher in normal compared to those of iron deficient anemic sheep (table 1). However, rams showed significantly higher differences ($P<0.05$) in serum Mg concentrations compared to other normal subgroups (table 3).

Table (1): Total serum Ca, Mg, and P concentrations in clinically normal and iron deficient anemic (IDA) Iraqi Awassi sheep (range and Mean ± SE).

Groups	No. of sheep	Serum Ca mmol/L	Serum Mg mmol/L	Serum P Mmol/L
Normal	181	0.82 – 2.56 1.97 ± 0.02 a	0.57 – 3.29 1.35 ± 0.05 a	0.82 – 3.87 2.31 ± 0.04 a
IDA	66	0.78 – 2.26 1.76 ± 0.04 b	0.53 – 2.79 1.02 ± 0.06 b	0.80 – 3.00 1.89 ± 0.05 b

The differences in letters vertically refer to presence of significant value at (P<0.05).

Table (2): Serum Ca, Mg, and P concentrations in clinically normal and IDA males and females' Iraqi Awassi sheep (range and Mean ± SE).

Gender	No.	Ions		
		Serum Ca mmol/L	Serum Mg mmol/L	Serum P mmol/L
Males	73	1.33 – 2.56 2.04 ± 0.03 a	0.57 – 3.29 1.91 ± 0.11 a	1.27 – 3.87 2.47 ± 0.06 a
Females	108	0.82 – 2.47 1.93 ± 0.03 ab	0.58 – 1.41 0.98 ± 0.01 c	0.82 – 3.60 2.21 ± 0.05 b
IDA Males	19	1.32 – 2.26 1.84 ± 0.07 bc	0.53 – 2.79 1.43 ± 0.18 b	1.31 – 2.94 2.06 ± 0.11 b
IDA Females	47	0.78 – 2.24 1.73 ± 0.05 c	0.57 – 1.24 0.86 ± 0.02 c	0.80 – 3.00 1.82 ± 0.06 c

The differences in letters vertically refer to presence of significant value at (P<0.05).

Table (3): Serum Ca, Mg, and P values according to the physiologic status in clinically normal and IDA Iraqi Awassi sheep (range and Mean ± SE).

Groups	No.	Ions		
		Serum Ca mmol/L	Serum Mg mmol/L	Serum P mmol/L
Ram lambs	36	1.33 – 2.48 AB 1.97 ± 0.04 a	0.57 – 1.39 B 0.97 ± 0.03 a	1.27 – 3.87 A 2.65 ± 0.10 a
IDA ram lambs	12	1.32 – 2.21 1.82 ± 0.08 a	0.53 – 1.08 0.85 ± 0.04 a	1.31 – 2.94 2.14 ± 0.15 b
Rams	37	1.62 – 2.56 A 2.11 ± 0.03 a	2.24 – 3.29 A 2.83 ± 0.04 a	1.38 – 3.29 AB 2.29 ± 0.06 a
IDA rams	7	1.35 – 2.26 1.86 ± 0.14 b	2.23 – 2.79 2.42 ± 0.07 b	1.34 – 2.45 1.92 ± 0.16 b
Ewe lambs	35	0.82 – 2.41 AB 1.93 ± 0.06 a	0.63 – 1.30 B 0.95 ± 0.03 a	1.44 – 3.60 A 2.59 ± 0.08 a
IDA ewe lambs	5	1.51 – 2.01 1.78 ± 0.09 a	0.60 – 0.88 0.79 ± 0.04 b	1.71 – 3.00 2.32 ± 0.21 a
Pregnant ewes	37	1.10 – 2.39 B 1.80 ± 0.05 a	0.58 – 1.41 B 1.02 ± 0.02 a	0.82 – 3.23 B 1.95 ± 0.08 a
IDA pregnant ewes	21	0.78 – 2.24 1.56 ± 0.09 b	0.58 – 1.24 0.91 ± 0.03 a	0.80 – 2.43 1.65 ± 0.09 a
Lactating ewes	36	1.48 – 2.47 A 2.07 ± 0.03 a	0.69 – 1.36 B 0.96 ± 0.02 a	1.28 – 2.82 B 2.11 ± 0.07 a
IDA lactating ewes	21	1.34 – 2.23 1.89 ± 0.05 a	0.57 – 1.03 0.82 ± 0.02 b	1.30 – 2.35 1.88 ± 0.08 a

Different capital letters vertically refers to the presence of significant differences at (P<0.05) between normal subgroups.
Different small letters vertically refers to the presence of significant differences at (P<0.05) between normal and anemic subgroups.

Moreover, serum Mg levels were significantly higher ($P<0.05$) in rams, ewe lambs and lactating ewes of healthy sheep compared to the respective anemic subgroups (table 3).

Serum P concentration in healthy sheep was 2.31 ± 0.04 mmol/L ranged 0.82– 3.87 mmol/L , 2.47 ± 0.06 mmol/L and 2.21 ± 0.05 mmol/L in males and females respectively with significant differences between them, while serum P in anemic sheep was 1.89 ± 0.05 mmol/L ranged from 0.80 – 3.00 mmol/L. It was 2.06 ± 0.11 mmol/L in males and 1.82 ± 0.06 mmol/L in females with a significant difference between them. Moreover, a significant increase of serum P in normal compared to anemic one (table 1). However, in normal sheep serum P concentration was significantly higher in ram lambs and ewe lambs compared to pregnant and lactating ewes. Finally, ram lambs and rams showed significantly higher values compared to the respective anemic subgroups.

DISCUSSION

The current study has been given a full idea about blood constituents of Ca, Mg, and P with reference to the effect of sex, age, and physiologic status in clinically healthy and iron deficient anemic Iraqi Awassi sheep, and the data recorded in this research shown significant differences between normal and iron deficient anemic sheep with some variation between them within subgroups.

The present study were noted some facts about Awassi sheep concerning three minerals and these were accepted by many investigators internationally (10-12, 18), as they recorded the normal values of Ca, Mg, and P in healthy sheep. Local studies in the neighboring countries also noticed relatively closed data to what we approached like (19) in Turkey, who studied the effects of the reproductive status on the serum chemistry and minerals of pregnant and lactating ewes and they found significant gradual decrease in Ca and P with the progression of pregnancy, while Mg did not affected by pregnancy or lactation.

In Iraq, few researches were done in this line as (20) in Mosul (Northern of Iraq) who estimated Ca and Mg levels in the blood of healthy and infested with tapeworms lactating ewes; their results in healthy one were closely nearer to what we found in this study. In contrast, a recent study to (21) applied to evaluate 5 minerals (Ca, Mg, P, Cl, and K) in 150 sheep divided according to weight, they were registered very low values (when compared to our results) without referring to sex, age, and physiologic status.

Another recent study to (22) who studies the effect of propolis on several serum minerals levels like (Ca, P, Mg, Na, and K) on Awassi lambs and they found higher results than our data in Awassi male lambs.

The results of the present study were in accordance with (23) who studied blood profile of 10 Dubrovnik sheep (Croatian endangered breed aged 3 years) including serum minerals like (Ca and P), and they found partially constant levels of Ca and P in serum of rams as we noticed.

The results of the current study showed declined Ca ion concentrations with development of pregnancy and even after parturition and lactation perhaps due to the lack of Ca supplement in food, and this was proved by (24)(25) who implicated hypocalcaemia in ewes to many factors including poor diet with Ca or its resources. Also, in the same manner in Serbia, (13) confirmed our opinion by their results in the pasture at which 100 Zapata sheep were grazed, as they found low levels of Ca, Mg, and P in the soil and in the grass, corn grits and hay of sheep food.

Furthermore, our results in lactating sheep were accepted by (26) who studied 30 sheep in Serbia analyzing them for serum Ca, Mg, and P and he found nearer results to what we obtain attributed that deficiency to the low levels of these minerals in food as well as excessive release with milk (especially Ca).

In contrast, (27) referred that there was no significant changes of Ca, P, and Mg values in grazing sheep fed on *Anagallis arvensis* plants in the Saudi Arabia Kingdom, and he found no effect of those ions on anemic sheep. In the same way, the Indian scientist (28) was studied the infection of *Trypanosoma congolense* in sheep which produced just alterations in protein and iron metabolism, but no significant effects on plasma calcium, magnesium, zinc, copper, and inorganic phosphate.

We might be agreed with a Romanian scientist (29) who studied 40 healthy sheep dealt in a good manner and grazing forages, they realized that season had significant effect on the concentrations of Ca, Mg, and P in blood suggesting lower levels during Spring than in Autumn with less favourable conditions during winter.

In a point of view of some investigators like (30) who implicated the fluctuated results

especially in minerals like Ca, Mg, and inorganic P to the addition of anticoagulants which affect on plasma biochemistry of sheep; they found that there were significant differences of their values between blood plasma and serum, when sodium citrate or EDTA as anticoagulants were used.

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قسم الدراسات العربية

ARABIC SECTION

تأثير الرش بتراكيز مختلفة من مستخلص جـذور عـرق الـسوس *Glycyrrhiza glabra* وكبريتـات المغنيسيوم المائية ومؤشرات النمو لنبات السفندر *Ruscus* sp

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الملخص باللغة العربية

نفذ البحث في احد المشاتل الخاصة في محافظة البصرة للفترة من 2011/10/16 ولغاية 16/6/ 2012 لدراسة تأثير الرش بثلاثة تراكيز من مستخلص جذور عرق السوس هي (0 و 2.5 و 5 غم /لتر) وأربعة تراكيز من كبريتات المغنيسيوم المائية ($MgSO_4.2H_2O$) هي (0 و 32 و 64 و 96 ملغم /لتر) والتداخل فيما بينها في تحسين نمو نباتات السفندر (الصالون) . نفذ البحث كتجربة عامليه بتصميم القطاعات العشوائية الكاملة (R.C.B.D) بثلاثة مكررات بعاملين , اختبرت المتوسطات حسب اختبار اقل فرق معنوي المعدل (R.L.S.D) وعلى مستوى احتمال 0.05.

أظهرت النتائج أن رش مستخلص جذور عرق السوس بتركيز 5 ملغم\لتر أو كبريتات المغنسيوم المائية بتركيز 96 ملغم\لتر زاد معنويا من عدد وطول الفروع , عدد الأوراق/ الفرع , مساحة الورقة الواحدة , النسبة المئوية للمادة الجافة , ومحتوى الأوراق من الكلوروفيل الكلي والكاربوهيدرات الكلية الذائبة والبروتين , بالإضافة إلى زيادة تركيز الفسفور البوتاسيوم والمغنيسيوم في الأوراق , وحدوث انخفاض معنوي في نسبة الكاربوهيدرات الى النتروجين (C/N) في الأوراق.

وقد أظهرت نتائج التداخل بين العاملين زيادة معنوية عدد الفروع وطولها , عدد الأوراق ومساحتها ومحتوى الأوراق من الكلوروفيل الكلي والبروتين بالإضافة إلى تركيز البوتاسيوم والمغنيسيوم في الأوراق .

يستنتج من البحث ان الرش بمستخلص جذور عرق السوس بالتركيز 5 غم/لتر او كبريتات المغنيسيوم المائية بتركيز 96 ملغم/لتر اثر معنويا في تحسين مؤشرات النمو لنبات السفندر(الصالون) .

ABSTRACT

This study was conducted in a private nursery in Basrah governorate during 16th. October / 2011 - 16th. June / 2012 on Ruscus plant to study the effect of three concentrations of liquorices extract (i.e . 0, 2.5 and 5 gm /l and four concentrations of magnesium fertilizing (I .e . 0, 32, 64 and 96 mg/l) on the growth of this plant growing in pots.

The experiment was conducted in as factorial experiment , Complete Randomized Block Design (R . C . B . D) with three replicates.

Results showed a significant increase in number of branches per plant , average branch length , average number of leaves per branch , average leaf length , average leaf area , percentage of dry matter, chlorophyll content in the leaf , leaf content of carbohydrates , protein content, phosphorus, potassium and magnesium content in leaves, while (C/N) ratio was decreased significantly as the liquorices extract was increased. A significant increase in the same growth parameters was obtained as magnesium concentration was raised except the content of phosphorus in leaves was not raised significantly and the decreased of (C/N) ratio was not significant either .

The interaction between the two factors was significant in increasing number of branches per plant , average branch length , average number of leaves per branch , average leaf area , chlorophyll content in the leaf , protein content, potassium and magnesium content in leaves.

The study concluded that spraying with liquorices extract at 2.5 or 5 gm/l had a significant increase in growth parameters of plant and spraying with magnesium at , 64 or 96 mg/l raised these parameters significantly except phosphorus content in leaves .

It also concluded that the interaction between the two factors significantly improved some of these parameters in this plant, and the study recommended that Ruscus plant should be treated with liquorices extract at 5 gm/l and magnesium at 64 mg/l to improve its growth.

المقدمة

نبات السفندر (الصالون) *Ruscus* sp. هو أحد نباتات العائلة الزنبقية Liliaceae , وهو من النباتات الجميلة التي تصلح للتربية داخل المنازل والأماكن الظليلة ونصف الظليلة، وهو نبات عديم الأشواك أوراقه الأصلية متحورة الى حراشف سمراء صغيرة وسيقانه الى اوراق عريضة ذات لون أخضر (1).

اتجه الباحثون الى ايجاد اساليب وطرائق فنية حديثة لغرض اعتمادها في تجهيز النباتات بالمغذيات الضرورية لاستمرار وتحسين نموها ، وذلك عن طريق التقليل او الحد من المعوقات التي تواجهها العناصر الغذائية في التربة والتي تقلل من جاهزيتها للنبات , ومن هذه الطرائق طريقة التغذية الورقية والتي تعني رش العناصر الغذائية بشكل محاليل مغذية أو مستخلصات نباتية مختلفة على النبات , والتي تعتبر فعالة في إيصال العناصر الغذائية مباشرة الى الورقة بصورة جاهزة خاصة تحت ظروف محددات الامتصاص من قبل الجذور والمتمثلة بظروف التربة غير الملائمة الامتصاص مثل الجفاف الارتفاع والانخفاض الحادين في درجات حرارة التربة (2) فضلا عن سرعة الاستجابة لامتصاص المغذيات بهذه الطريقة (3) . هذا وان الاتجاه الحالي في الزراعة هو الابتعاد عن استعمال الأسمدة والمواد الكيميائية والمبيدات باختلاف أنواعها في العمليات الزراعية وذلك لتأثيراتها السامة والضارة على حياة الإنسان وتلوث البيئة ، ولهذا فقد استعملت عدة بدائل لهذا الغرض ومنها رش النباتات بالمستخلصات النباتية الطبيعية , ومنها مستخلص جذور عرق السوس *Glycyrrhiza glabra* , وهو نبات عشبي معمر يكثر وجوده بالعراق بصورة برية (4). ويعتبر مستخلص عرق السوس من المستخلصات الرخيصة والمتوفرة في الأسواق . وقد اشار Anita الى ان المادة الفعالة (Glycerrhizin) هي المادة الرئيسة في جذور عرق السوس حيث توجد بشكل املاح الكالسيوم او البوتاسيوم لحامض الكليسيرايزك (Glycyrrhizic acid) وان هذه المادة في هذا الحامض تتصف بكونها مشابهة لعمل الجبرلين (5) . بالاضافة الى احتوائه على البروتين , السكريات المختزلة , التانين والالياف الخام , كما انه يحتوي على العديد من المغذيات منها الفسفور , البوتاسيوم , الحديد , الزنك , المنغنيز والنحاس (6) فضلاً على احتوائه على حامض الميفالونك Mevalonic acid الذي له دور في البناء الحيوي للجبرلين (7) .

ومن خلال البحوث التي أجريت على نباتات الزينة وجد ان رش مستخلص جذور عرق السوس عليها ادى الى تحسين مؤشرات النمو فيها , فقد ذكر العبدلي في دراسة قام بها حول تأثير الرش بمستخلص جذور عرق السوس بتركيز 0 و1.5 و3 غم/لتر في مؤشرات النمو لنبات القرنفل *Dianthus hybrida* L. ، وجد ان النباتات المعاملة بـ 3 غم/لتر قد تفوقت على باقي المعاملات في ارتفاع النبات وقطر الساق والمساحة الورقية ومحتوى الاوراق من الكلوروفيل الكلي (8)، وبينت الربيعي عند رش مستخلص عرق السوس بتركيز 2.5 غم/لتر على نبات الفريزيا *Freesia hybrida* حدوث زيادة معنوية في ارتفاع النبات وعدد الاوراق والمساحة الورقية والمحتوى النسبي للكلوروفيل الكلي بلغت 70.23% مقارنة بنباتات المقارنة والتي اعطت 58.93%. كما انه ادى الى زيادة معنوية في محتوى النبات من النتروجين والنسبة المئوية للفسفور وللبوتاسيوم في الاوراق (9)، كما وجدت العلوي ان رش نبات الداودي *Dendranthema grandiflorum* ب 4 غم/ لتر من مستخلص جذور عرق السوس أدى إلى زيادة ارتفاع النبات وقطر الساق وعدد الاوراق معنويا مقارنة بمعاملة المقارنة والتي اعطت اقل القيم (10) . وقامت ساهي بدراسة تأثير الرش بعرق السوس بتراكيز مختلفة (صفر و 5 و 10 غم/ لتر) وكلوريد الكالسيوم في صفات النمو لنبات حنك السبع *Antirrhinum majus* , واوضحت النتائج أن رش النباتات بتركيز 5 أو 10 غم/ لتر مع 1 غم/ لتر من كلوريد الكالسيوم أعطى أفضل النتائج للصفات الخضرية للنبات (11). وفي دراسة اجراها الزرفي على نبات الايرس الاسباني *Iris xiphium* L بين ان لرش مستخلص عرق السوس بتركيز 3 غم/لتر سبب زيادة معنوية في عدد الاوراق , المساحة الورقية , الوزن الجاف للاوراق ومحتوى الاوراق من الكلورفيل الكلي والفسفور (12)، واكدت ناصر ان رش مستخلص جذور عرق السوس على نبات الجرانيوم Pelargonium zonale L زاد معنويا من ارتفاع النبات , عدد الافرع الكلية والاوراق , المساحة الورقية والوزن الجاف للنبات , بالاضافة الى زيادة محتوى الاوراق من النتروجين والفسفور معنويا مقارنة مع النباتات الغير مرشوشة(المقارنة) والتي اعطت اقل القيم (13) .

ان الانسجة النباتية تحتوي على المغنيسيوم بمعدل 0.5% من المادة الجافة وان ما يقرب من 70% من المغنيسيوم الكلي في انسجة النبات يكون مرتبطا مع الايونات السالبة غير العضوية والايونات السالبة للاحماض العضوية مثل Malate وCitrate ويحتل المغنيسيوم المركز في جزيئة الكلورفيل وله دور مهم في تنشيطه الانزيمات التي تشترك في عمليات الفسفرة كما ان له اهمية في عملية تثبيت CO_2 في تفاعلات الظلام في التركيب الضوئي (14) , ولقد درس Hugh وSeeley تاثير اضافة المغنيسيوم الى المحلول المغذي لثلاثة أجناس من الاوركيد هي *Phalaenopsis* ، *Cattleya* ، *Cymbidium* بالتراكيز 25 ، 50 ، 100 ملغم/لتر , ووجد ان اعلى عدد للاوراق واكبر طول للورقة نتج في النباتات النامية في المحلول الحاوي على تركيز 25 ملغم/لتر وللاجناس الثلاثة (15).

وعند تنمية نبات بنت القنصل *Euphorbia pulcherrima* في محاليل مغذية وجد ان افضل ارتفاع للنبات نتج من النباتات النامية المحلول الحاوي على تركيز 24 ملغم/لتر من المغنيسيوم (16) . وبين Donald ان تسميد نبات الكاميليا *Camellia sasanqua* L بكبريتات المغنسيوم المائية وبالتركيز (0 , 4 , 10 , 20 و 30) غم/لتر زاد من عدد الاورارق والسيقان (17) ، كما وجد انه بزيادة تركيز ايون المغنيسيوم في المحلول المغذي يزداد محتوى الأوراق من المغنيسيوم (18) , وذكرت ساهي عند رش نبات الجربيرا *Gerbera jamesonii* L بكبريتات المغنسيوم المائية ($MgSO_4.2H_2O$) وبمعدل 2 غم/لتر زاد معنويا من عدد الاوراق , المساحة الورقية , الوزن الجاف للنمو الخضري , محتوى الاوراق من الكلوروفيل الكلي (19) . واوضحت دراسة عبد العزيز واخرون ان رش نبات Eustoma ruselliانum L. (Lisianthus) بالمغنيسوم المخلبي بالتركيز (0.5 و 1.0) غم/لتر اديا الى

زيادة معنوية في ارتفاع النبات ونسبة الكلوروفيل والمساحة الورقية والوزن الجاف للنبات (20).
ولأهمية نبات السفندر الجمالية والتنسيقة وندرة الابحاث علية , تم اجراء تجربة لبيان تاثير بثلاثة تراكيز من مستخلص عرق السوس واربعة تراكيز من كبريتات المغنيسيوم المائية في صفات النمو لنبات السفندر.

المواد وطرائق العمل

نفذ البحث في احد المشاتل الخاصة في محافظة البصرة للفترة من 2011/10/16 ولغاية 6/16/ 2012 لدراسة تأثير الرش بثلاثة تراكيز من مستخلص جذور عرق السوس هي (0 و 2.5 و 5 غم /لتر) واربعة تراكيز من كبريتات المغنيسيوم المائية ($MgSO_4.2H_2$) هي (0 و 32 و64 و 96 ملغم /لتر) والتداخل فيما بينها في صفات النمو لنباتات السفندر (الصالون) .
جلبت شتلات السفندر وبعمر 8 أشهر من أحد المشاتل الخاصة وانتخبت منها النباتات المتجانسة قدر الإمكان وتمت زراعتها في اصص قطرها 35 سم , بها وسط النمو متكون من الزميج والبتموس والبرلايت بنسبة 1:1:2 , اذ تمت زراعة نبات واحد في كل اصيص. حضر المستخلص المائي لمستخلص عرق السوس وذلك باحضار مسحوق جذور عروق السوس من السوق المحلية ومن ثم نخله واستخراج المسحوق الناعم منه لتحضير التراكيز المطلوبة , اذ حضر التركيز الاول باذابة 5 غم في لتر من الماء المقطر على درجة حرارة 50 °م في زجاجة خلاط كهربائي وخلط المزيج لمدة 15 دقيقة وبعد الانتهاء ترك المزيج لمدة 30 دقيقة ثم رشح باستخدام قماش الململ ولعدة مرات ليكون جاهزا لاستعماله بعمليات الرش وحضر التركيز الثاني بالتخفيف للتركيز الاول بالماء المقطر.

تمت الرشة الأولى بعد 3 أسابيع من الزراعة والرشة الثانية بعد 3 أسابيع من الرشة الأولى ووضع مع محلول الرش مادة ناشرة Tween-20 بتركيز (1مل/لتر) لتقليل الشد السطحي لجزيئات محلول الرش ولغرض احداث البلل الكامل للأجزاء الخضرية ومن ثم رفع مقدرة النبات على الاستفادة من المحلول وبمعدل أصيصين لكل مكرر وبثلاثة مكررات . وتم رش النباتات وحسب التراكيز المثبتة في البحث (21).

استخدمت كبريتات المغنيسيوم المائية $MgSO_4.7H_2O$ كمصدر للتسميد بعنصر المغنيسيوم , اذ تم تحضير المستخلص المائي للمغنيسيوم بأربعة تراكيز هي 0 و 32 و 64 و 96 ملغم /لتر (22، 23) وتم رش نباتات السفندر بعد 15 يوما من زراعتها في الاصص، وكانت عملية الرش تجري بمرشة يدوية حتى البلل الكامل وحتى سقوط أول قطرة كما رشت نباتات المقارنة بالماء المقطر وبعد مرور شهر على الرشة الاولى تم تكرار الرش مرة اخرى بنفس الطريقة السابقة.
وفي نهاية التجربة الحقلية أي في 6/30 تم اخذ القياسات التجريبية والتي اشتملت على عدد الأفرع ومعدل طول الفرع (سم) ومعدل عدد الأوراق على الفرع ومعدل طول الورقة (سم) ومعدل مساحة الورقة (سم^2) فقد تم حسابها كما هو موصوف في (24) في أوراق مكتملة النمو (بالغة) وبوحدة (سم^2) , وتم حساب النسبة المئوية للمادة الجافة في الاوراق حسب الطريقة الموصوفة من قبل (25) وتم تقدير محتوى الكلوروفيل الكلي في الأوراق حسب ما ذكر في الطريقة التي وصفها (26). ومحتوى الاوراق من الكاربوهيدرات الذائبة الكلية (ملغم/غم وزن جاف) , اذ تم اخذ العينات من أوراق النبات حسب الطريقة التي وصفها(27) فقد قدرت الكاربوهيدرات بطريقة الفينول-حامض الكبريتيك المعدلة Phenol-Sulphuric Acid, اما البروتين في الأوراق فقد تم احتساب النسبة المئوية للبروتينات في أوراق النباتات على أساس الوزن الجاف (A.O.A.C. ، 1975) وحسب المعادلة الآتية :
نسبة البروتين % = النسبة المئوية للنتروجين × 6.25
كذلك فقد قدرت نسبة الكاربوهيدرات الى النتروجين (C/N) في الأوراق فقد تم احتساب هذه الصفة من المعادلة الآتية :

$$\text{C/N} = \frac{\text{النسبة المئوية للكاربوهيدرات}}{\text{النسبة المئوية للنتروجين}}$$

اما محتوى الاوراق من الفسفور فقد قدر باستعمال طريقة مولبيدات الامونيوم وعلى وفق طريقة (28) , ومحتوى الاوراق من البوتاسيوم قدر حسب طريقة (29) , ومحتوى الاوراق من المغنيسيوم فقد تم تقديره في مستخلصات الأوراق وحسب الطريقة التي وصفها (30) بوساطة جهاز المطياف اللهبي Flame-photometer (ألماني الصنع) .
نفذت التجربة باستعمال تصميم القطاعات العشوائية الكاملة (R.C.B.D) بثلاثة مكررات ونباتين للوحدة التجريبية. واستعمل البرنامج الإحصائي Statistical Package for Social Sciences SPSS (11.0) لاختبار الفروق بين المتوسطات كما عمد إلى استخدام اختبار اقل فرق معنوي المعدل (R.L.S.D) لتحديد معنوية تلك الفروق تحت مستوى احتمال 0.05(31).

النتائج والمناقشة

يتضح من الجدول (1) ان الرش بمستخلص جذور عرق السوس ادى الى زيادة معنوية في عدد الافرع لنبات السفندر مع زيادة تركيز مستخلص عرق السوس , وبلغ 14.78 في معاملة الرش 5غم/لتر في مقابل 12.50 في معاملة الرش بـ 2.5 غم/لتر و 9.60 في نباتات المقارنة. وقد تعزى هذه الزيادة الى احتواء مستخلص عرق السوس على حامض الميفالونك (Mevalonic acid) بادئ البناء الحيوي للجبرلين الداخلي وهذا يشكل عاملا مساعدا في عمليات انقسام واستطالة الخلايا (7) . وهذا نفس ما وجدته (13) على نبات الجرانيوم .
كما يوضح الجدول نفسه ان الرش بكبريتات المغنيسيوم المائية قد ادى الى زيادة عدد الافرع معنويا مع زيادة التركيز المرشوش , ووصل الى 12.77 في النباتات المعاملة بالتركيز 96 ملغم/لتر في مقابل 11.76 فقط لنباتات المقارنة، ربما يعزى ذلك الى كون المغنيسيوم عنصراً مهماً في انتقال وتوزيع النشا وكذلك في تكوين السكريات فهو يعمل كعامل مساعد في كثير من التفاعلات الداخلة في عمليات التخمر الكحولي للكاربوهيدرات وتفاعلات الظلام للبناء الضوئي (32).

جدول (1): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسيوم المائية في عدد الافرع على نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز كبريتات المغنيسيوم المائية (ملغم/لتر)				متوسط المعاملة بمستخلص عرق جذور السوس
	0	32	64	96	
0	9.0	9.6	9.8	10.0	9.60
2.5	12.1	12.3	12.6	13.0	12.50
5	14.2	14.6	15.0	15.3	14.78
متوسط المعاملة بكبريتات المغنيسيوم المائية	11.76	12.17	12.47	12.77	12.30

لتركيز عرق 0.292 , $RLSD_{(0.05)}$ لتركيز الكبريتات = 0.337
$RLSD_{(0.05)}$السوس=
$RLSD_{(0.05)}$ للتداخل= 0.693

واظهر التداخل بين العاملين اختلافات معنوية فيما بين النباتات في هذه الصفة. اذ تبين ان رش النباتات ب 5غم/لتر مستلخص جذور عرق السوس مع الرش ب 96 ملغم/لتر كبريتات المغنيسيوم اعطى اعلى عدد معنويا بلغ 15.3 فرع/نبات مقارنة بنباتات المقارنة التي اعطت اقل عدد بلغ 9.0 فرع/نبات (جدول 1) .

أظهرت نتائج التحليل الاحصائي في جدول (2) ان الرش بمستخلص جذور عرق السوس ادى الى زيادة معدل طول الفرع معنويا , ووصل الى 36.63 سم في النباتات المعاملة بـ 5 غم / لتر في حين كان معدل طول الفرع في تلك المعاملة بـ2.5 غم / لتر قد بلغ 32.48 سم وفي نباتات المقارنة بلغ 23.28 سم . وقد ترجع هذة الزيادة الى احتواء مستخلص جذور عرق السوس على حامض الميفالونك Mevalonic acid الذي له دور في البناء الحيوي للجبرلين (7) . والذي تؤدي المعاملة به الى انتاج نباتات اكثر طولا نتيجة لزيادة استطالة السلاميات دون ان تؤثر في عددها كما انه يساعد على استطالة الخلايا واتساعها (33، 34) .

كما يوضح الجدول نفسه الزيادة المعنوية الحاصلة في معدل طول الفرع عند الرش بكبريتات المغنيسيوم المائية , اذ وصل الى 31.70 سم في مقابل 30.00 سم للنباتات المعاملة بالتركيز صفر من المغنيسيوم ، وهذا يتفق مع ما توصلت اليه دراسة (23) على نبات بنت القنصل.

هذا وقد ازداد عدد الاوراق معنويا مع زيادة الرش بمستخلص جذور عرق السوس , فبعد ان كان 8.68 في نباتات المقارنة (النباتات غير المرشوشة) وصل الى 15.08 في معاملة الرش بـ 5 غم/لتر (جدول 3). وقد تعزى هذه الزيادة الى احتواء مستخلص جذور عرق السوس على سكريات مختزلة وغير مختزلة ومركبات عضوية وعناصر معدنية مثل الفسفور والبوتاسيوم والكالسيوم وعناصر صغرى مثل الحديد والزنك والمنغنيز وغيرها (6) وان لهذه المركبات والعناصر دوراً في تنشيط الأنزيمات الخاصة بفعاليات النمو المختلفة ومنها عملية التمثيل الضوئي وكذلك دخول العناصر المعدنية في تركيب الأحماض النووية (RNA و DNA) الضرورية لانقسام الخلايا (35) ومن ثم الزيادة في عدد الأوراق وكبر المساحة الورقية للنبات . وهذا نفس ما اوضحه الزرفي (12) على نبات الايرس الاسباني وناصر (13) على نبات الجرانيوم.

كما يبين الجدول نفسه ان النباتات المعاملة بـ96 ملغم/لتر من كبريتات المغنيسيوم المائية قد تفوقت معنويا في عدد الاوراق على تلك المعاملة بالتركيز 32 ملغم/لتر او النباتات غير المرشوشة بالكبريتات . وتتشابه هذه النتائج مع ما وجده (23) على نبات بنت القنصل و (19) على نبات الجربيرا .

وكان لتداخل تراكيز مستخلص عرق السوس والرش بالمغنيسيوم تأثيراً في زيادة عدد الاوراق على الفرع فقد تفوقت المعاملة بـ 5غم/لتر مع الرش بـ96 أو 64 ملغم/لتر من كبريتات المغنيسيوم المائية معنوياً على تلك المعاملة بالتركيز صفر كبريتات ، دون فرق معنوي فيما بينها .

يتضح من الجدول (4) أن رش بمستخلص جذور عرق السوس أعطى فروقا معنوية بين المعاملات في معدل طول الورقة ، إذ تفوقت النباتات المعاملة بالتركيز 5 غم /لتر معنويا على النباتات غير المرشوشة بمستخلص جذور عرق السوس . كذلك يبين الجدول نفسه ان رش كبريتات المغنيسيوم المائية قد احدث اختلافات معنوية بين المعاملات اذ تفوقت معنويا معاملتا التسميد 96 ملغم/لتر و 64 ملغم/لتر على المعاملة 32 ملغم/لتر وعلى المعاملة بالتركيز صفر كبريتات . وربما تعزى هذه الزيادة الى دور هذا عنصر المغنيسيوم في تنشيط أنزيمات الخاصة بتثبيت جزيئة ثاني أوكسيد الكاربون في دورة كالڤن في تفاعلات الظلام مما قد يعمل على زيادة انتاج المواد الغذائية المصنعة الخاصة ببناء المجموع الخضري للنبات وكذلك دوره في تصنيع جزيئة الكلوروفيل وتجميع الرايبوسومات لتصنيع البروتينات (35) . مما يعمل بالنهاية على زيادة طول الورقة . وهذا نفس ما وجده (15) على نبات الاوركيد . هذا ولم يكن للتداخل بين العاملين اي تأثير معنوي في طول الورقة .

تفوقت معاملات الرش بمستخلص جذور عرق السوس في اعطاء اكبر مساحة بلغت 13.45 على معاملة المقارنة والتي اعطت اقل مساحة للورقة الواحدة بلغت 6.99 (جدول 5) , وهذه النتيجة تتشابه مع ما ذكره (8) على نبات القرنفل و (10) على نبات الداودي . ويبين الجدول نفسه ان معدل مساحة الورقة قد ازداد مع زيادة تركيز المغنيسيوم الا ان تلك الزيادة لم تكن معنوية . في حين كان للتداخل بين العاملين تأثيرا معنويا في هذه الصفة. اذ كانت اعلى مساحة في النباتات المرشوشة ب 5غم/لتر مستخلص جذور عرق السوس مع 96ملغم/لتر كبريتات المغنيسيوم المائية وبلغت (14.00 سم^2) مقارنة مع اقل مساحة (6.83 سم^2) في نباتات المقارنة .

جدول (2): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسيوم المائية في معدل طول الفرع (سم) في نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز كبريتات المغنيسيوم المائية (ملغم/لتر)				متوسط المعاملة بمستخلص جذور عرق السوس
	0	32	64	96	
0	22.9	23.2	23.4	23.6	23.28
2.5	31.2	32.6	32.6	33.5	32.48
5	35.9	36.2	36.3	38.1	36.63
متوسط المعاملة بكبريتات المغنيسيوم المائية	30.0	30.7	30.8	31.7	30.80

$RLSD_{(0.05)}$ لتركيز عرق السوس= 0.467 , $RLSD_{(0.05)}$ لتركيز الكبريتات = 0.539

$RLSD_{(0.05)}$ للتداخل= 0.934

جدول (3): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسيوم المائية في معدل عدد الاوراق على الفرع في نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز كبريتات المغنيسيوم المائية (ملغم/لتر)				متوسط المعاملة بمستخلص جذور عرق السوس
	0	32	64	96	
0	7.1	8.8	9.2	9.6	8.68
2.5	11.3	11.4	12.8	13.0	12.13
5	14.2	15.3	15.3	15.5	15.08
متوسط المعاملة بكبريتات المغنيسيوم المائية	10.86	11.83	12.43	12.70	511.9

$RLSD_{(0.05)}$ لتركيز عرق السوس= 0.451 , $RLSD_{(0.05)}$ لتركيز الكبريتات = 0.497

$RLSD_{(0.05)}$ للتداخل= 1.114

جدول (4): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسيوم المائية في معدل طول الورقة (سم) في نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز كبريتات المغنيسيوم المائية (ملغم/لتر)				متوسط المعاملة بمستخلص جذور عرق السوس
	0	32	64	96	
0	5.0	5.3	6.0	6.1	5.6
2.5	6.6	6.8	6.9	7.0	6.8
5	7.5	8.0	8.7	8.8	8.3
متوسط المعاملة بكبريتات المغنيسيوم المائية	6.4	6.7	7.2	7.3	6.9

$RLSD_{(0.05)}$ لتركيز عرق السوس= 0.257 , $RLSD_{(0.05)}$ لتركيز لكبريتات = 0.283

التداخل غير معنوي

جدول (5): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسيوم المائية في معدل مساحة الورقة (سم^2) في نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز كبريتات المغنيسيوم المائية (ملغم/لتر)				متوسط المعاملة بمستخلص جذور عرق السوس
	0	32	64	96	
0	6.83	6.96	7.00	7.20	6.99
2.5	8.90	8.93	9.60	10.03	9.37
5	12.80	13.07	13.91	14.00	13.45
متوسط المعاملة بكبريتات المغنيسيوم المائية	9.51	9.65	10.17	10.41	9.94

$RLSD_{(0.05)}$ لتركيز عرق السوس= 1.592 , $RLSD_{(0.05)}$ لتركيز الكبريتات = غير معنوي

$RLSD_{(0.05)}$ للتداخل= 3.184

يتضح من النتائج المبينة في جدول (6) ان الرش بمستخلص جذور عرق السوس قد ادى الى حدوث زيادة معنوية في النسبة المئوية للمادة الجافة في اوراق نبات السفندر ، فقد وصل معدلها الى 35.86% عند الرش بـ 5غم/لتر وبفارق معنوي عن مثيلاتها المعاملة بـ 2.5غم/لتر التي وصل معدلها الى 33.95% والذي اختلف هو الآخر معنويا عن عن معدل النسبة المئوية للمادة الجافة في نباتات المقارنة والذي بلغ 32.52% .

قد تعزى الزيادة في هذه الصفة الى زيادة (جدول 3 و 4) ومساحة الورقة الواحدة (جدول 5) مما ادى الى زيادة الوزن الطري للمجموع الخضري وبالنهاية زيادة النسبة المئوية للمادة الجافة , وهذا مشابه لما توصل اليه كل من (11) على نبات حنك السبع و (13) على نبات الجرانيوم

ويوضح الجدول نفسه ان الرش بالمغنيسيوم بتركيز 64 أو 96 ملغم/لتر قد ادى الى زيادة النسبة المئوية للمادة الجافة في الاوراق معنويا مقارنة بالنباتات غير المرشوشة والتي اعطت اقل النسب . ويعزى ذلك الى زيادة عدد الاوراق وطول ومساحة الورقة (3 , 4 و 5) والذي ادى الى زيادة الوزن الطري للورقة وبالتالي زيادة النسبة المئوية للمادة الجافة فيه. وهذه النتائج مشابه لما اشار اليه كل من (19) على نبات الجربيرا و(20) على نبات (Lisianthus) . هذا وقد كان التداخل بين العاملين غير معنوي .

جدول (6): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسيوم المائية في نسبة المادة الجافة في الاوراق (%) في نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز المغنيسيوم (ملغم/لتر)				متوسط المعاملة بمستخلص جذور عرق السوس
	0	32	64	96	
0	32.19	32.50	32.66	32.71	32.52
2.5	33.84	33.90	33.95	34.12	33.95
5	34.75	35.17	36.67	36.85	35.86
متوسط المعاملة بكبريتات المغنيسيوم المائية	33.59	33.85	34.42	34.56	34.11

$RLSD_{(0.05)}$ لتركيز عرق السوس= 0.419 , $RLSD_{(0.05)}$ لتركيز الكبريتات = 0.462
التداخل غير معنوي

جدول (7): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسسوم المائية في محتوى الاوراق من الكلوروفيل الكلي (ملغم/غم وزن طري) في نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز كبريتات المغنيسيوم المائية (ملغم/لتر)				متوسط المعاملة بمستخلص جذور عرق السوس
	0	32	64	96	
0	10.74	10.92	11.06	11.30	11.00
2.5	12.11	12.36	12.37	12.46	12.33
5	12.31	12.48	12.51	12.54	12.46
متوسط المعاملة بكبريتات المغنيسيوم المائية	11.72	11.92	11.98	12.10	11.93

$RLSD_{(0.05)}$ لتركيز عرق السوس= 1.476 , $RLSD_{(0.05)}$ لتركيز الكبريتات = 1.704
$RLSD_{(0.05)}$ للتداخل= 2.952

جدول (8): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسسوم المائية في محتوى الاوراق من الكاربوهيدرات الذائبة الكلية (%) في نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز كبريتات المغنيسيوم المائية (ملغم/لتر)				متوسط المعاملة بمستخلص جذور عرق السوس
	0	32	64	96	
0	21.56	21.70	22.23	23.00	22.12
2.5	24.03	25.63	26.57	26.90	25.78
5	27.00	28.45	28.56	28.81	28.21
متوسط المعاملة بكبريتات المغنيسيوم المائية	24.19	25.26	25.78	26.23	25.37

$RLSD_{(0.05)}$ لتركيز عرق السوس= 0.625 , $RLSD_{(0.05)}$ لتركيز الكبريتات = 0.627
التداخل غير معنوي

يبين الجدول (7) أن رش مستخلص جذور عرق السوس المستخدم قد أثر معنوياً في زيادة محتوى اوراق النبات من الكلوروفيل الكلي , إذ تفوق الرش بالتركيزين 2.5 و 5 غم/لتر معنويا على التركيز صفر . وقد يعزى سبب تفوق معاملات الرش على المعاملة من دون رش الى وجود العناصر الغذائية في تركيب مستخلص عرق السوس ومنها عنصر النيتروجين واللذي يدخل في تركيب جزئية الكلوروفيل . واتفقت هذه النتائج مع ما وجدته (9) من أن الرش بمستخلص عرق السوس سبب زيادة محتوى الاوراق من الكلوروفيل في نبات الفريزيا. ومع ما توصلت اليه(13) على نبات الجرانيوم .

ويتضح من الجدول نفسه ان الرش بكبريتات المغنيسيوم المائية كان له تأثير ايجابي في زيادة محتوى اوراق النبات من الكلوروفيل الكلي , إذ تفوقت معاملة الرش بكبريتات المغنيسيوم المائية بتركيز 96ملغم/لتر في زيادة محتوى اوراق النبات منه وبلغت 12.10 ملغم/غم وزن طري مقارنة مع اقل محتوى بلغ 11.72 ملغم/غم وزن طري في نباتات المقارنة . وربما يعزى سبب الزيادة الى دخول المغنيسيوم في تركيب جزيئة الكلوروفيل واحتلاله مركز هذه الجزيئة لذا فهو يسبب محتوى الاوراق من الكلوروفيل الكلي (24). وهذا نفس ما وجدته (19) على نبات الجربيرا . ومع ما وجدته دراسة (18) من ان محتوى الكلوروفيل الكلي قد ازداد معنويا في أوراق نبات البزرنكوش مع زيادة تركيز المغنيسيوم في المحلول المغذي.

وكان للتداخل بين الرش بمستخلص جذور عرق السوس والرش بكبريتات المغنيسيوم المائية تأثيراً معنوياً في زيادة محتوى اوراق النبات من الكلوروفيل الكلي ، إذ تفوقت معاملات الرش بمستخلص عرق السوس بتركيز 5غم/لتر مع الرش بكبريتات المغنيسيوم المائية بتركيز 96 ملغم/لتر في اعطاء اعلى محتوى بلغ 12.54 ملغم/ غم وزن طري . في حين بلغت عند الرش بالتركيز صفر لكل منهما 10.74 ملغم/غم وزن طري .

يبين الجدول (8) أن الرش بمستخلص عرق السوس بالتركيزين 2.5 و 5 غم/لتر قد اثر معنوياً في زيادة محتوى أوراق النبات من الكاربوهيدرات الذائبة الكلية و بلغتا 28.21 و 25.78% وعلى التوالي ، في حين بلغ ذلك المحتوى في اوراق نباتات المقارنة 22.12% . وقد تعزى تلك الزيادة الى احتواء مستخلص جذور عرق السوس على الكربوهيدرات اضافة الى أنه سبب زيادة عدد الاوراق ومساحة الورقة (جدولي 3 و 5) مما اثر ايجابياً في زيادة كفاءة عملية البناء الضوئي وبالتالي زيادة تصنيع وتمثيل المواد الغذائية والمركبات العضوية ومنها الكربوهيدرات . وهذا نفس ما وجدته دراسة (13) على نبات الجرانيوم .

كما يتضح من الجدول (8) ان النسبة المئوية للكاربوهيدرات الذائبة الكلية قد ازدادت مع زيادة التركيز , اذ اعطت النباتات المرشوشة بتركيز 96 ملغم/لتر اعلى نسبة مئوية بلغت 26.23 مقارنة مع النباتات غير المرشوشة والتي اعطت اقل نسبة بلغت 24.19 معنويا , وربما تعزى زيادة الكاربوهيدرات عند الرش بكبريتات المغنيسيوم المائية الى دور عنصر المغنيسيوم في تنشيط العديد من الأنزيمات المصاحبة لأيض الكربوهيدرات ومنها glucokinase و fructokinase و galactokinase و Hexokinase (35) او ربما تعزى الى كون المغنيسيوم عنصراً مهماً في انتقال وتوزيع النشا وكذلك في تكوين السكريات . اذ انه يعمل كعامل مساعد في كثير من التفاعلات الداخلة في عمليات التخمر الكحولي للكاربوهيدرات (32) مما ادى بالنهاية الى زيادة النسبة المئوية للكاربوهيدرات الذائبة الكلية في الاوراق . هذا ولم يكن للتداخل بين العاملين تأثير معنوي في هذه الصفة.

ازداد محتوى الأوراق من البروتين في النبات مع زيادة تركيز الرش بمستخلص جذور عرق السوس معنويا (جدول 9) , اذ بلغ 18.48 و 13.54% عند الرش بالتركيزين 5 و 2.5 غم/ لتر من مستخلص , وعلى التوالي , في حين بلغت تلك النسبة في نباتات المقارنة 9.99%. وقد يعزى ذلك الى احتواء مستخلص جذور عرق السوس على عنصر النتروجين (31). وتشابهت هذه النتائج مع (9) على نبات الفريزيا .

وادت المعاملة بكبريتات المغنيسيوم المائية بتركيز 96 ملغم/لتر الى زيادة محتوى الاوراق من البروتين معنويا بلغت 18.48 بالمقارنة مع نباتات المقارنة والتي اعطت اقل قيمة بلغت 9.99 (الجدول 9) . وقد تعزى هذه الزيادة الى دور عنصر المغنيسيوم في تمثيل البروتينات فهو يعمل كمنشط للأنزيمات التي تدخل في تمثيل الأحماض النووية كما انه يعمل على ثبوتية الرايبوسومات والتي تكون مهمة لتمثيل البروتينات وان نقصه يعمل على انفصال تلك الرايبوسومات الى وحداتها الأصغر ، كما وان العمليات الحيوية للنتروجين تتأثر بالتغذية بعنر المغنيسيوم اذ يشترك في عملية اختزال النترات داخل النبات (14) .

واظهرت نتائج التداخل بين العاملين حدوث زيادة معنوية في محتوى الاوراق من البروتين. اذ اعطت معاملة الرش ب 5غم/لتر مستخلص جذور عرق السوس مع الرش ب 96 ملغم/لتر كبريتات المغنيسيوم المائية اعلى محتوى بلغ 19.43 , في حين اعطت النباتات غير المعاملة (المقارنة) اقل محتوى بلغ 9.35 .

اوضحت نتائج التحليل الإحصائي في جدول (10) حدوث انخفاض معنوي في نسبة الكاربوهيدرات الى النتروجين مع زيادة تراكيز مستخلص جذور عرق السوس المرشوشة به النباتات . اذ اعطت معاملة الرش ب 5 غم/لتر اقل نسبة بلغت 9.35 مقارنة مع معاملة المقارنة والتي اعطت اعلى نسبة بلغت 13.95 . ويمكن ان تعزى هذه النتيجة الى زيادة انتاج البروتين اكثر من انتاج الكاربوهيدرات في النبات , بالاضافة الى احتواء مستخلص جذور عرق السوس على عنصر النتروجين (6) .

ولم تظهر نتائج الرش بكبريتات المغنسيوم المائية او التداخل بين العاملين أي تاثير معنوي في هذه النسبة (جدول 10) .

جدول (9): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسسوم المائية في محتوى الاوراق من البروتين (%) في نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز كبريتات المغنيسيوم المائية (ملغم/لتر)				متوسط المعاملة بمستخلص جذور عرق السوس
	0	32	64	96	
0	9.35	9.74	10.47	10.43	9.99
2.5	11.93	13.24	14.37	14.62	13.54
5	16.93	18.12	19.43	19.43	18.48
متوسط المعاملة بكبريتات المغنيسيوم المائية	12.74	13.70	14.75	14.82	14.00

$RLSD_{(0.05)}$ لتركيز عرق السوس= 0.371 , $RLSD_{(0.05)}$ لتركيز الكبريتات = 0.429

$RLSD_{(0.05)}$ للتداخل= 0.884

جدول (10): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسيوم المائية في نسبة الكاربوهيدرات الى النتروجين (C/N) في الأوراق (%) في نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز كبريتات المغنيسيوم المائية (ملغم/لتر)				متوسط المعاملة بمستخلص جذور عرق السوس
	0	32	64	96	
0	14.59	13.90	13.39	13.77	13.91
2.5	12.60	12.08	11.56	11.48	11.93
5	9.95	9.80	9.18	9.26	9.55
متوسط المعاملة بكبريتات المغنيسيوم المائية	12.38	11.92	11.37	11.50	11.79

$RLSD_{(0.05)}$ لتركيز عرق السوس= 0.591 , تركيز الكبريتات غير معنوي

التداخل غير معنوي

تبين النتائج ان محتوى الفوسفور في اوراق نبات السفندر ازداد معنويا مع زيادة تركيز مستخلص جذور عرق السوس المعاملة به النباتات (جدول 11). ووصل اعلى محتوى في النباتات المعاملة ب 5 غم/لتر مستخلص اذ بلغت 1.20 , اما اقل محتوى فكان في النباتات غير المعاملة (المقارنة) بلغ 0.75 , وقد تعزى هذة الزيادة الى احتواء مستخلص جذور عرق السوس على كميات لا باس بها من الفسفور, فعند رشة على الاوراق يمتص من قبل الاوراق مما يسبب ارتفاع نسبته , او ان المستخلص سبب زيادة في عدد الاوراق ومساحة الورقة (جدولي 3،5) ومن ثم زيادة التمثيل الغذائي وزيادة امتصاص العنصر من قبل الاوراق (6) وبالنهاية زيادة محتوى الاوراق من الفسفور , وهذه النتائج هي نفس مع ما وجدته دراسة (9) و (13) من أن الرش بمستخلص جذور عرق السوس سبب زيادة النسبة المئوية للفسفور في أوراق نباتي الفريزيا والجرانيوم . هذا ولم تبين نتائج والمعاملة بكبريتات المغنيسيوم المائية او التدخل بين عاملي التجربة أي تاثير معنوي على محتوى الاوراق من الفسفور في النبات .

كذلك فقد ازداد محتوى الأوراق من البوتاسيوم مع زيادة تركيز مستخلص جذور عرق السوس المرشوشة به النباتات ، وكان اعلى محتوى في النباتات المرشوشة ب 5 غم/لتر مستخلص بلغ 3.94 في حين اعطت النباتات غير المرشوشة اقل محتوى بلغ 2.68 , وربما يعزى السبب في زيادة محتوى الأوراق من البوتاسيوم الى نفس الاسباب التي ذكرت سابقا حول زيادة محتوى الاوراق من الفسفور نتجة الرش بمستخلص جذور عرق السوس .

ويتضح من الجدول (12) انه كان لرش كبريتات المغنيسيوم المائية تأثيرا معنويا في زيادة محتوى الأوراق من البوتاسيوم , اذ بلغ اعلى محتوى (3.45) في النباتات المرشوشة ب 96 ملغم/لتر كبريتات مقارنة بالنباتات غير المرشوشة والتي اعطت اقل محتوى بلغ (3.08) , وهذا مشابه لنتائج دراسة (9) على نبات الفريزيا .

هذا وقد كان للتداخل بين العاملين المدروسين تأثيرا معنويا اذ وصل اعلى محتوى في النباتات المرشوشة ب 5 غم/لتر مستخلص مع 96 ملغم/لتر كبريتات وبلغ 4.01 في حين وصل اقل محتوى في نباتات المقارنة اذ بلغ 2.57 .

جدول (11): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسيوم المائية في معدل محتوى الاوراق من الفسفور (%) في نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز كبريتات المغنيسيوم المائية (ملغم/لتر)				متوسط المعاملة بمستخلص جذور عرق السوس
	0	32	64	96	
0	0.60	0.78	0.80	0.80	0.75
2.5	0.91	0.95	1.02	1.06	0.99
5	1.10	1.17	1.27	1.28	1.20
متوسط المعاملة بكبريتات المغنيسيوم المائية	0.87	0.97	1.03	1.05	0.97

$RLSD_{(0.05)}$ لتركيز عرق السوس= 0.210 , تركيز المغنيسيوم غير معنوي
التداخل غير معنوي

جدول (12): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسيوم المائية في معدل محتوى الاوراق من البوتاسيوم (%) في نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز كبريتات المغنيسيوم المائية (ملغم/لتر)				متوسط المعاملة بمستخلص جذور عرق السوس
	0	32	64	96	
0	2.57	2.69	2.73	2.74	2.68
2.5	2.81	2.97	3.31	3.60	3.17
5	3.86	3.89	4.00	4.01	3.94
متوسط المعاملة بكبريتات المغنيسيوم المائية	3.08	3.18	3.34	3.45	3.26

$RLSD_{(0.05)}$ لتركيز عرق السوس= 0.059 , $RLSD_{(0.05)}$ لتركيز الكبريتات = 0.072
$RLSD_{(0.05)}$ للتداخل= 0.946

جدول (13): تأثير رش مستخلص جذور عرق السوس وكبريتات المغنيسيوم المائية في معدل محتوى الاوراق من المغنيسيوم في (%) في نبات السفندر

تركيز مستخلص جذور عرق السوس (غم/لتر)	تركيز كبريتات المغنيسيوم المائية (ملغم/لتر)				متوسط المعاملة بمستخلص جذور عرق السوس
	0	32	64	96	
0	5.2	5.9	5.9	6.3	5.82
2.5	6.4	6.5	6.5	6.7	6.53
5	7.4	7.6	7.6	7.7	7.58
متوسط المعاملة بكبريتات المغنيسيوم المائية	6.33	6.66	6.66	6.90	6.64

$RLSD_{(0.05)}$ لتركيز عرق السوس= 0.190 , $RLSD_{(0.05)}$ لتركيز الكبريتات = 0.355
$RLSD_{(0.05)}$ للتداخل= 1.702

اظهرت نتائج التحليل الاحصائي في جدول (13) ان الرش بمستخلص جذور عرق السوس زادت من محتوى الاوراق من المغنيسيوم وبلغت 7.58 و 6.53 % عند استخدام التركيز 5 و 2.5 غم/لتر وعلى التوالي , واللذان اختلفتا معنويا عن معاملة المقارنة اذ بلغ 5.82 %. وقد تعزى هذه الزيادة نتيجة الرش بمستخلص جذور عرق السوس الى نفس الاسباب التي ادت الى زيادة محتوى الاوراق من الفسفور والبوتاسيوم نتيجة الرش بهذا المستخلص . وهذا نفس ما وجده .

كذلك فان الرش بكبريتات المغنيسيوم المائية بتركيز 96 ملغم/لتر زادت من محتوى الاوراق من المغنيسيوم ووصلت الى 6.99 في ان نباتات المقارنة اعطت اقل محتوى بلغ 6.33 (جدول 13) , وقد تعود هذة الزيادة أن رش كبريتات المغنيسيوم المائية الحاوية على المغنيسيوم على المجموع الخضري تعمل على زيادة كمية المغنيسيوم الجاهز للامتصاص من قبل المجموع الخضري للنبات ومن ثم زيادة تركيزه في اجزاء النبات المختلفة ولاسيما أن حركته تكون بواسطة المسافات البينية ثم تنفذ الى اللحاء (24) . كما قد تعزى زيادة تركيز المغنيسيوم في الاوراق الى امتصاصه من محلول الرش عن طريق الأوراق . وقد تشابهت هذة النتائج مع (18) على نبات البزرنكوش .

كما ادى الرش ب5 غم/لتر مستخلص عرق السوس مع الرش ب 96 ملغم/لتر كبريتات المغنيسيوم المائية الى اعطاء اعلى محتوى معنويا مقارنة مع نباتات المقارنة والتي اعطت اقل محتوى من المغنيسيوم في الاوراق (جدول 13) .

يستنتج من التجربة ان الرش بمستخلص جذور عرق السوس بتركيز 5 غم/لتر او الرش بتركيز 96 ملغم من كبريتات المغنيسيوم المائية حسن معنويا من صفات النمو لنبات السفندر ضمن ظروف التجربة .

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تشخيص بعض المركبات الفعالة في بذور الحبة السوداء (*Nigella sativa*)

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الملخص باللغة العربية

تضمنت هذه الدراسة تشخيص المكونات الكيمائية الفعالة الموجودة في بذور الحبة السوداء المتمثلة بالقلويدات (Alkaloids)، العفصيات (Tannins)، الكليكوسيدات (Glycosides)، الراتنجات (Resins)، السابونينات (Saponins) والكومارينات (Coumarins). مع تقدير نسب المكونات الأساسية: الرطوبة, الرماد، البروتين، الزيت والكاربوهيدرات. وتقدير نسب الأحماض الدهنية الأساسية في الزيت. وتقدير محتوى البذور من الأحماض الأمينيه الأساسية وغير الأساسية. كما تم تشخيص أثنى عشر عنصر كيميائي في تركيب تلك البذور وقياس تركيز كل منها.

أظهرت النتائج أن هذه البذور تحتوي على: الرطوبة 5.31%، الرماد 4.75%، البروتين 23.4%، الزيت 36.8% والكاربوهيدرات 29.74%.

ونسب الأحماض الدهنية الأساسية في الزيت بلغت 81.95% وتشمل حامض الاوليك (Oleic) 24.45%، اللينوليك (LinoLeic) 56.71%، اللينولينك (Linolenic) 0.67% والاراكيدونك (Arachidonic) 0.12% وكذلك نسب الاحماض الدهنية غير الأساسية التي بلغت 18.05% وكالآتي: مايرستيك (Myristic) 0.17%، بالمتك (Palmitic) 12.11% وستيريك (Stearic) 5.77% .

وتحتوي على تسعة أحماض أمينيه أساسية بنسبة 66.51% إذ تم قياسها بتركيز(ملغرام/100غرام) وشملت ليوسين (Leucine) 1253، لايسين (Lysine) 853، فنيل الالنين (Phenyl alanine) 813، ميثيونين (Methionine) 740، ايزوليوسين (Isoleucine) 651، ارجنين (Arginine) 603، فالين (Valine) 602، ثريونين (Threonine) 553، هستدين (Histidine) 516 بالإضافة إلى الأحماض الامينية غير الأساسية وبنسبة 33.49% التي شملت ثمانية احماض منها حامض الكلوتاميك (Glutamic acid) 797، حامض الاسبارتك (Aspartic acid) 612، برولين (Proline) 486، كلايسين (Glycine) 462، سيرين (Serine) 306،الانين (Alanine) 262، تايروسين (Tyrosine)247، وسستين (Cystine)143.

وفيها العناصر الكيميائية التالية بتركيز (ملغرام/100 غرام):- بوتاسيوم 835.0، فسفور 486.6،كبريت 393.2، كالسيوم 214.3، مغنيسيوم 172.7، صوديوم 38.0، حديد 4.3، زنك3.6، منغنيز2.8، نحاس 1.7، كروم0.7 وسلينيوم 0.04.

وتم استخلاص الزيت من البذور الطازجة بطريقتين الأولى بالعصر الميكانيكي إذ بلغت نسبة الزيت 22%، أما الطريقة الثانية باستخدام الهكسان في عملية الاستخلاص بلغت نسبة الزيت 36.8% بالإضافة إلى تحضير المستخلص المائي واستخلاص القلويدات والزيوت العطرية من تلك البذور ولابد من الإشارة إلى أن نسبة الزيت في البذور المحمصة بلغت 36.9%.

وأظهرت التحليلات الخاصة بالتقدير الكمي لنسب بعض المكونات الفعالة الداخلة في تركيب بذور الحبة السوداء احتواء تلك البذور على 1.3% من الزيوت العطرية، 1.0% من القلويدات، 0.8% من التانينات و 0.6% من الكليكوسيدات.

وتم فصل البروتينات الذائبة في الماء من بذور الحبة السوداء وقدر وزنها الجزيئي بـــ 64000 دالتون باستعمال كروموتوكرافيا الترشيح الهلامي (Gel Filtration chromatography).

ABSTRACT

This study includes Identification of the active chemical components which are present in the black seed that include alkaloids, tannins, glycoside, resins, saponins and coumarins.Estimation of the major components of these seeds like moisture 5.31%, ash 4.75% protein 23.40% and oil 36.8% and carbohydrate 29.74%.

Estimation of the essential fatty acids in seeds oil which were 81.95%: oleic acid 24.45%,Linoleic acid 56.71%, linolenic acid 0.67% and arachidonic acid 0.12%. The percentage of non-essential fatty acids found to be 18.05%: myristic 0.17%, palmitic 12.11% and stearic 5.77%.The seeds were include 9 essential amino acids with a percent off 66.51% measured by (mg/100 gm) include leucine 1253, lysine 853, phenyl alanine 813, methionine 740, isoleucine 651, arginine 603, valine 602, threonine 553, histidine561 in addition to the non-essential amino acids with a percent of 33.49% and it includes 8 acids as follows: glutamic acid 797, aspartic acid 612,proline 486, glycine 462, serine 306, alanine 262, tyrosine 274 and cystine 143. The diagnosis of 12 trace elements in the seeds measured by (mg/100 gm) and as follows: potassium 853, phosphor 486.6, copper 393.2, calcium 214.3, magnesium 172.7, sodium 38.0, iron 4.3 zinc 3.6, manganese 2.8, cupper 1.7, chromium 0.7 selenium 0.04.

Oil was extracted from the fresh seeds by two methods :Extract by mechanical pressure , the percentage of oil was 22% and by hexane , the percentage extracted oil was 36% in addition to the preparation of the aqueous solution and extraction of the alkaloids and volatile oils from these seeds. As a point of view it is important to mention the percent of oil in the fried seeds that reaches to 36.9%. Investigations that are related to the quantitative evaluation of the percentage of some active components are present in the structure of the black seeds includes 1.3% of the volatile oils, 1% of alkaloids, 0.8 % of tannins and 0.6% from glycosides.

A porteinious compound which was separated from the black seed as molecular weight was 64000 dalton by gel filtration chromatography.

المقدمة

يطلق على الحبة السوداء عدد من الأسماء في الأقطار العربية منها الحبة المباركة أو الكمونة السوداء وذكرها المصريون في رواياتهم أنها نبات طبي وكانوا يسمونها (شنقت) وتدعى في مصر حبة البركة أو البشمة (1) والحبة السوداء في العراق (2) والخردل أو القزحة في بلاد الشام (3) وفي اليمن تسمى القحطة (4) وفي الهند تسمى منجرل (Mangral) (5) وأما في الباكستان فتسمى كلونجي (klonji) (6) والشونيز في لغة الفرس (6) إن اختلاف هذه التسميات في أغلب بلدان العالم يرجع إلى الأهمية الغذائية والعلاجية لهذه النبتة التي تتبع العائلة الشقائقية (Ranunculaceae) التي تتصف ثمارها بكونها بذور سوداء اللون بيضوية الشكل (7).

استخدمت النباتات الطبية لمعالجة الكثير من الأمراض وحتى المستعصية منها ، فقد استخدمت لمعالجة الأمراض السرطانية (8) ومخفضاً لنسبة سكر الدم(Hypoglycemic effect) (9) ولمعالجة أمراض القلب (10) والكبد (11) والأمراض الجلدية (12) فضلا عن زيادة إدرار الحليب عند الأمهات المرضعات (13) ، وتم اختيار بذور الحبة السوداء في هذه الدراسة لأهميتها العلاجية و الغذائية فقد أظهرت الدراسات الحديثة (6) إمكانية استخدامها بمفردها أو مع مواد أخرى للمعالجة بتناولها مسحوقا يومياً أو إضافة قليل من زيت الحبة السوداء إلى كوب من العسل أو الليمون يساعد على تقوية جيش الدفاع الدموي ضد الإصابة الجرثومية أو البكتيرية وتعزى معظم الخاصية العلاجية للحبة السوداء إلى المواد الفعالة الموجودة فيها ، إضافة إلى ذلك وجد أن لها تأثيراً مسكناً للألم (Analgesic effect) (14) ومضاداً للالتهابات (Anti-inflammatory effect) (15) ومضاداً للأحياء الدقيقة (Anti-microbial effect) (16) ومضاداً للتشنج (Anti-spasmotic effect)، و موسعاً للقصبات (Bronchodilator effect) (17، 18) مدرا وخافضا للضغط (Diuretic and hypotensive effect) (19) و مضاداً للهستامين (Anti-histaminic effect) (20)، و مضاداً للطفيليات (Anti-parasitic effect) (21)، والوقاية من القرحات الهضمية المحرضة بالايثانول (Ethanol induced ulcers) لدى الجرذان (22)، وتأثيراً مضاداً لبعض الأورام (Anti-neoplastic effect) (8)، بالإضافة إلى ذلك أن لبذور الحبة السوداء فعالية مقوية لجهاز المناعة(Immunopotentiating effect) (23) ولم تشر أي من الدراسات السابقة الذكر إلى احتمال أن يكون لبذور الحبة السوداء تأثيراً سميا (24) وقد تبين أن الإعطاء الحاد والمزمن لتلك البذور لا يسبب أية تبدلات في أنسجة الكبد أو أنزيماته الرئيسة عند حيوانات التجارب (19)، وهنالك دراسات أشارت إلى أن خلاصة بذور الحبة السوداء تحمي من سمية عقار السيسبلاتين (Cisplatin) (مركبات البلاتين المستوية) (Cis-DDP) (Cis-Diamine dichloro platinum) (مضاد للسرطان) وتمنع تخريبه لأنسجة الكبد ولها أيضا تأثير واق ضد التهتك الكبدي والكلوي المحدث بوساطة العوامل الكيميائية والممرضة. (25)

أجريت دراسة عملية من قبل الباحثين EL-Kadi and Kandil (26) حول تأثير الحبة السوداء على المناعة الطبيعية في الإنسان في معهد أكبر للطب الإسلامي للتعليم والأبحاث بمدينة بناما وثبت أن تناول حبوب الحبة السوداء بالفم بجرعة غرام واحد مرتين يومياً ولمدة أربعة أسابيع متتالية له أثر مقو على وظائف المناعة ويكون لذلك دور في علاج السرطان والإيدز وبعض الظروف المرضية الأخرى التي ترتبط بحالات نقص المناعة.

ووجد .Topozada وآخرون (27) بان زيت الحبة السوداء يوقف نمو العديد من أنواع البكتريا وأثبتوا أثرها الايجابي في علاج التهابات الإذن الخارجية المزمنة عند استعمالها بتراكيز 0.2%، 0.5% ، 1% وكذلك أثبتوا تأثيرها في التهابات الجيوب الأنفية المزمنة وأضافوا بان هذا العلاج أقوى في تأثيره من المضادات الحيوية المعروفة وأقل منها سمية وليس له تأثير ضار على القلب أو الدم أو الجهاز التنفسي ، وفي مجال تأثير مسحوق الحبة السوداء على بعض الفطريات الجلدية وجد Abdel Kader وآخرون (28) أن استعمال مسحوق الحبة السوداء بتراكيز 2.5% ، 5% ، 10% له تأثير مثبط لكل من الفطريات *Trichophyton mentagrophyes* ، *M. gypeum* ، *Microsporum canis* ، *T. rubrum* ، *T. soudanense* بنسب تتراوح بين 35.13% إلى 100%.

المواد وطرائق العمل

الأجهزة المستخدمة:

تم قياس أطياف الأشعة فوق البنفسجية – المرئية بجهاز , Double-beam UV-visible recording spectrophotometer shimadzu UV . 160 / Japan.

. جهاز محلل الأحماض الامينية Amino acid analyser model 415 ; ALPHA, LKB , Bromma . , Sweden , 1983.

كما تم تعيين نسب العناصر في المعقدات بواسطة جهاز الامتصاص الذري نوع Atomic absorption . spectrophotometer , shimadzu AA-670/Japan.

مطياف الانبعاث الذري– بلازما الحث المقترن Inductively complied plasma- Atomic Emission Spectrophotometer المزود من شركة Hilger Analytical / England

المواد المستعملة:

جميع المواد المستخدمة في البحث تم تجهيزها من شركة BDH-Chemicals أما المذيبات الايثانول المطلق , ثنائي اثيل ايثر وثنائي مثيل سلفوكساييد فقد تم تجهيزها من شركة Aldrich .

الكواشف والمحاليل المستخدمة:

تم تحضير الكواشف الآتية (29–32):

كاشف بندكت (Benedect reagent)

كاشف ماير (Mayer's reagent)

كاشف فهلنك (Fehling reagent)

كاشف كيد (Kedde reagent)

كاشف واكنر (Wagner's reagent)

بذور الحبة السوداء: تم الحصول على بذور الحبة السوداء من الصنف المحلي (*Nigella sativa*) من الأسواق المحلية. تم تنظيف البذور من الشوائب ومن ثم طحنها وحفظها في قناني زجاجية جافة لحين الاستعمال .

تحضير المستخلص المائي لبذور الحبة السوداء

حضر المستخلص المائي لبذور الحبة السوداء بإتباع طريقة Harborne (33) وذلك بإضافة 150 مل من الماء المقطر إلى 50 غرام من مسحوق تلك البذور ووضع المزيج على المحرك المغناطيسي لمدة 24 ساعة في 4 ^{o}م ثم رشح المحلول وتم تركيز المحلول باستعمال المبخر الدوار تحت درجة حرارة 45 ^{o} م وضغط مخلخل للتخلص من الماء والحصول على المستخلص الخام الجاف. كان وزن الناتج الجاف 20 غرام (40%). أذيب المستخلص في 100 مل من الماء المقطر وحفظ في 4 ^{o}م لحين الاستعمال.

الكشف الكيميائي لبعض المكونات الفعالة لبذور الحبة السوداء (34):

الكشف عن الكليكوسيدات
الكشف عن العفصيات
الكشف عن السابونينات
الكشف عن القلويدات
الكشف عن الراتنجات
الكشف عن الكومارينات

تقدير النسب المئوية لبعض المكونات الفعالة في بذور الحبة السوداء:

تقدير نسبة الكليكوسيدات (35)
تقدير نسبة التانينات (36)
تقدير نسبة الزيوت العطرية (37)
تقدير نسبة القلويدات (38)

تقدير المكونات الرئيسية لبذور الحبة السوداء:

تقدير نسبة الرطوبة (39)
تقدير مسبة الرماد (40)
تقدير نسبة البروتين (40)

استخلاص الزيت:

الاستخلاص باستخدام جهاز العصر الميكانيكي (41)
الاستخلاص بواسطة المذيب العضوي (40)

الأحماض الدهنية الحرة:

تم تقدير الأحماض الدهنية في زيت بذور الحبة السوداء وذلك بوضع 30 ملغراما من الزيت في أنبوبة خاصة مع مل واحد من كاشف استر المثيل (Methyl ester) [تم تحضير الكاشف بإضافة 0.1 مل من كلوريد الاستيل إلى 25 مل ميثانول (42). سخن المزيج بعد إحكام غلق الأنبوبة في حمام مائي مغلي لمدة 25 دقيقة وترك النموذج ليبرد في درجة حرارة الغرفة وبهذا يكون جاهزا للكشف] وتقدير الأحماض الدهنية بوساطة جهاز الكروماتوكراف الغاز السائل المزود بكاشف التأين الحراري (FID) (Flame Ionization Detector) وعمود الفصل الزجاجي (2.1 متر طولا X 2 ملم قطرا) والمعبأ بمادة 3% سيلار (3% Silar Loc) المحملة على مادة كروموسورب (Chromosorp DMCS-DWA) بقطر حبيبة 80–100 مايكروميتر ، واستخدم غاز الهليوم بسرعة 30 مل/دقيقة بوصفه غاز ناقل للنموذج في عمود الفصل ، أما الهيدروجين والهواء فقد استخدما بسرعة 30 و 300 مل / دقيقة على التوالي لغرض الحصول على اللهب اللازم للتأين وكانت حرارة الفرن الابتدائية 160 ^{o} م ترتفع بمعدل 5 ^{o} م/دقيقة لتصل إلى درجة حرارة نهائية قدرها 200 ^{o} م، أما حرارة موقع حقن النموذج وكاشف التأين فقد نظمت على درجة حرارة 270 ^{o} م وتم حقن مايكرولتر واحد من العينة في الجهاز لغرض الفصل والحصول على منحنيات للأحماض الدهنية وتم حساب زمن الظهور (Retention time) و قورن مع زمن ظهور الأحماض الدهنية القياسية التي جرى حقنها بالجهاز وتحت ظروف الفصل نفسها (43).

الأحماض الامينية الحرة

تم تقدير الأحماض الامينية الحرة بإتباع الظروف القياسية المستخدمة من قبل Sharif (44) باستعمال جهاز محلل الأحماض الامينية ، وتم التعرف على الأحماض الامينية الموجودة في بذور الحبة السوداء بمقارنة أوقات ظهورها(Retention time) مع أوقات ظهور الأحماض الامينية القياسية ، حيث تم تحضير النموذج المعد للتحليل بإذابة 0.2 غرام من الزيت في 3 مل من حامض الهيدروكلوريك (6N) ثم سخن النموذج في أنبوبة زجاجية مغلقة في حمام زيتي لمدة 16 ساعة تحت درجة حرارة 110 ^{o} م ، بعدها ترك النموذج ليبرد قبل إجراء عملية التحليل .

تشخيص العناصر الكيميائية الموجودة في بذور الحبة السوداء:

أتبعت الطريقة المستخدمة من قبل Hammed وآخرون (45) في تشخيص وتقدير كمية العناصر الموجودة في بذور الحبة السوداء بوساطة جهاز مطياف الامتصاص الذري (Atomic absorption Spectroscopy) ، إذ حضر النموذج المعد للتحليل بإذابة (1) غرام من مسحوق بذور الحبة السوداء في 10 مل من خليط الأحماض الآتية (النتريك ، البركلوريك، الكبريتيك) بنسب (5 : 2 :1)، تم تركيز المحلول إلى 0.5 مل بتسخين النموذج إلى 70 ^{o} م بعدها خفف إلى 25 مل بوساطة ماء مقطر خالٍ من الأيونات (Deionized Water) في حين استعملت تقنية جهاز البلازما المقترن بالحث (ICP) الموجود في منظمة الطاقة الذرية لتشخيص وتقدير كمية العناصر الموجودة بكميات ضئيلة في تلك البذور .

استخلاص البروتينات الذائبة في الماء من بذور الحبة السوداء:

تم إتباع الطريقة المستخدمة من قبل Harborne (33) وذلك بإذابة 100 غرام من مسحوق بذور الحبة السوداء في لتر واحد من الماء المقطر مع التحريك المستمر بوساطة المحرك المغناطيسي لمدة 24 ساعة ثم نبذ المحلول باستخدام جهاز الطرد المركزي بقوة 6000 دورة / دقيقة. فصل الراشح وشبع بكبريتات الامونيوم (70%) ثم ترك المحلول لمدة 24 ساعة لغرض ترسيب البروتينات بعد ذلك فصلت تلك البروتينات باستخدام جهاز الطرد المركزي بقوة 6000 دورة / دقيقة ولمدة 30 دقيقة.

أذيبت البروتينات المترسبة في الخطوة السابقة في الماء المقطر وأجريت لها عملية الديلزة مقابل الماء المقطر لمدة 48 ساعة لغرض التخلص من المركبات ذات الأوزان الجزيئية الواطئة علماً أن جميع الخطوات السابقة أجريت

عند 4° م ثم جفف المحلول بوساطة جهاز المبخر الدوار تحت الضغط المخلخل لغرض الحصول على البروتينات للاستفادة منه في التجربة اللاحقة .

كروموتوكرافيا الترشيح الهلامي (Gel Filtration Chromatography)

تم إتباع الطريقة المستخدمة من قبل Robyt and White (46) وذلك باستخدام عمود الفصل الكروموتوكرافي (27X1.2) سم. تم تعبئة عمود الفصل بهلام السفادكس (Sephadex G-75) والمحضر بتنقيعه (Swelling) في الماء المقطر لمدة ثلاثة أيام وغسل عمود الفصل لعدة مرات بالماء المقطر وحدد معدل السريان بواقع 0.5 مل / دقيقة.

أذيب 0.00157 غرام من البروتينات في 1 مل من الماء المقطر وأضيف إلى عمود الفصل واسترد بالماء المقطر وقد جمعت بواقع 5 مل لكل عينة وتم قياس الامتصاص الضوئي عند طول موجي قدره 280 نانوميتر واستعملت البروتينات القياسية (Chymotrypsinogen A (M.wt 25000 و Ovalbumin (M.wt 34000) و Albumin (M.wt 67000) لاستخراج الوزن الجزيئي للبروتينات المستخلصة من بذور الحبة السوداء.

النتائج والمناقشة

المكونات الفعالة في بذور الحبة السوداء:

الجدول (1) يبين نتائج الكشف الكيميائي للمكونات الفعالة في بذور الحبة السوداء (*Nigella sativa*) ومنه يتضح احتوائها على الكليكوسيدات، الراتنجات، القلويدات، الكومارينات، السابونينات والعفصيات .

الجدول (1): الكشوفات الكيميائية للمركبات الفعالة الموجودة في بذور الحبة السوداء

المركب الفعال	نوع الكشف	نتيجة الكشف الموجب
الكليكوسيدات (Glycosides)	كاشف كيد	لون أزرق بنفسج
القلويدات (Alkaloids)	كاشف واكنر	راسب بني
السابونينات (Saponins)	كلوريد الزئبقيك	راسب أبيض
العفصيات (Tannins)	خلات الرصاص (1%)	راسب أبيض
الراتنجات (Resins)	كحول أثيلي+تسخين+ماء مقطر	ظهور تعكر
الكومارينات (Coumarins)	كحول أثيلي + ورقة ترشيح مبللة بـ NaOH + تسخين + UV	لون أخضر مزرق

أن وجود هذه المركبات الفعالة يفسر أهمية تلك البذور ويفسر سبب استخدامها في الطب القديم والذي جاء متطابقاً مع نتائج الدراسات الحديثة وما تم التوصل إليه من النتائج الايجابية لاستخدام تلك البذور في معالجة معظم الأمراض المستعصية لاسيما مرض السرطان (8)، مرض السكري (9)، ومرض الإيدز (29) بالإضافة إلى الأمراض الشائعة الأخرى .

تتفق النتائج التي تم الحصول عليها من حيث احتواء بذور الحبة السوداء على القلويدات مع ما هو موجود في الأدبيات (47–49) ، كما تشير البحوث والدراسات السابقة إلى احتواء الحبة السوداء على السابونينات (50،51) في حين تمكن النجار (52) من فصل مركبين كومارينين من بذور الحبة السوداء .

النسب المئوية لبعض المكونات الفعالة الموجودة في بذور الحبة السوداء

تبين من التحليلات الخاصة لتقدير النسب المئوية لبعض المكونات الفعالة في بذور الحبة السوداء احتواء تلك البذور على نسبة 1.3% من الزيوت العطرية، 1.0% من القلويدات، 0.8% من التانينات و0.6% من الكليكوسيدات وكما موضح في الجدول (2) في حين لم تشير الأدبيات السابقة إلى تقدير نسبة تلك المكونات الفعالة في بذور الحبة السوداء.

الجدول (2) : النسب المئوية للمكونات الفعالة لبذور الحبة السوداء

المكونة الفعالة	النسبة المئوية
الزيوت العطرية volatile oils	1.3
القلويدات Alkaloids	1.0
التانينات Tannins	0.8
الكليكوسيدات Glycosides	0.6

المكونات الأساسية في بذور الحبة السوداء

الجدول (3) يبين النسب المئوية للمكونات الأساسية لبذور الحبة السوداء ومنه يتضح احتواء تلك البذور على نسبة 5.31% من الرطوبة وهذه النتيجة مقاربة للدراسة المعدة من قبل Babayan وآخرون (53) إذ بلغت 5.52% في حين أشار AL-Jassir (54) إلى أن النسبة المئوية للرطوبة في تلك البذور تبلغ 4.6% وتختلف هذه النسبة تبعاً لظروف النضج والجني والخزن والبلد الذي تشترى منه.

أما نسبة البروتين المستخلصة من تلك البذور فكانت 23.40% وهي تتفق مع ما هو موجود في الدراسات السابقة (53، 54) التي أشارت إلى أن نسبة البروتين تبلغ أكثر من 20% في تلك البذور.

يظهر أيضاً من الجدول (3) إن نسبة الزيت بحدود 36.8% من المكونات الأساسية لتلك البذور إذ جرى الاستخلاص بطريقتين : الأولى (بالعصر الميكانيكي) كانت نسبة الزيت 22% ، والثانية (الاستخلاص بالهكسان) كانت نسبة الزيت 36.8% وترجح النسبة العالية في الطريقة الثانية مقارنة بالطريقة الأولى إلى الإذابة الكلية لجميع جزيئات الزيت في الوسط العضوي (الهكسان) وبالتالي استخلاص الزيت كلياً وهنالك دراسات سابقة (39، 50)

نتائجها مقاربة مع ما تم التوصل إليه وكذلك أشار Babayan وآخرون (53) إلى أن نسبة الزيت في بذور الحبة السوداء تبلغ 35.49% و لا بد من الإشارة إلى أن نسبة الزيت في البذور المحمصة بلغت 36.9%.

تعتبر الكاربوهيدرات ذات مصدر الطاقة الحيوية والبنائية التركيبية بالنسبة للجسم من العناصر الغذائية المهمة وتم احتساب تلك الكمية طبقاً للمعادلة المستخدمة من قبل AL-Jassir (54) وكما موضح:

Total carbohydrates = 100 - (%Moisture + %Crude protein + % Crude fat + %ash content)

إذ بلغت تلك النسبة 29.74% في حين كانت نسبة المواد الكاربوهيدراتية في بذور الحبة السوداء السعودية 31.9% عند الدراسة المعدة من قبل AL-Jassir (54)، أما Babayan وجماعته (53) فقد أشاروا إلى أن نسبة المواد الكاربوهيدراتية في بذور الحبة السوداء المصرية تبلغ 33.96% ولابد من الإشارة إلى أن اختلاف منشأ بذور الحبة السوداء قد يؤدي إلى اختلافات هامة وجذرية في الخواص العلاجية لكونها تختلف في المحتوى الكيميائي (23).

الجدول (3) : النسب المئوية للمكونات الأساسية لبذور الحبة السوداء

المكونة الأساسية	النسبة المئوية
الرطوبة (Moisture)	5.31
الرماد (Ash)	4.75
الزيت (Oil)	36.80
البروتين (Protein)	23.40
الكاربوهيدرات (Carbohydrate)	29.74

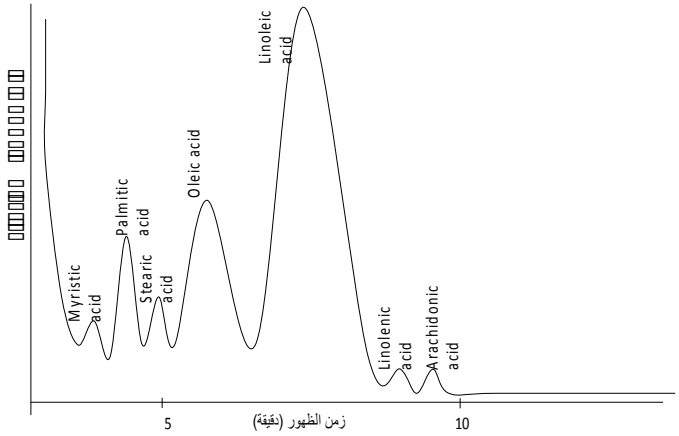
الأحماض الدهنية الحرة في بذور الحبة السوداء

أظهرت التحليلات الخاصة ببذور الحبة السوداء (الجدول 4) احتواء زيت تلك البذور على نسبة 81.95% من الأحماض الدهنية الأساسية ذات الأهمية الحيوية للعمليات الايضية التي لا يمكن بناءها داخل الجسم في حين تحتوي تلك البذور على نسبة 18.05% من الأحماض الدهنية غير الأساسية التي يمكن بناءها داخل الجسم اذ تمثلت الأحماض الدهنية الأساسية في حامض الاوليك، اللينوليك، اللينولينك والاراكيدونك.

أما الأحماض الدهنية غير الأساسية فشملت حامض المايرستيك ، البالمتك والستيريك اعتماداً على تسلسل ظهور تلك الأحماض في البولوغراف (الشكل 1).

الجدول (4): النسب المئوية للأحماض الدهنية الحرة الموجودة في زيت بذور الحبة السوداء

تسلسل ظهور الحامض الدهني		النسب المئوية للحامض
1	مايرستيك (Myristic)	0.17
2	بالمتك (Palmitic)	12.11
3	ستيريك (Stearic)	5.77
4	أوليك (Oleic)	24.45
5	لينوليك (Linoleic)	56.71
6	لينولينك (Linolenic)	0.67
7	اراكيدونك (Arachidonic)	0.12



الشكل (1): تحليل الأحماض الدهنية الحرة الموجودة في زيت الحبة السوداء باستخدام كروموتوغرافيا الغاز السائل (GLC)

لقد أشارت الدراسات السابقة إلى اختلاف تلك الكمية حيث أشار AL-Jassir (54) إلى أن نسبة الأحماض الدهنية الأساسية تبلغ 83.70% في حين تبلغ نسبة الأحماض الدهنية غير الأساسية 16.30% في بذور الحبة السوداء السعودية وكذلك أشار Babayan وآخرون (53) إلى أن نسبة الأحماض الدهنية الأساسية تبلغ 84.65% والأحماض الدهنية غير الأساسية 15.35% في بذور الحبة السوداء المصرية وتتفق جميع الدراسات على أن نسبة الأحماض الدهنية الأساسية هي أعلى من الأحماض الدهنية غير الأساسية في حين أشارت بعض الدراسات (55) إلى عدم وجود حامض اللينولينك في تركيب بذور الحبة السوداء ذات النسبة الواطئة في دراستنا في حين أشارت دراسات سابقة إلى وجود هذا الحامض بنسبة قليلة (53، 54) وبالإضافة إلى ذلك أشار Ustun وآخرون (41) إلى اختلاف نسبة هذا الحامض في تركيب بذور الحبة السوداء التركية وهذا يفيد بان النسبة تعتمد على طبيعة المنطقة التي زرع فيها النبات من حيث خصوبتها والظروف الجوية.

تعتبر الأحماض الدهنية الأساسية مهمة للعمليات الايضية التي تحصل في الجسم حيث يساهم حامض اللينوليك في خفض ضغط الدم وتوسيع المجاري التنفسية (56) في حين أشار Holmes and Bortz (57) إلى أهمية حامض اللينوليك في بناء كريات الدم الحمر، أما Wilson وآخرون (58) فقد أشار إلى أهمية هذا الحامض في مقاومة الجسم للأمراض الجلدية وتساقط الشعر.

وقد أشارت بعض الدراسات (59، 60) إلى دور حامض اللينوليك في خفض كولسترول الدم وبالتالي الحد من احتمالية الإصابة بأمراض القلب وأمراض تصلب الشرايين، كما أشار الزهيري (61) إلى أن نقص حامض اللينوليك يؤدي إلى وقف نمو الأطفال وظهور الالتهابات الجلدية والاكزما فضلاً عن ارتفاع نسبة الكولسترول في الكبد والدم.

الأحماض الامينية الحرة في بذور الحبة السوداء

يظهر من الجدول (5) احتواء بذور الحبة السوداء على الأحماض الامينية الأساسية ذات الأهمية الحيوية لبناء البروتينات بنسبة 66.51% في حين احتوت على نسبة 33.49% من الأحماض الامينية غير الأساسية ولابد من الإشارة إلى أن اختلاف العوامل البيئية والوراثية تلعب دوراً رئيساً في اختلاف هاتين النسبتين.

أظهرت الدراسات السابقة مثل AL-Jassir (54) بان نسبة الأحماض الامينية الأساسية بلغت 30.19% في حين بلغت نسبة الأحماض الامينية غير الأساسية 69.81% عند دراسته لبذور الحبة السوداء السعودية وكذلك أشار إلى عدم وجود أية نسبة للحامض الاميني التربتوفان في تركيب تلك البذور فيما أشار Babayan وجماعته (53) إلى عدم وجود الحامضين الامينيين الأساسيين التربتوفان والهستدين في تركيب بذور الحبة السوداء المصرية والى احتواء تلك البذور على ثمانية أحماض أمينية أساسية وهذا يعزز أهمية تلك البذور كقيمة غذائية وعلاجية للجسم ولابد من الإشارة إلى أن نسبتي الأحماض الامينية الأساسية وغير الأساسية كانت مقاربة لما تم الحصول عليه في دراستنا حيث بلغت نسبة الأحماض الامينية الأساسية 60.43% في حين بلغت نسبة الأحماض الامينية غير الأساسية 39.57% .

الجدول (5): الأحماض الأمينية الحرة الموجودة في زيت بذور الحبة السوداء

الجدول (5) (A): الأحماض الامينية الأساسية (Eessential amino acids)

الحامض الاميني الأساسي	الكمية (ملغرام / 100 غرام)
ليوسين (Leucine)	1253
لايسين (Lysine)	853
فنيل الانين (Phenyl alanine)	813
ميثونين (Methionine)	740
ايزوليوسين (Iso Leucine)	651
ارجنين (Arginine)	603
فالين (Valine)	602
ثريونين (Threonine)	553
هستدين (Histidine)	516

الجدول (5) (B) الأحماض الامينية غير الأساسية (Non-essential amino acids)

الحامض الاميني غير الأساسي	الكمية (ملغرام / 100 غرام)
حامض الكلوتاميك (Glutamic acid)	797
حامض الاسبارتك (Aspartic acid)	612
برولين (Proline)	486
كلايسين (Glycine)	462
سيرين (Serine)	306
الانين (Alanine)	262
تايروسين (Tyrosine)	247
سستين (Cystine)	143

العناصر الكيميائية في بذور الحبة السوداء

الجدول (6) يبين العناصر الكيميائية الموجودة في بذور الحبة السوداء والتي تعتبر من العناصر الأساسية التي يحتاجها الجسم لأهميتها في فعالية الانزيمات والهرمونات وكذلك في العمليات الايضية التي تحصل داخل الجسم إذ أن هنالك (17) عنصراً كيميائياً على الأقل تعتبر عناصر أساسية لإدامة العمليات الحيوية.

يعتبر الزنك من العناصر الضرورية لفعالية الأنزيمات حيث يدخل في تركيب أنزيم (Carbonic anhydrase) الموجود في كريات الدم الحمر وجدران الأمعاء ويكون هذا الأنزيم مهماً في نقل ثاني أوكسيد الكاربون في الدم وفي توليد حامض الهيدروكلوريك في المعدة كما يدخل الزنك في تركيب أنزيمات أخرى مثل (Carboxy peptidase) لذا فان له أهمية في التمثيل البروتيني (62) ويساهم في عملية التئام الجروح (56) يدخل الحديد في تركيب هيموكلوبين الدم ويعتبر النحاس من العناصر الضرورية لتخليق صبغة الميلامين (63) ويكون عنصر النحاس مهم في عملية تكوين الهيموكلوبين إذ يساعد على امتصاص الحديد كما يدخل النحاس في تركيب مجموعة من الأنزيمات التي تلعب دوراً في عمليات الأكسدة والاختزال التي تحصل داخل الجسم مثل أنزيم (Tyrosinase) الذي يؤكسد المركبات الفينولية (64) وتلعب بعض العناصر دوراً مهماً في أيض الكاربوهيدرات مثل عنصر المنغنيز ويعتبر أيضاً عاملاً ناقلاً للدهون (Lipotropic) في الدم.

يظهر من الجدول (6) أن أعلى نسبة كانت لعنصر البوتاسيوم ويتطابق ذلك مع الدراسات السابقة[54,45] لتقدير كمية العناصر الموجودة في بذور الحبة السوداء.

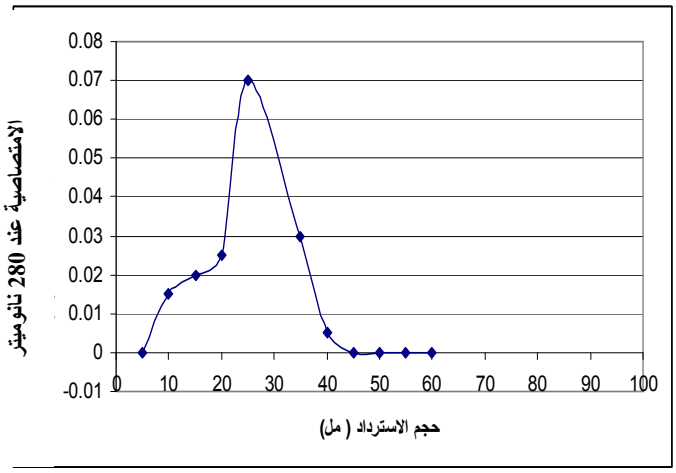
الجدول (6) : العناصر الكيميائية الموجودة في بذور الحبة السوداء

العنصر الكيميائي	الكمية (ملغرام/ 100غرام)
بوتاسيوم	835.0
فسفور	486.6
كبريت	393.2
كالسيوم	214.3
مغنيسيوم	172.7
صوديوم	38.0
حديد	4.3
زنك	3.6
منغنيز	2.8
نحاس	1.7
كروم	0.7
سلينيوم	0.04

استخلاص البروتينات الذائبة في الماء من بذور الحبة السوداء وتقدير وزنها الجزيئي بطريقة كروموتوكرافيا الترشيح الهلامي:

تم استخلاص البروتينات من بذور الحبة السوداء باستعمال الماء المقطر ورسبت تلك البروتينات بواسطة كبريتات الامونيوم وتم تنقيتها وتقدير وزنها الجزيئي باستعمال طريقة الترشيح الهلامي .

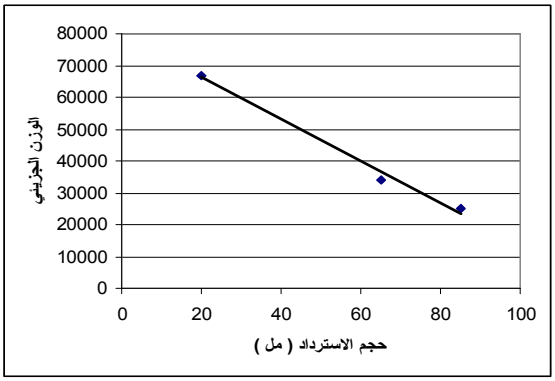
يوضح الشكل (2) أن البروتينات استردت عند 25 مل وهذا ما نتوقع أن تكون لتلك البروتينات وزن جزيئي عالٍ ومن خلال رسم علاقة بين حجم الاسترداد للبروتينات القياسية مقابل الأوزان الجزيئية لتلك البروتينات (الشكل 3) بالاعتماد على الجدول (7) تبين أن الوزن الجزيئي للبروتينات المستخلصة من بذور الحبة السوداء كان بحدود 64000 دالتون تقريباً.



الشكل (2): المنحنى البياني للبروتينات المستخلصة من بذور الحبة السوداء باستخدام كروموتوكرافيا الترشيح الهلامي

الجدول (7): العلاقة بين الاوزان الجزيئية للبروتينات القياسية مع حجم الاسترداد لتلك البروتينات

البروتينات القياسية	الوزن الجزيئي (دالتون)	حجم الاسترداد (مل)
Albumin	67000	20
Ovalbumin	34000	65
Chymotrypsinogen	25000	85



الشكل (3): المنحنى البياني لتقدير الوزن الجزيئي للبروتينات المستخلصة من بذور الحبة السوداء باستخدام كروموتوكرافيا الترشيح الهلامي

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تحضير ودراسة الخصائص الفيزيائية لأغشية (In_2O_3)النقية والمشوبة بطريقة الترذيذ بالبلازما

امل حسين داود (1) طالب زيدان الموسوي (2)

وزارة العلوم والتكنولوجيا – بغداد (1) كلية العلوم / الجامعة المستنصرية(2)- العراق

الملخص باللغة العربية

تمت دراسة الخواص الكهربائية والبصرية والتركيبية لأغشية In_2O_3 المحضرة بطريقة الترذيذ باستخدام منظومة البلازما ذات الأقطاب الثنائية المتوازية (Diode Sputtering).وتم تحضير عينات ذات تركيب بلوري نانومتري من أغشية مركب In_2O_3 النقية والمشوبة بالقصدير بالنسب الحجمية (2%,4%)على قواعد زجاجية .تم اخذ نتائج فحوصات جهاز الأشعة السينية (XRD)وقد أظهرت النتائج إن الأغشية المحضرة ذات تركيب متعدد التبلور و أدى التشويب إلى زيادة تبلور الاتجاه (110) . تم فحص النماذج بجهاز مجهر القدرة الذرية (AFM)وقد تبين حصولنا على الحجم الحبيبي النانومتري .و.تم دراسة الخصائص البصرية للاغشيةالمحضرة من خلال تسجيل طيفي النفاذية والامتصاصية لمدى الأطوال الموحية (300-900nm) .حيث وجد ان الامتصاصية تزداد بزيادة التشويب.كما تم ايجاد فجوة الطاقة التي تقل بزيادة التشويب وتتراوح قيمتها بين (3.5-3.32eV) وقد وجد ان اعلى قيمة للنفاذية تبلغ(85%) كما تم ايجاد الخواص الكهربائية والتي تضمنت دراسة تغير التوصيلية الكهربائية المستمرة مع درجة الحرارة ضمن المدى الحراري (300-423 K)وقد تبين ان التوصيلية الكهربائية تقل بزيادة التشويب .

ABSTRACT

Electrical characteristics were studied with structul and optical of (In2O3)films prepared by sputtering method using plasma system with parallel electrodes (Diode Sputtering) and prepare samples of crystalline nanomtri installation of composite (In2O3)film pure and doping with (Sn) volumetric proportions (2%, 4%) glass rules. results of tests taken x-ray (XRD) has shown that membranes prepared with multiple installation take shape and the contamination caused by increased crystallization direction ((110) The samples were examined with a microscope, atomic power (AFM) showed grain nanometric size. &. optical properties were studied by recording the spectral transmittance and absorbance of wavelengths (300-900nm). where the absorbance increasing doping caused by increase. as a smaller energy gap by increasing doping caused by and between ((3.5-4.25)eV and found that the highest value of permeability (85%) It was finding the electrical characteristics, which included the study of electrical conductivity changes with the temperature within the range (300-423 K) and electrical conductivity was found to be decreasing by increasing doping .

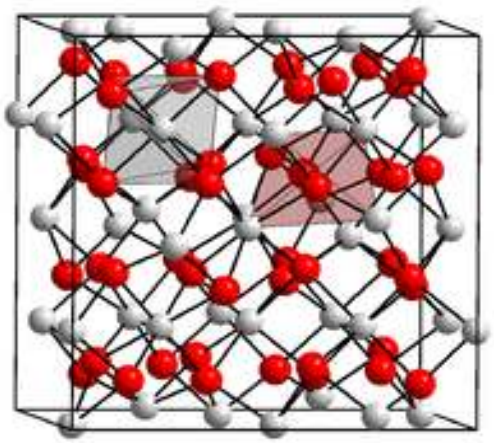
المقدمة

انّ الموصلات التي تسمى بأكاسيد التوصيل الشفافة و يطلق عليها اختصاراً (TCOs) (Transparent Conductive Oxides)، هي عبارة عن أشباه موصلات مركبة مكونة من معدن متحد مع الأوكسجين مثل In_2O_3 ,ZnO SnO_2, [1]، وتجمع هذه المواد بين ميزتين إذ تتميز بارتفاع توصيليتها ونفاذيتها البصرية العالية(شفافة) فيمتد طيف النفاذية فيها ما بين nm(1500 – 400) ويعتمد ذلك على ظروف تحضير المادة وبالرغم من كبر فجوة طاقة هذه الاغشية نرى أنّ حزمة التوصيل مليئة بالإلكترونات الحرة (ويظهر ذلك واضحاً من خلال التركيز العالي لحاملات الشحنة) [2]، من كل هذا نرى ان البحوث الحديثة اتجهت الى حقل (TCOs) نظرا لاهميته التكنولوجية وتعدد استعمالاته في الخلايا الشمسية وكترانزستورات شفافة (Transparent Transistors) [3].

تعتبر اغشية اوكسيد الانديوم من صنف اغشية اشباه الموصلات النفاذة (Transparent)والتي لها مدى واسع من التطبيقات منها لانتاج الاغشية النفاذة العاكسة للحرارة (Transparent heat reflecting films)كما تدخل هذه الاغشية في صناعة الخلايا الشمسية المتعددة الطبقات وبوجود طبقة موصلة نفاذة امكن تصنيع خلايا شمسية بكلفة قليلة وباداء مناسب حيث ان هذه الاغشية تسمح بنفاذ الاشعاع الشمسي مباشرة الى المنطقة الفعالة مع توهين قليل للطاقة الاشعاعية كما هو الحال مع خلايا In_2O_3/Siالشمسية كما تستعمل الاغشية كمتحسسات غازية (Gas sensor) ومن التطبيقات الاخرى استخدامها كعناصر حرارية نفاذة على زجاج السيارات والطائرات لازالة الجليد(Deieing)والضباب (Defoging)المتكون عليها.(جدول 1، شكل 1).

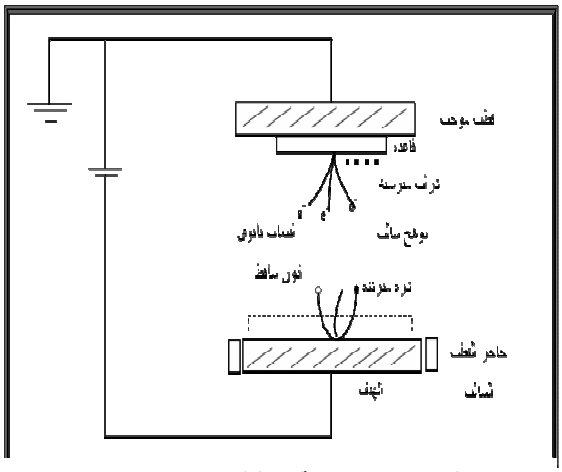
الجدول (1): بعض الخصائص الكيميائية والفيزيائية لـ(In_2O_3).[4]

ت	الزمرة	
1	درجة حرارة الانصهار (K)	2273
2	اللون	اصفر مخضر
3	الكثافة (g/cm^3)	6.99
4	الوزن الجزيئي (g/mol)	277.638
5	الشكل	cubic



الشكل(1): يوضح التركيب البلوري لمادة انديوم اوكساید (In_2O_3). [4].

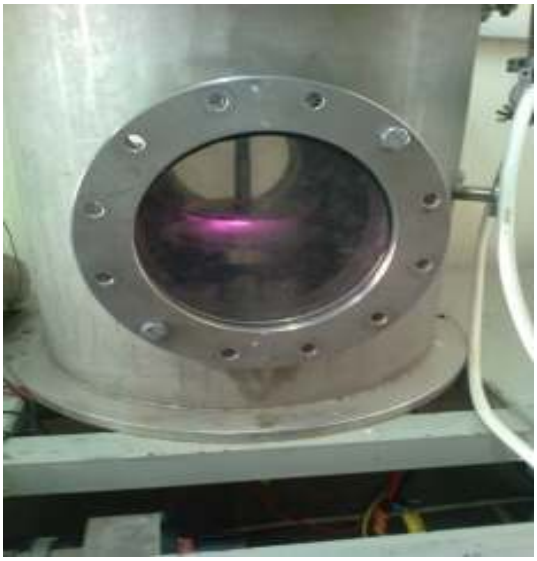
ان عملية خلع مادة من السطح وذلك بتبادل الزخم (Momentum exchange)بين الدقائق المشحونة والمعجلة نحو الهدف تدعى بالترذيذ وتعد طريقة الترذيذ واحدة من طرق الترسيب باستخدام البلازما حيث يتم تحضير الاغشية الرقيقة بواسطة قصف سطح المادة المراد تحضير الغشاء منه بجسيمات مشحونة وثقيلة او باشعاع [5].ذو تردد عالي مؤدية الى خلع ذرات السطح من مواقعها وانطلاقها وبالتالي ترسيبها على الارضية كمافي الشكل.(2)



شكل رقم (2):يوضح عملية الترذيذ لبلازما التفريغ التوهجي

المواد وطرائق العمل

تم تحضير اغشية(In_2O_3)الرقيقةوذلك عن طريق وضع هدف الترذيذ عند القطب السالب (الكاثود) والذي كان قطره بحدود (14.5cm)من خلال تجهيز اقطاب التفريغ بفولتية تتراوح(2000-3000v.H)من مجهز قدرة مصنع محليا ووضع عينة الترسيب على حامل عند القطب الموجب الذي يوازي ويشابه القطب السالب .بعد تفريغ الحاوية الى حدود ضغطtorr (10^{-4}) تم ادخال غاز الاركون عن طريق صمام ابري وذلك لغرض الحصول على الضغط النوعي المطلوب لاجراء تفريغ التوهج والذي كان بحدودtorr (10^{-2})بعدها يتم تسليط جهد كهربائي عالي بحدود (2kv)يؤدي الى توليد ايونات في تفريغ التوهج والتي تمتلك طاقة عالية نوعا ما تؤدي الى خلع ذرات هدف عند اصطدامها بها لتترسب على القواعد الزجاجية عند القطب الموجب والشكل (3)يوضح منظومة الترذيذ المصنعة محليا تم اجراء الفحوصات البصرية لاطياف النفاذية والامتصاصية البصرية باستخدام جهاز المطياف (optima-3000uv-vis).اجريت فحوصات حيود الاشعة السينية للاغشية المحضرة باستخدام جهاز (Schemadzu XRD-Culcx) في حين حصلت صور الحبيبات البلورية لاغشية (In_2O_3) باستخدام مجهرالقوة الذرية نوع(A° AFM) 2000 .قياسات التوصيلية الكهربائية تم استخراجها باستخدام المنظومة الموضحةا بالشكل (3).



شكل (3)يوضح حلقة البلازما التفريغ التوهج

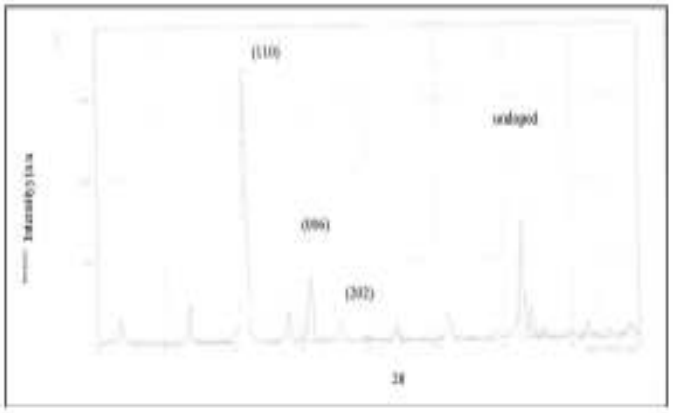
النتائج والمناقشة

حيود الأشعة السينية (X-Ray Diffraction (XRD:

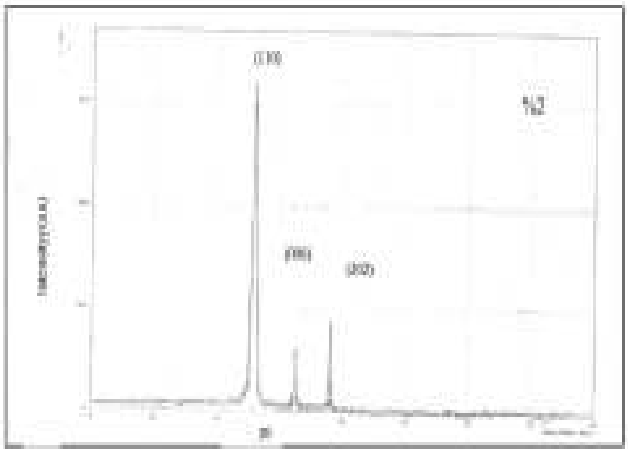
أظهرت نتائج التشخيص بتقنية حيود الأشعة السينية للأغشية المحضرة غير المشوبة والمشوبة بالقصدير(Sn) بنسب تشويب مختلف(2%,4%) ، أنها ذات تركيب متعدد التبلور (Polycrystalline) شكل(5و6و7) كما حصلنا عليها من الجهاز . وحُسبت المسافة بين المستويات البلورية باستخدام قانون براك (Bragg's Law) وبموجب العلاقة الآتية [6].

$$n\lambda = 2d \sin\theta_B \quad \ldots\ldots (1)$$

n: تمثل مرتبة الحيود
(λ): الطول الموجي للأشعة السينية الساقطة .
(d): المسافة البينية بين مستويين متتاليين
(θ_B): زاوية براك (Bragg's angle)



شكل (5)يوضح منحني حيود الاشعة السينية للغشاء النقي



شكل (6)يوضح منحني حيود الاشعة لنسية تشويب 2%

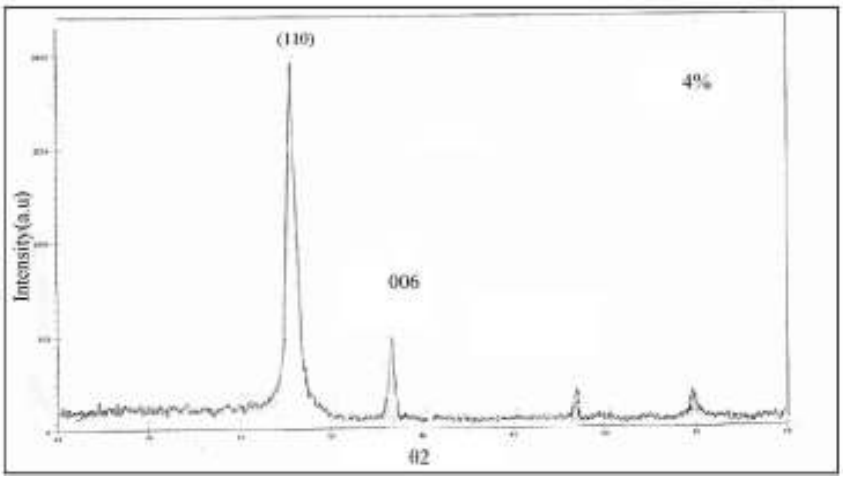
ووجــد أن هذه النتائج متفقة الى حدٍ ما عــند مقارنتها ببطاقة (ASTM) (American Standard of Testing Materials) . ونلاحظ أن الشدة للقمة (110) تزداد بزيادة التشويب بالقصدير مقارنة بأغشية (In_2O_3)غير المشوبة، وهذا يتفق مع ما توصل إليه الباحث[7,13].تم حساب معدل الحجم الحبيبي (G_z) باستخدام علاقة شرر(2)، وقد وُجد أنه يقل بزيادة التشويب با (Sn) ، ويمكن تفسير ذلك بسبب صغر نصف قطر ايون المادة الشائبة لل (Sn) والذي يساوي (0.58Å) ، عند مقارنته مع نصف قطر ايون المادة المضيفة (In) والذي يساوي (0.71Å) فإن ذلك يؤدي الى دخول شائبة القصدير في بلورة(In_2O_3)[10] على شكل شائبة بينية (interstitial impurities) مما يؤدي الى تناقص الحجم الحبيبي وبالتالي زيادة (2θ) و عرض القمة لمنتصف المنحني [16,14] (FWHM).و كما موضح في الجدول (2) ،إذ يتناسب الحجم الحبيبي (G_z) عكسيا مع عرض القمة لمنتصف المنحني (FWHM) كما مبين في علاقة شرر (2)

$$G_z = \frac{\square}{\beta \cos\theta_{\square}} \quad \ldots\ldots\ldots\ldots\ldots (2)$$

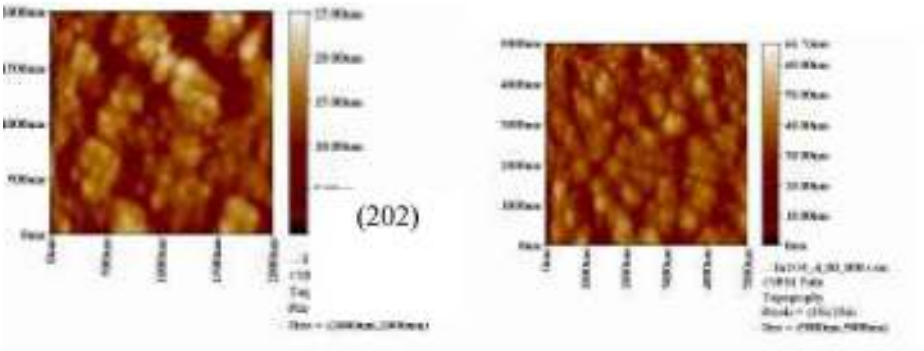
$B_{hk\ell}$: عرض النصف للقمة (Full width at Half Maxiuman)

الجدول (2): **النتائج التي تم الحصول عليها من حيود الأشعة السينية**

Sample	2Θ **(deg)**	**d(110) (Å)**	FWHM (110) **(deg)**
Pure	*32.7633*	*2.73123*	*1.3000*
2%Sn	*32.9606*	*2.71533*	*1.5971*
4%Sn	*33.2341*	*1.78002*	*1.7125*

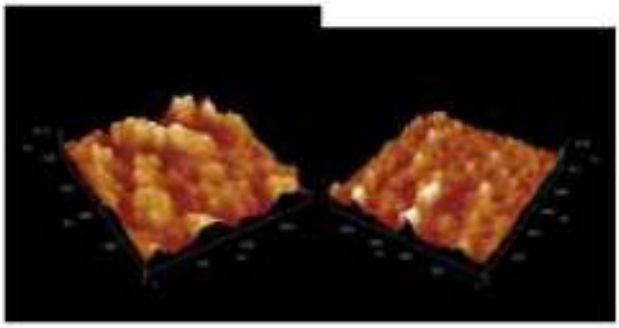


شكل (7)يوضح منحني حيود الاشعة السينية لنسبة تشويب 4%



صورة[8-A] ثنائيةالابعاد للعينات

ثنائيةالابعاد للعينات



صورة[8-B] ثلاثيةالابعاد للعينات

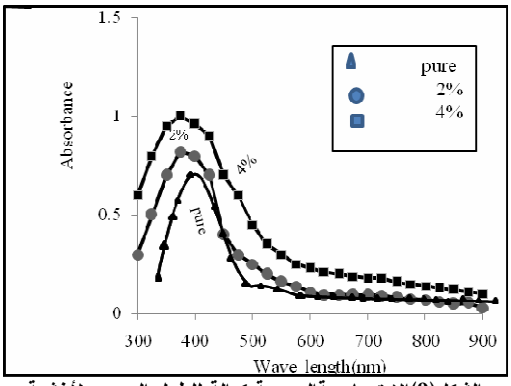
شكل (8) النماذج المرسبة بجهاز مجهرالقوة الذرية

الفحص بمجهر القوة الذرية Atomic Force (Microscope)(AFM)

تم فحص النماذج المرسبة بجهاز مجهرالقوة الذرية وقد تبين ان النماذج التي تم فحصها قد حققت الحجم النانومتري ومتوافقة مع نتائج البحوث [15,16] كما في الشكل (8).

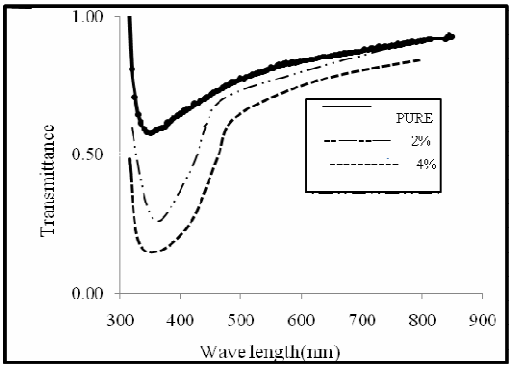
نتائج القياسات البصرية Optical Measurement

تمت دراسة الخصائص البصرية لأغشية (In_2O_3)غير المشوبة والمشوبة بالقصدير(In_2O_3:Sn)من خلال طيفي النفاذية والامتصاصية ضمن مدى الأطوال *الموجية -(350 nm (900 .كما تضمنت هذه الخواص حساب الثوابت البصرية ، كمعامل الامتصاص الامتصاصية_Absorptance والنفاية Transmission* . تتأثر امتصاصية المواد بعوامل عدة مثل نوع المادة و السمك ، وطول موجة الإشعاع الساقط.ولقد تم قياس الامتصاصية بوصفها دالة للأطوال الموجية -(350 900)nm كما واضح في الشكل(9). وقد تبين ان الامتصاصية تقل مع زيادة الطول الموجي للأغشية كافة. ويعني هذا فيزيائياً أن الفوتون الساقط لم يستطع أن يهيج الإلكترون وينقله من حزمة التكافؤ إلى حزمة التوصيل لأن طاقة الفوتون الساقط أقل من قيمة فجوةالطاقة لشبه الموصل [17] ، ونلاحظ أيضا أن الامتصاصية تزداد كلما ازدادت نسبة التشويب بالقصدير وهذا يؤكد دخول القصدير ضمن التركيب البلوري للغشاء المحضر ، بتكوين مستويات موضعية داخل فجوة الطاقة أدت بدورها الى امتصاص الفوتونات ذات الطاقة المنخفضة.



الشكل(9)الامتصاصيةالبصرية كدالة للطول الموجي لأغشية (In_2O_3) غير المشوبة والمشوبة Sn بنسب مختلفة

ولكن طيف النفاذية كما في الشكل (10) للاغشية المحضرة فقد ابدى سلوكاً معاكساً للامتصاصية ، اذ تقل النفاذية للأغشية المحضرة بزيادة التشويب بالقصدير ، وإن زيادة نسبة التشويب تؤدي إلى تكوّن مستويات موضعية جديدة أسفل حزمة التوصيل وهذه المستويات مهيأة لاستقبال الإلكترونات وتوليد ذيول في فجوة الطاقة البصرية وهذه الذيول تعمل باتجاه التقليل من فجوة الطاقة. وهي أحد العيوب البلورية.وهذا يتفق مع البحوث السابقة.



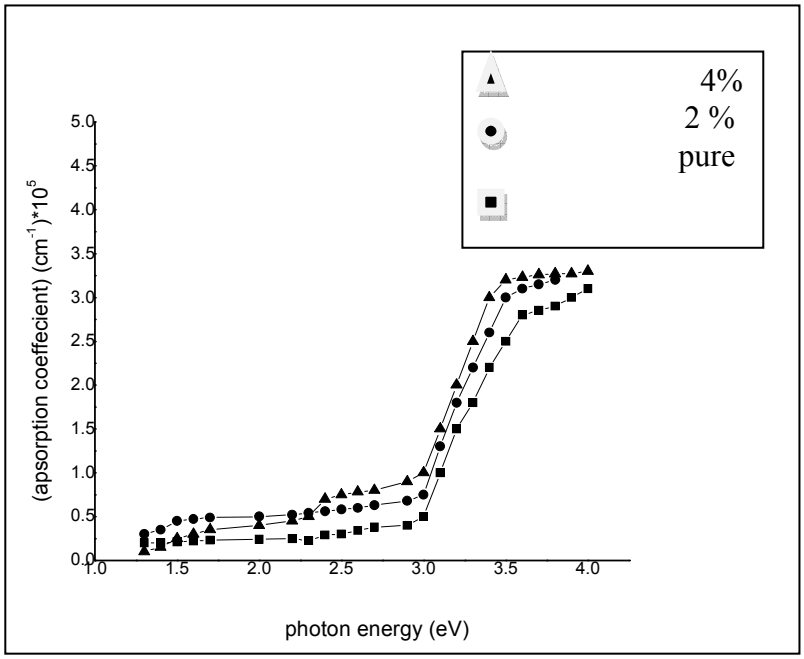
الشكل(10) النفاذيةالبصرية كدالة للطول الموجي لأغشية (In_2O_3) غير المشوبة والمشوبة

معامل الامتصاص Absorption Coefficient

لقد تم حساب معامل الامتصاص للأغشية غير المشوبة والمشوبة كافة من طيف الامتصاصية لهذه الاغشيةباستخدام المعادلة

$$\alpha\ \Delta t=2.303\Delta A \ldots\ldots\ldots\ldots\ldots(3)$$

ان الشكل (11) يبين تغير معامل الامتصاص للأغشية بوصفه دالة لطاقة الفوتون، ونلاحظ أن معامل الامتصاص يزداد بزيادة طاقة الفوتون، وكذلك يزداد بزيادة التشويب بالقصدير ، وتعزى الزيادة في معامل الامتصاص الى قلة فجوة الطاقة البصرية، وحصول الانتقالات المباشرة . ويؤكد ذلك القيم الكبيرة لمعامل الامتصاص التي تكون ($\alpha>10^5 cm^{-1}$) .[18,19].



الشكل (11) معامل الامتصاص كدالة لطاقة الفوتون لأغشية (In_2O_3) غير المشوبة والمشوبة بالقصدير (Sn) بنسب مختلفة

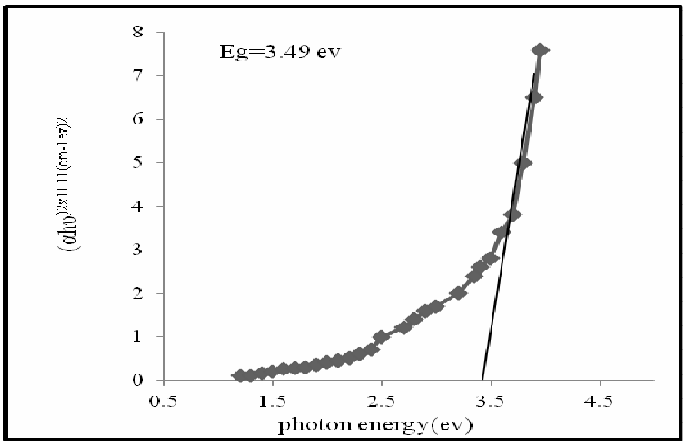
تعد فجوة الطاقة البصرية (E_g^{opt}) ذات أهمية كبيرة في تحديد أمكانية استعمال الاغشية الرقيقة في صناعة الخلايا الشمسية والخلايا الضوئية ، إذ إنها تعطي فكرة واضحة عن الامتصاص البصري ، أذ يكون الغشاء شفافا للاشعاع الذي تكون طاقته أقل من فجوة الطاقة ($E_g^{opt} > h\nu$) وماصا للاشعاع الذي تكون طاقته أكبر منها ($E_g^{opt} < h\nu$) . هناك الكثير من العوامل التي تؤثر في فجوة الطاقة منها نوع المادة الغشاء المحضر وطريقة تحضير الغشاء وكذلك تتأثر بشكل كبير بعمليتي الأشابة والتلدين ، فضلا عن ذلك تتأثر فجوة الطاقة بظروف التحضير وطبيعة البنية التركيبية للأغشية المحضرة ومدى الانتظام البلوري . يمكن حساب فجوة الطاقة البصرية للانتقال المباشر المسموح من المعادلة

$$\alpha\, h\nu = B_o\,(h\nu - E_g^{opt})^r \quad \ldots\ldots(4)$$

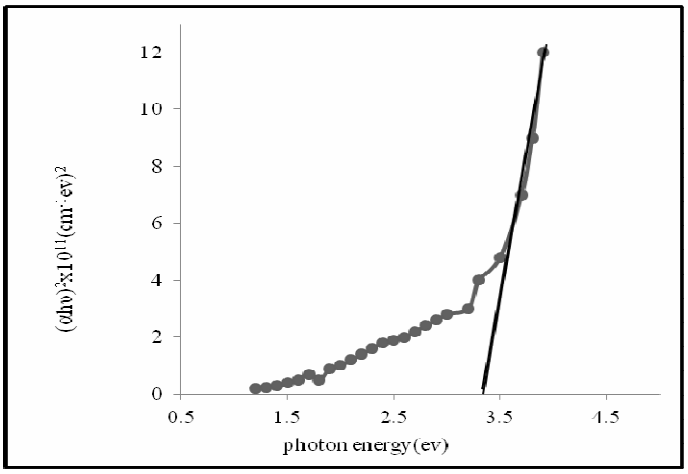
اذ تكون قيمة $\left(r = \frac{1}{2}\right)$ ، ذلك برسم العلاقة الخطية بين $(\alpha h\nu)^2$ وبين طاقة الفوتون الساقط $(h\nu)$ وبمد الجزء المستقيم من المنحني ليقطع محور طاقة الفوتون عند النقطة $(\alpha h\nu)^2 = 0$ وحيث تتحقق المعادلة (4) وبمعنى آخر أن $(E_g = h\upsilon)$ أي أن نقطة القطع تمثل قيمة فجوة الطاقة البصرية (E_g^{opt}) للانتقال المباشر المسموح. وكما هو موضح في الشكل (14,13،12) لأغشية (In_2O_3) عند جميع نسب التشويب ويبين الجدول (3) قيم فجوة الطاقة البصرية للانتقالات المباشرة المسموحة لأغشية (In_2O_3) عند نسب تشويب مختلفة. وقد وجد أنها تتناقص مع زيادة التشويب بالقصدير ، أي إزاحة حافة الامتصاص إزاحة حمراء (red shift) (باتجاه الطاقات الفوتونية الواطئة) [21,22].

الجدول (3) قيم فجوة الطاقة البصرية للانتقال المباشر المسموح للأغشية المحضرة

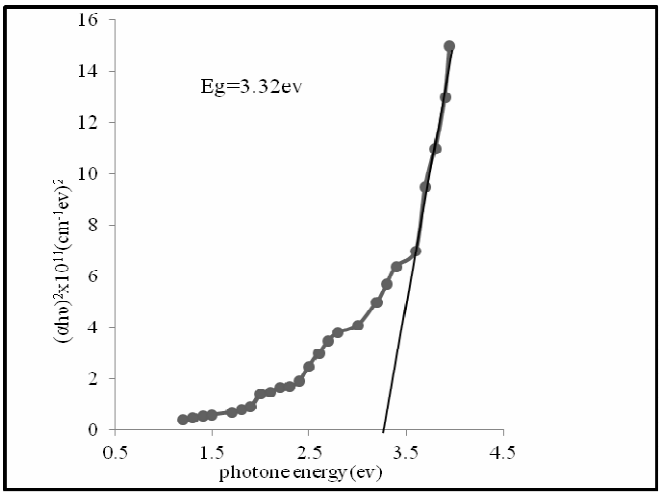
الغشاء ونسبة الشائبة	قيم فجوة الطاقة البصرية للانتقال المباشر المسموح (eV) E_g^{opt}
In_2O_3	3. 49
In_2O_3:Sn 2%) (	3.43
In_2O_3:Sn (4%)	3.32



شكل (12)يمثل فجوة الطاقة البصرية للانتقال المباشر للغشاء النقي



شكل (13) يمثل فجوة الطاقة البصرية للانتقال المباشر للغشاء المشوب 2%



شكل (14)يمثل فجوة الطاقة البصرية للانتقال المباشر للغشاء المشوب 4%

D.C الخواص الكهربائية التوصـيلية المـستمرة ($\sigma_{d.c}$) Conductivity

ان قابلية توصيل المادة الكهربائية تعتمد على كثافة حاملات الشحنة في أشباه الموصلات الالكترونات والفجـوات هـي المسؤولة عن نقل التيار الكهربائي داخـل المـادة ، وتعـد معرفة التوصيلية الكهربائية ضرورية ومهمة لمعرفة بعض صفات شبه الموصل مثل نوعية شـبة الموصـل ويمكـن معرفة التوصيلية من خلال

$$\sigma_{d.c} = \frac{1}{\rho} \quad \text{.....5.}$$

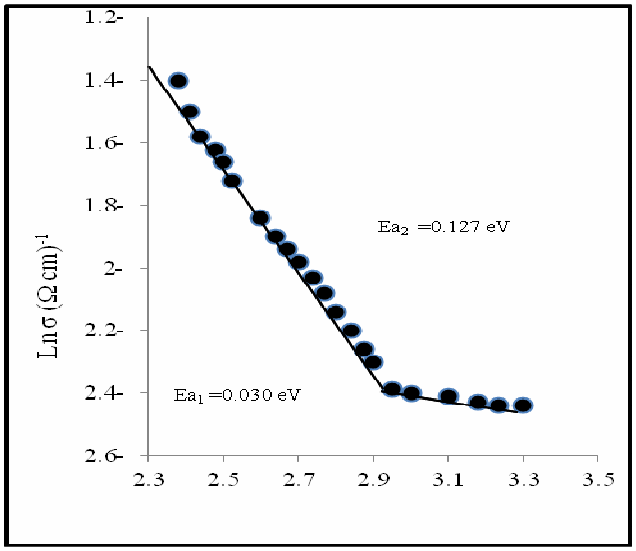
$$\rho = R\frac{s}{L} \quad \text{.....6}$$

$$s = a.t$$

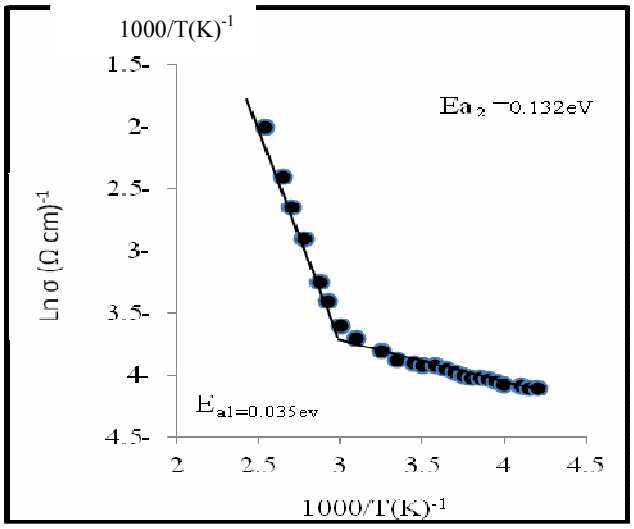
$$\rho = R\frac{a.t}{L} \quad \text{.....7..}$$

$\sigma_{d.c}$: التوصيلية (Conductivity) ، ρ : المقاوميـة R(Resistivity): مقاومة الغشاء المقاسة عمليا بوحـدات(Ohm) ، s : مساحة المقطع العرضي لحركة الالكترونات من خلال الغشاء ، L: المسافة بين قطبي الألمنيوم وعرض القطـب(a) بوحـدات (mm)، t : سـمك الغـشاء بوحدات(Å).[11 و12]حسبت في هذا البحث التوصـيلية الكهربائية المـستمرة ($\sigma_{d.c}$) لاغـشية انـديوم اوكـسايد غيرالمشوب والمشوب بالقصديرولجميع النسب ودرس تأثير التشويب على قيم التوصيلية اذ استخدمت المنظومة كما في الشكل(5) لقياس التوصـيلية الكهربائيـة المـستمرة وان القياسات تمت ضمن مدى من الدرجات الحـراري وعنـد ملاحظة الشكل(15و16و17) نرى منحني العلاقة لأغـشية انديوم اوكسايدغير المشوب والمشوب بالقـصديراذ نـرى عندزيادة درجة الحرارة تزداد قيمة التوصيلية وهذه صـفه مميزة لأشباه الموصلات[17,19] وكـذلك لاحـظ وجـود طاقتي تنشيط وهذا يتفق مع (Kasap) [13] للاغشية غيـر المشوبـة. وان لهـذه الاغشية توصيليـة عاليـة بحـدود ($S\ .cm^{-1}$)(30)ان القيمة لهذه التوصـيلية ضـمن مـدى توصيلية اشباه الموصلات ويمكن ايجادالتوصيلية($\sigma_{d.c}$) من خلال معرفة قيمة المقاومية (ρ) اعتماداً على المعادلـة[5] في درجة حرارة الغرفة و من خلال رسم المنحني البيـاني بين $Ln\sigma_{d.c}$ على محـور الـصادات و (1000/T) علـى محور السينات نجد أن الميل مـضروباً بثابـت بولتزمـان (K_B) يمثل مقدار طاقة التنشيط وكما في المعادلةالاتية:

$$E_a = slope \times K_B \quad \text{.....(8)}$$



شكل (15)يبين التغير في التوصيلية الكهربائية المستمرة مع تغير درجة الحرارة للغشاء 4%

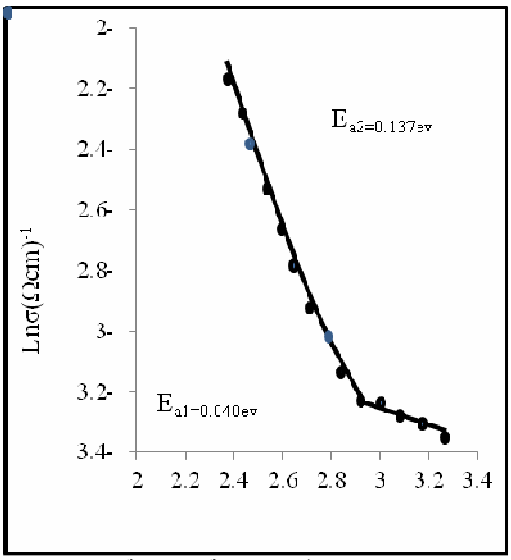


شكل(16) يبين التغير في التوصيلية الكهربائية المستمرة مع تغير درجة الحرارة للغشاء2%

ان وجود طاقتي تنشيط للاغشية المحـــضرة ($E_{a1}<E_{a2}$) [20,21]و يدل على وجود اليتين للانتقال الالكتروني فـــي درجات الحرارة الواطئة والعاليـــة نـــسبيا [20,21] .كمـــا يتضح من الجدول بان التوصيلية الكهربائية تزداد بتنـــاقص فجوة الطاقة البـــصرية بـــسبب زيـــادة كثافـــة المـــستويات الموضعية داخل فجوة التحركية وهذا ما تؤكده نتائج حساب فجوة الطاقة البصرية والذي يؤدي الى حاجـــة الحـــاملات الموجودة في حزمة التكافؤ الى طاقة اصغر لعبور الفجـــوة ووصولها الى حزمة التوصيل .

جدول (4)يبين قيم التوصيلية الكهربائية المستمرة وطاقات التنشيط لاغشية (In_2O_3)النقية والمشوبة

Sample	Dc electrical conductivity(300)(Ω.cm)$^{-1}$ $\sigma_{d.c}$	**Dc electrical conductivityy(334)(Ω.cm)$^{-1}$** $\sigma_{d.c}$	Activation energy E1(ev)	Temperature range (K	Activationenergy E2(ev)	Temperaturerange (K)
Pure	*1.8505*10^{-2}*	*7.93*10^{-2}*	*0.04*	*300-334*	*0.137*	*334-423*
2%Sn	*4.0996*10^{-2}*	*10.734*10^{-2}*	*0.035*	*300-328*	*0.132*	*328-418*
4%Sn	*8.2241*10^{-2}*	*21.654*10^{-2}*	*0.030*	*300-337*	*0.127*	*334-423*



شكل(17) يبين التغير في التوصيلية الكهربائية المستمرة مع تغير درجة الحرارة للغشاء النقي

النتائج

1. أغشية (In_2O_3) غير المشوبة والمشوبة بالقصدير(Sn) للظروف المنتخبة هي ذات تركيب متعدد التبلور.

2. أدى التشويب بالقصدير إلى زيادة عرض المنحني لمنتصف القمة في منحنى حيود الأشعة السينية مما يعني نقصان الحجم الحبيبي(G)، ونقصان المسافة بين السطوح البلورية (d).

3. إن قيم معامل الامتصاص للأغشية المحضرة أكبر من ($10^5 cm^{-1}$) مما يؤدي الى حصول انتقالات الكترونية مباشرة ، وإن حافة الامتصاص لـ(In_2O_3) تزحف نحو الطاقات الواطئة بزيادة نسب التشويب بالقصدير

4. إن زيادة التشويب بالقصدير يؤدي الى زيادة الامتصاصية وكذلك زيادة معامل الامتصاص ، ولذلك يمكن استعمال الأغشية المحضرة في تصنيع الخلايا الشمسية.

5. اوضحت نتائج التوصيلية الكهربائية المستمرة لاغشية (In_2O_3) الرقيقة ان التوصيلية تزداد بزيادة درجة الحرارة وهذه سمة من سمات اشباه الموصلات حيث يزداد تركيز حاملات الشحنة بزيادة درجة الحرارة.

6. وجد ان هناك قيمتين لطاقة التنشيط حيث (E_{a1})عند درجات الحرارة الواطئة نسبياو(E_{a2})عند درجات الحرارة العالية نسبيا .

7. اوضحت نتائج التوصيلية الكهربائية المستمرة لاغشية (In_2O_3) الرقيقة ان التوصيلية قد ازدادت مصاحبا لذلك انخفاض طاقة التنشيط .

8. اوضحت نتائج الفحص بمجهر القوة الذرية (AFM)ان الحجم النانومتري للاغشية قد تحقق.

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