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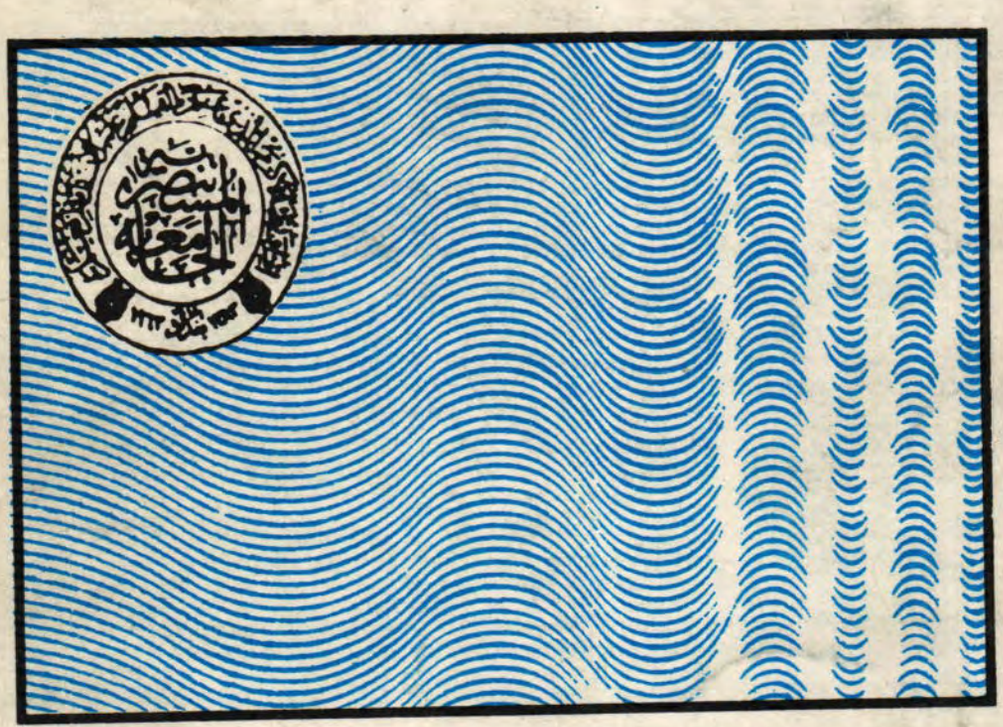

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المحتويات

البحوث الانكليزية

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Page No.

First Occurance of The Anchor Worm *Lernaea cyprinacea* L. in Fishes of Shatt Al-Arab River, Basrah, Iraq.

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(Recieved Oct. 9, 1994; Accepted Apr. 4, 1995)

الخلاصة

اثناء فحص ٧٥٧ نموذجا من الاسماك العائدة الى عشرة انواع من نهر شط العرب تم العثور على الدودة الكلابية *Lernaea cyprinacea* في اربعة انواع من الاسماك هي الحمري والبني والكارب الاعتيادي والقوبيون الملطخ . ويمثل هذا اول ظهور لهذه الافة في اسماك المياه الطبيعية لنهر شط العرب واضافة مضيف جديد (القوبيون الملطخ) للدودة الكلابية في العراق . تم اعطاء قائمة مفصلة بتسجيلات المضيفات السابقة وتمت مناقشة كل من موقع ومصدر الاصابة .

ABSTRACT

Upon exanining 757 fish specimens, belonging to 10 fish species, from Shatt Al-Arab river, the anchor worm *Lernaea cyprinacea* was found on four fish species namely *Barbus luteus, B. sharpeyi, Cyprinus carpio* and *Boleopghthalmus dussumieri*. This is the first occurrence of this pest in fishes taken from natural waters of Shatt Al-Arab river and adds a new host (*B. dussumieri*) for *L. cyprinacea* in Iraq. Detailed list of previous host records in Iraq is provided. Both site and source of infection were discussed.

INTRODUCTION

The genus *Lernaea* includes some of the mosst dangerous crustacean parasites of economically freshwater fishes which have been responsible for serius losses of the fishing industry (1). According to Hoffman (2), *L. cyprinacea*, which lacks host specificity, can probably infect all freshwater fishes and even frog tadpoles and salamanders.

The anchor worm was reported for the first time in Iraq in April 1969 by Al-Hamed & Hermis (3) from seven fish species at Al-Zaafaraniya Fish Culture Station at Baghdad. Later on, it was reported from different fish farms of Iraq (4-22). In addition to fish farms, Abdullah (23) and Balasem et al. (24) had reported this parasite from natural waters of Iraq, viz. Dokan Lade and Tigris river at Baghdad, respectively. The present paper is concerned with the occurrence of the anchor worm for the first time in natural waters of Shatt Al-Arab river, south of Iraq.

MATERIALS AND METHODS

During the period from September 1991 to January 1993, fish specimens were collected either freshly from Garmat Ali Fish Market of fished from Shatt Al-Arab river at the site of Basrah University in Garmat Ali. Cill nets, Castnets and hand nets as well as line and hook were used to catch fishes.

Fishes were inspected as soon as possible for shaft-like projections issued from their bodies which indicate *Lernaea* infestations. Parasites were removed from their hosts with the aid of $KMnO_4$ solution as stated by Mhaiden (6) and examined in lactic acid using wooden slide method (25).

Parasite sites of attachment on host body were recorded. Identification of th parasite was carried out according to Yamaguti (26).

RESULTS AND DISCUSSION

A total of 757 fish specimens belonging to 10 fish species were inspected for *L. cyprinacea*. Only five specimens (four species) were infected (Table 1).

Table 1: Anchor worm ingstation of fishes of Shatt Al-Arab river

Fish family and species	No. fish examined	No. fish infested	Sex and total length (mm) of infested fish
Family Cyprinidae			
Barbus luteus	248	1	♀175
Barbus sharpeyi	12	1	♂ 108
Barbus xantho-pterus	7	-	
Carassius auratus	1	-	
Cyprinion kais	45	-	
Cyprinus carpio	166	2	♀150 ♀ 98
Family Siluridae			
Silurus tiostegus	5	-	
Family Mugilidae			
Liza abu	252	-	
Family Cobiidae			
Boleo-phthalmus dussumieri	15	1	♀ 127
Family Masta-cembelidae			
Masta-cembelus masta-cembelus	6	-	

The previous records of *L. cypricacea* from *B. luteus*, *B. sharpeyi* and *C. carpio* in Iraq were mainly from fish farms (3, 4, 6-10, 16, 19-22). In addition, this parasite was also reported twice on fishes taken from natural water bodies of Iraq. These fishes included *B. luteus*, *C. carpio* and *Leuciscus lepidus* from Dokan Lade (23) and *Chomdrostoma regium* from Tigris river at Gaghdad (24). Therefore, the occurrence of *L. cyprinacea* of the present investigation in Shatt Al-Arab river, represents its first record in Shatt Al-Arab river, first record on both *B. sharpeyi* and *B. dussumieri* from Iraqi natural waters and the second record on both *B. lluteus* and *C. carpio* from Iraqi natural waters. On the other hand, *B. dussumieri* of the present study represents a new host to be added to the previous hosts reported for *L. cypricacea* in Iraq. This brings the total number of such hosts to 16 species (Table 2). In this table, the names of such fish species are alphabetically arranged and the references are chronologically arranged.

During the late seventies and earlly eighties of this century, th authorities of Iraqi State Enterprise for Fisheries had released millions of carp fingerlings in some lakes, marshes and rivers of Iraq. As stated by Mhaisen (27), these fingerlings, reared in Al-Zaafaraniya Fish Culture Sstation and Al-Wahda Fish Hatchery at Al-Suwaira, were not carefully inspected for parasites before their release into the natural water bodies. Also, the flood of 1988 in Iraq had covered many fish farms in mid Iraq and hence helped in commin carp liberation into Iraqi natural water bodies. Above all, the accidental and possible escape of some carp fingerlings and juveniles from some fish farms via outlet waters to the drainage systems may also be responsible for the spread of *L. cyprinacea* to the natural waters of Iraq. For the above mentioned surveys from different localities are expected to reveal lernaeosis and thus may assert the present assumptions.

The infestation of the gobiid fish *B. dussumieri* with *L. cyprinacea* can be explained on the bases of anchor worm lack

of host specificity (2) on one hand and as a result of the sluggish movement of this fish which also spends long time of the day by the edge of the muddy shore. This may facilitate the attachment of anchor worm larval infective stages.

Table 2: *Lernaea cyprinacea*- host list in Iraq.

Aphanius dispar	Mhaisen (12)
Aspius vorax	Khalifa et al. (4)
Barbus esocinus	Khalifa et al. (4)
Barbus grypus	Al-Hamid & Hermiz (3), Khalifa et al. (4)
Barbus luteus	Al-Hamid & Hermiz (3), Khalifa et al. (4), Abddullah (23), the present study
Barbus sharpeyi	Khalifa et al. (4), Sarsam (7), Khalifa(16), the present study
Barbus xanthopterus	Al-Hamed & Hermiz (3), Sarsam (7), Khalifa (16)
Boleophthalllmus dussumieri	The present study
Carassius auratus	Al-Hamed &Hermiz (3), Khalifa et al. (4), Mhaisen (6), Ali (11), Mhaisen (12)
Chondrostoma regium	Balasem et al. (24)
Ctenopharyngodon idella	Al-Hamed & Hermiz (3), Ali et al. (14), Mhaisen et al. (18).
Cyprinus carpio	Al-Hamed & Hermiz (3), Khalifa et al. (4), Mhaisen (6), Sarsam (7), Mhaisen (8), Ali & Shaaban (9), Ali (10, 11), Mhaisen (12), Ali et al. (13,14), Khalifa (16), Mhaisen & Abul-Eis (19), Al-Dabhawi (20), Abdullah (23), Balasem et al . (24), Mhaisen et al. (21, 22), the present study.
Cambusia affinis	Mhaisen (12)
Hypophthalmichthys molitrix	Al-Hamed & Hermiz (3), Ali et al. (15).
Leuciscus lepidus	Abdullah (23), Mhaisen et al. (17).
Liza abu	Mhaisen et al. (17).
Unspecified pond fishes	Khlifa et al. (7).

The ten anchor worms found on *B. dussumieri* were scttered on different parts of fish body especially th head and the base of dorsal, pectoral and anal fins. Al-Hamed & Hermiz (3) and Hgaisen (6) concluded that tha anchor worm prefers the anterior parts of its host body. As far as *B. luteus, B. sharpeyi* and *C. carpio* are concerned, only one to two anchor worms were detected on each fish. Sites of infection for these later cases were the dorsal and caudal fins.

ACKNOWELEDGEMENT

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seedling

Effect of Post-Irradiation Treatments o Indole Acetic Acid (IAA) and Benzyl Amino Purine (BAP) on Ceeklings Developed from Rice Caryposes *Oryza sativa* L. var. Yareet.

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الخلاصة

تمت معاملة حبوب الرز باندول حامض الخليك (IAA) والبنزيل امينوبيورين (BAP) بتركيز 10^{-7}M لازالة الاثر الضار للاشعاع على النباتات الصغيرة الناتجة من حبوب الرز المشعة كان التاثير الترميمي (التصحيحي) معنويا عند معاملة الحبوب بخليط من IAA و BAP بينما كان التاثير ضعيفا عند معاملة الحبوب باحد المنظمين كل على انفراد . ومن الاهمية بمكان ان نذكر بان معاملة الحبوب باندول حامض الخليك لم يعكس ضرر الاشعاع الناتج على المجموع الخضري بينما كان تاثيره ايجابيا على المجموع الجذري وبالمقابل فان البنزيل امينوبيورين كان ذو تاثير جيد في ازالة ضرر الاشعاع الحادث في المجموع الخضري . واستنتج ان تاثير كلا المادتين في زيادة طول النبتة ونسبة البقاء وزيادة الوزن الطري للمجموع الخضري والجذري كان عن طريق التازر او الاضافة .

ABSTRACT

Post-irradiation treatments of rice caryopses with 10^{-7}M. of IAA and BAP to overcomethe extent of injury to the young seddlings developed from germination of irradiated caryopses, was investigated. The restoration effect was signiigicantly manifested when the caryopses were treated with IAA and BAP combined together. The effect on the other hand, was mediocre when the two growth sustances were applied individually. It is important to mention also that IAA alone did not reverse the shoot injury but it did reverse the root injury caused by irradiation. BAP, on the other hand, was more effective on restoration of shoot system. It is concluded that the two growth sustances might acted edditively of synergistically to increase seedling height, percentage of survival and fresh weight of shoot and root.

INTRODUCTION

High doses of gamma irradiation is known to induce mutation, but the problem is that physiological and genetical effects are correlated (1, 2, 3). That means if we raise to some extent the dose the percntage of survival decreases.

Since th presence of high concentration of extractable cytokinins in tissues composed of rapidly dividing cells has been used as evidence that cytockinin is a promotor or a product of cell dividion and since irradiation stops of delays cell dividion, a part of this study was undertaken to determine whether BAP applied after irradiation, is a radiorestoration material for rice caryopses.

Ussuf and Nair (5) obsereved a 50 percent decrease in the level of treptophan, the substrate for IAA synthesis, after irradiation that means tryptopan is radiolabile amino acid. Also ionizing radiation interferes, with the synthesis of the enzyme involving in the formation of IAA, from tryptophan. Thus

exogenous application of IAA is supposed to trigger the resumption of the synthesis of this protein.

The failure of BAP or IAA indvidually to restore rice embyos irradiated with high doses of gamma irradiation, had been previously declared while combination of both growth substaces gave an excellent results (2).

In the present study, therefore an attempt has been made to find out how far IAA and BAP used separately or combined togethyer, affect certain morphological characters of irradiated rice caryopses.

MATERIALS AND METHODS

Hand selected yareet rice caryopses of uniform size (13% moisture) were divided int 44 groups (48 caryopsis each) and exposed to 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 krad. of gamma irradiation at a dose rate of about 0.233 $krad^{-1}$ from a Co^{oo} source.

The irradiated and unirradiated caryopses were sterelized by shaking for 30 minutes in 13% chlorox solution (fas) containing 2 drops to Tween 20, and then rinsed three times with sterile distilled water. The caryopses to be used as irradiated or unirradiated controls were kept in distilled water for 48 hr., while those to be treated with growth substances were removed from distilled water and placed for 48 hr. iin solution of IAA or BAP applied alone or combined to each other at a concentration of 10^{-7}M.

All samples are then spreaded on wet filter paper in petri dishes and kept at room temperature for three days. The seedlings were then transfered into pots containing sterelized soil in rows four centimeters appart and ten centimeters between each, and kept for 30 days under green house conditions. Statistical analysis based on blocks methods (7) were carried out.

The following morphological characteristics were observed and recorded: percentage of survival, seedling height (shoot length) and fresh weight of both shoots and roots.

RESULTS AND DISCUSSION

Previous studies have shown that post-irradiation treatments of rice embryos with 10^{-7}M. of IAA + BAP could reverse radiation damages induced by ionizing radiation (2). We have extended these studies to determine whether a similar effects would occur on the caryopses.

Survival

The results of seedling survival, as a function of irradiation and addition of growth substances are shown in table (1) and Figure (1). The effect of IAA+BAP in raising percentage of survival was clearly observed on seedlings grown from caryopses, irradiated with relatively high doses (30 krad, 35 krad).

Since exposure to sublethal doses of 40 and 45 krad caused seedling death after germination and exogenous application of IAA+BAP increased th number of survivors, thus it is probable that endogenous growth substances are completely destructed by these doses. Such high doses might cause reduction of both RNA and protein synthesis (8) preventing cell division (4). It can also be seen from table (1) that the dose of 50 krad is a lethal dose because addition of growth substances did not reverse the harmful effect of ionizing radiation (9).

Seedling Height

It is known that seedling height decreases as radiation exposure increases (10 and 11). Table (2) and Figure (2) show that treatment with BAP and IAA overcomes the radiation effects. This radiorestoration was discernble only when the two growth substaces were added together. (see analysis of variance in Table 3). The accumulative effects of BAP+IAA was obvious at low dose, (5 krad), relatively high doses (30 and

35krad) and sublethal doses (40 and 45 krads). We noticed that the BAP or IAA applied seperately has a limited restoration effect. The failure of BAP or IAA alone to restore rice caryopses, by increasing seedling height may be due to failure of the material to reach the sensitive site due to impermeability or inactivation (7) or because the ratio of cytokini/auxin is disturbed.

Table1: Survive of rice seedlings after 30 days following irradiation of caryopses by different doses of gamma rays and post-irradiation treatments with IAA, BAP or IAA+BAP.

Radiation Dose (krads)	Number of Survivors			
	Post-irradiation treatment			
	0	IAA	BAP	IAA+BAP
0	47	48	48	48
5	48	48	48	48
10	43	45	48	48
15	42	45	47	47
20	33	39	38	43
25	25	35	34	37
30	20	27	25	33
35	11	15	16	28
40	7	12	12	18
45	zero	4	6	10
50	zero	zero	zero	zero

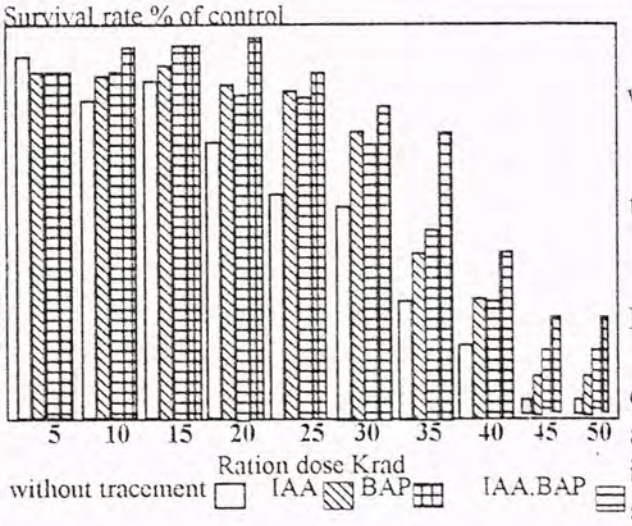

Figure 1: Influence of post-irradation treatment on percentage of survival of rice seedlings grown from irradiated rice caryopses

Table 2: Sum of seedlings heights (shoot length) expressed in centimeters as a function of radiation doses and post-irradiation treatments with IAA, BAP or IAA+BAP

Radiation Dose (krads)	Seedling hieght in centemeters			
	Post-irradiation treatment			
	0	IAA	BAP	IAA+BAP
0	564	720	696	708
5	614	790	819	953
10	559	585	648	648
15	450	515	459	575
20	312	379	418	473
25	258	301	357	402
30	175	181	180	280
35	60	109	115	205
40	43	73	68	177
45	Zero	24	42	142
50	Zero	Zero	Zero	Zero

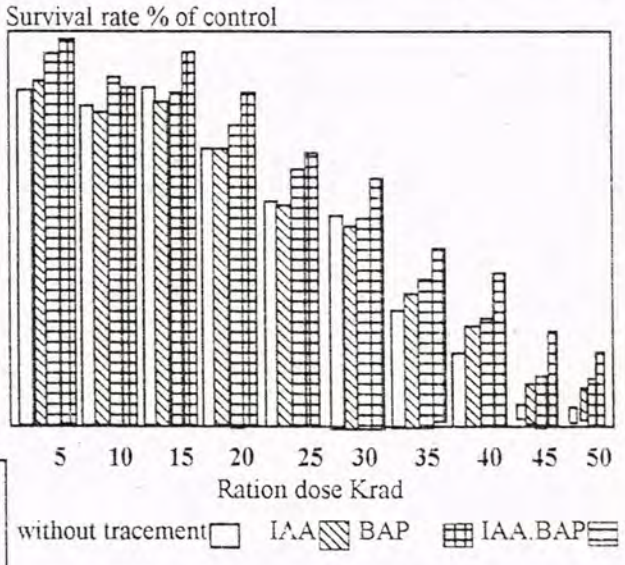

Figure2: Influence of post-irradiation treatment on seedlings height developed from irradiated rice catyopses.

Fresh Weight of Shoot and Root

Figures (3 and 4) illustrate the result of fresh weights of shoots of rice seedlings as a function of irradiation doses and post-irradiation treatments with growth substances. Analysis of variance for these criteria are shown in Tables 4 and 5.

Table 3 Analysis of Variance for tabe 2

Source of Variation	Sum of squares	Degree of freedom	Variance	Calculated Value of F	Critical Value of F	
					5%	1%
Total	655144.44	159				
BLocks	1.22	3	0.41	0.13	8.53	26.12
Treatment	654774.19	39	16789.08	5329.87	1.53	1.84
Error	369.03	117	315			

C.V = 1.89%

LSD 5% =2.5
1%=3.3

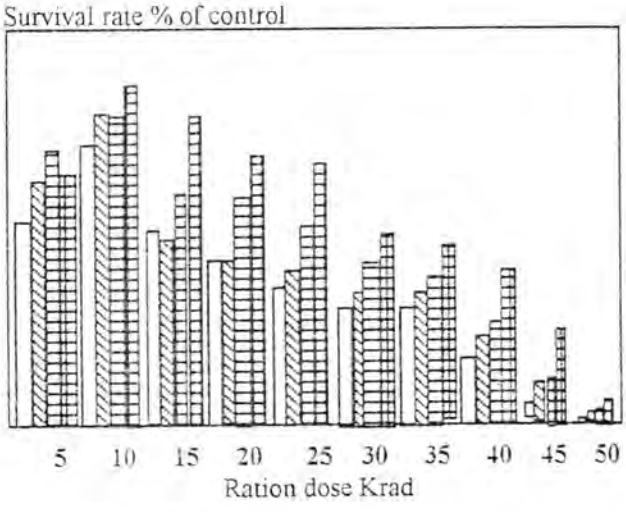

Figure 3: Influence of post-irradiation treatment on fresh weight of shoot of rice seedling devekoped from irradiated or non irradiated caryopses

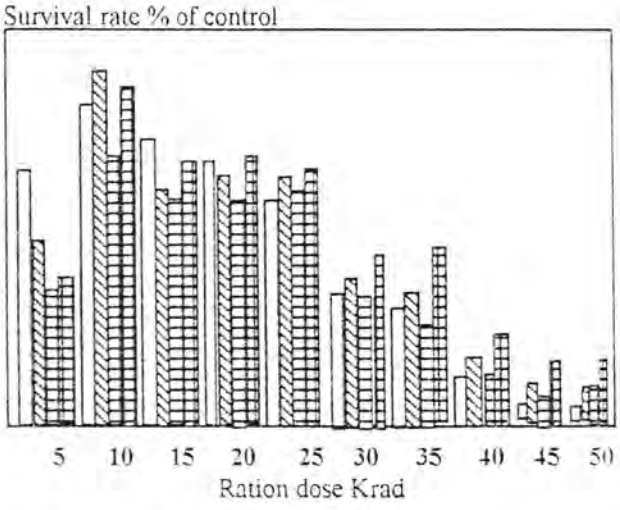

Figure 4:Influence of post-irradiation treatments on fresh weight of root of rice seedlingsgrown from irradiated or non irradiated Caryopses.

Table 4 Analysis of Variance for Figure 3

Source of Variation	Sum of squares	Degree of freedom	Variance	Calculated Value of F	Critical Value of F	
					5%	1%
Total	175227	159				
BLocks	3.07	3	1.02	0.36	8.53	26.12
Treatment	174889.74	39	4484.35	1595.85	1.53	1.84
Error	328.19	117	2.81			

C.V = 3.4%

LSD 5% =2.4
1%=3.2

Table 5 Analysis of Variance for Figure 4

Source of Variation	Sum of squares	Degree of freedom	Variance	Calculated Value of F	Critical Value of F	
					5%	1%
Total	68518.77	159				
BLocks	1.32	3	0.44	0.14	8.53	26.12
Treatment	68158.77	39	1747.66	569.27	1.53	1.84
Error	358.68	117	3.07			

C.V = 6.2%

LSD 5% =2.5
1%=3.3

Caryopses exposed to relatively high doses (10, 15, 20, 30, and 35 Krad) and tracted with BAP+IAA exhibited a significant increase in fresh weight of shoot and root in comparison with caryopses irradiated and not treated with growth substances. This indicates that ionizing radiation imposes a reduction effect on fresh weight of shoot and root, and suggests the ability of auxine plus cytokini to restore rice caryopses (12 and 13). Although exposure to a sublethal dose of 40 krad represented a threshold dose for maximum reduction of fresh weight of shoot and root, yet addition of BAP+IAA alleviated this reduction.

Application of IAA alone increased significantly fresh weight of roots but not shoots. This proves that auxin alone raised the level of root emergences and reverses the effects of radiation (14).

It is of interst that, in agreement with the observations of Gordon and Buess (12) the present experiments revealed that the recovery processes affecting survival, seedling height of fresh weight of shoot and roots has different sensitivities to exogenous substances.

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Characterization of a New Cell Line Derived from Mouse Melanocarcinoma

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الخلاصة

صممت هذه الدراسة لتوصيف خط خلوي جديد اشتق من ورم في جلد الفئران احدث عن معاملة الجلد بمادة 7-12 dimethylbenz(a) anthracene وزيت حب الملوك (Croton oil) لاحداث سرطان الجلد المسمى Melanocarcinoma استعمل في زرع المستنبت الاولي والمستنبتات الثانوية الوسط الزرعي Minimum Essential Medium الغني بمصل عجل البقر حديث الولادة ، واستعمل التربسين في تفكيك الخلايا . ان الدراسة قد انجزت على الخلايا الماخوذة من المستنبتات بعد الجيل الخامس عشر . اظهرت النتائج بان الخلايا المستنبتة في الاجيال الاولى تكون دائرية الشكل وتظهر بطأ في التصاقها في قعر الزجاج لتصل الى ٤٨ ساعة بينما تقل هذه المدة في الاجيال المتاخرة لتنخفض الى ٢٤ ساعة وتستقر ثم تلتصق في قعر الزجاج . ان الخلايا النامية غالبا ما تحتوي انويتها على اكثر من نوية واحدة . كما وقد احتسب زمن تضاعف الخلايا ليكون ٣٦ ساعة بينما كان التضاعف في الطور الاسي ٨ ايام . نستنتج من ذلك باننا حصلنا من هذه الدراسة على نتائج مشجعة لدراسات لاحقة تعتمد عليها دراسات متقدمة لاستبيان المزيد من خواص هذه الخلايا واختبارها ببعض العقاقير ذات الصلة في الدراسات السرطانية .

ABSTRACT

This study was designed to characterize a new cell line of mouse skin melanocarcimoma. A solid tumor was obtained by topical treatment with 7, 12 Dimethylbenz(a) anthracene and croton oil. Primary culture and subcultures were performed using Eagle modified Minimum Essential Medium enriched with fetal calf serum, and trypsin for separation of cells. The fifteenth transfer generation cells were studied. Results have shown that the early and late generation cells remained rounded, and took 48 and 24 hours respectively to attach to and flatten on the bottom of the culture fask. The nuclei contained predominantly more than one nucleolus. The growth doubling time and the time to reach exponential phase were found to be 36 hours and 8 days respectively. We conclude that these results are encouraging for a follow up study to elucidate further charactersistcs, and to test the pharmacological effects of some anticancer drugs on this cell line.

INTRODUCTION

Most normal and Cancer mammlian cells are immortak in tissue cultures in the presence of favourable conditionos, they can replicate and increase in number by cell division or mitosis (1, 2, 3). For several reasons, it is extremely difficult to carry out pharmacological and biochemical experiments, *In vivo* with human nioplasma (4). Primary

cultures of tumor origin and characteristics; thus, the use of this technique may help in the screening and development of new antineoplastic drugs, and irradiation dose which may contibute to the clinical management of malignant diseases (5, 6, 7).

This work was an attempt to characterize a new cell line of mouse skin melanocarcinoma, induced by topical treatment with 7, 12 Dimethylbenz (a) anthracene (DMBA), a cancer initiator, after application of croton oil, a cancer promoter (8), using simple biological methods.

MATERIALS AND METHODS

Animals and Tumor

Albino Swiss mice three months old were produced and maintained in our animal house. A tumor was induced by painting croton oil (Fluka), and ethanol saturated DMBA (Fluka) on a preshaved area of the mouse skin as described in a previous report (9). A solid tumor was obtained by monthly serial passage o subcutaneous implantation of tumor fragments, over a year, in mice of either sex.

The *In vitro* investigations were performed by modification of the method described by Paul (10). The tumor was carefully removed from the host animal under aseptic conditions and transferred to a sterile petri dish, and rinsed three times with Hanks balanced salt solutiion (HBSS), containing 100 unit/ml penicillin and 50 µg/ml streptomycin, to prevent bacterial growth. Non-cecrotic area of the tumor was transferred to another sterile petri dish containing 5 ml Eagle modified Minimum Essential Medium (MEM, Sigma) enriched with 20% fetal calf serum (FCS, Sigma), and miced finely with two scalpels in opposed directions to chop the tumor bulk in fine fragments (about 1 mm each).

Fragments were placed in a 250 ml conical flask containing about 100 ml of prewarmed 0.25%trypsin (1/250, Sigma) in Ca^{++} and Mg^{++} free HBSS. A sterile silicone-covered magnet was inserted into the flask which was placed over a magnetic stirrer arranged to run fairly slowly. Agter trypsinization, the collected cells were cetrifuged at 1000 rpm for five minutes, the supernatant was discarded. This procedure was repeated three tives to remove any trypsin excess. The settled cells were suspended in MEM enriched with 10% FCS, and the tumor cells were prepared and counted.

Cell Culture

The free cells, which were obtained from the solid tumor, were suspended in growth medium (a modified MEM) containing 10% FCS, and 100 unit/ml penicillin and 50 µg/ml streptomycin. The cells were dislodhed from the bottom of the culture flask (Bibby, England) using 0.25% trypsin. The final cell concentration in the medium was adjusted to be about 300.000 cell/ml, using a haemocytometer for cell counting, and placed in the culture flasks. Epithelial cells, fibroblasts and macrophages were observed in the first week, while only the former remained in the fifth week of the weekly subcultures. Early passages of the subcultures were found to be free of mycoplasma and other bacterial and fungal contaminants.

Histological Examination

Cells were fixed in Clarck's fixative and stained with haematoxylin and eosin. Other cells were examined directly, without staining, by a phase-contrast condenser.

Growth Curve

The fifteenth transfer generation cells were studied to estimate the population doubling time. The culture was initiated with $1x10^5$ cells/ml, after checking the vitality with neutral red and incubated in water jacket humidified CO_2 incubator at 37°C. The cells were grown in about 2 ml of the modified MEM as discribed in the cell culture. The growth curve was obtained by counting the number of cells per dish, every 24 hours.

RESULTS AND DISCUSSION

The mouse tumor cells were epithelial-like cells showing polymorphic shapes, having lrge nuclei and abundant cytoplasm with perinuclear small vacuoles and numerous mitotic figures (Figure 1 &2); these are characteristics of cancer cells (11). The direct observation of the early and late subcultures showed that the cells were rounded, and the time required for the cells to attach to and flatten on the bottom of the flask decreased with later generations; i.e. the time observed for the first and the fifteenth generation was 48 and 24 hours respectively (Figure 2). This reduction in the time for the cells to settle may indicate adaptation of the cells in late passages for the *In vitro* conditions; similar suggestions were made by Jacobson and Papadimitiou (12). Further, the rounded cells were weakly adherent to the bottom of the fask and, unlide the fattened cells, showed atendency to float upon agitating the fask. The nucei were found in the centre of the flattened cells which showed different shapes, e.g. trigonal with large projections and others with denderitic processes. These cells contained predominantly more than one nuceolus (Figure 2); this is consistent with the criteria for cancer cells (11). The growth curve showed that the population doubling time was 36 hours and the exponential phase was reached in about 8 days (Figure 3).

An interesting observation in this study was the formation of communicating bridges between flattened cells (Figure 2); a similar observation was made on colonies of human keratinocytes by Al-Ani (13). He suggested that transport of signals and materials necessary for the metabolism of cells. However, the presence of such bridges between cancer cells *In vitro* may not behave as they do *in vivo*.

In conclusion, this work showed that the *in vitro* culture of the chemically-induced mouse skin melanocarcinoma has been successful, and a characterization of this cell line has been described. However, a follow up work is suggested to elucidate further characteristics of later generations of this cell line enabling a firm establishment. Further, the effect of known and putative antineoplastic drugs can be studied with this cell line.

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Figure1 : A light micrograph of fixed and stained free cancer cells showing large nuclei and different phases of mitosis (arrows).x200

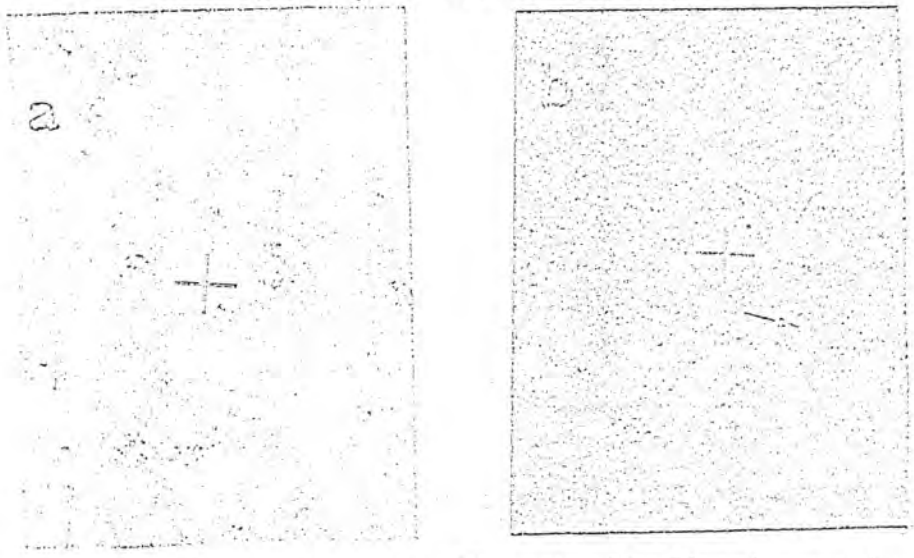
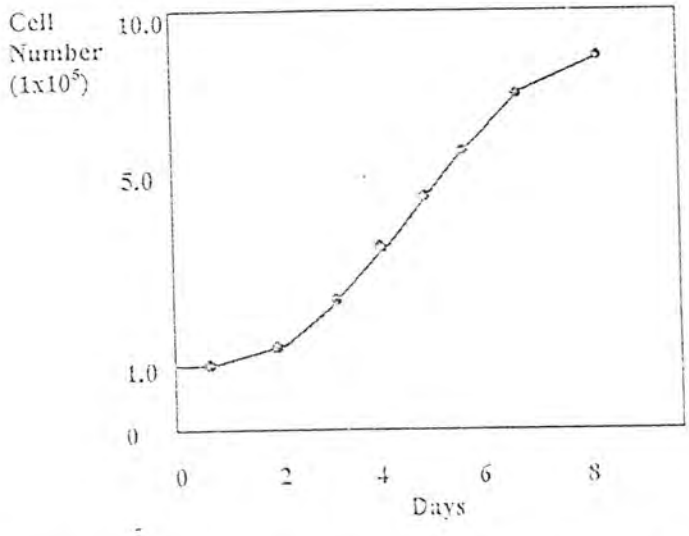


Figure 2: Phase contrast micrograph of free cells of the fifteenth generation subculture a. showing rounded cells, within first 24 hours of the subculture B. showing flattened cells attached to the bottom of the fask. Further, note the comunicating bridges between cells (arrow). x200



Figur 3: Growth curve of mouse tumor cells , cultured in modified MEM enriched with 10% FCS. Each value represents the mean value from triplicates.

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Diurnal Feeding Rythm of Gumbusia Affinis (Baird and Girard) and Aphanius Dispar (Rupple) In Al-Asafiah River. Basrah, Iraq.

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ABSTRACT

The composing periodicities of *Gambusia affinis* and *aphanius dispar* in Al-Asafiah River were carried out over 24 hrs during 14-15 October 1989. Two feeding peads per 24 hrs were found for *G. affinis*. on the other hand one peak was observed for *A. dispar*. There was no correlation detacted between feeding activity and tidal stage in both species. Catches of *G. affinis* were higher during day light than at night, and often correlated with the diyrbak cycles of feeding, while in *A. dispar* catches were greater at night. No correlation was observed between the daily catch and the daily feeding activity.

INTRODUCTION

Fish species differ widely in their daily patterns of feeding activity (1, 2, 3, 4, 5). Many factors have been suggested as reasons for these differences, such as tidal stage (6), temperature (7), daylight period (8, 9), dissolved oxygen (10), kind of food and interspecific competition (11). Diurnal cycles of feeding (i.e. hours of active feeding) are often correlated with locomotry activity in fish (12, 13, 14). This could explain the catch differences during the 24 hours fishing period (12, 8). The feeding habits of *G. affinis* and *A. dispar* inhabiting the Iraqi waters have been studied by (2, 15, 16). However little work has been done on the feeding rythms of these species (16). The aim of the present work is to find out the dailly feeding tythms, the feeding habits and the occurrence of *G. affinis* and *A. dispar* in Al-Asafiah River.

MATERIALS AND METHODS

G. affinis and *A. dispar* were sampled, with a seine net for 24 h period at 2 h intervals. The duration of each sample was 30 minutes. The samples were taken in October 14-15, 1989. The study area was under the influence of the tidal regime of Shatt Al-Arab River. The water temperature at sampling period varied from 26-34°C. The total number of specimens per haul in each period was recorded. Total length, weight and sex for each fish were also recorded. Since the stomachs of both *G. affinis* and *A. dispar* are not well defined, the contents of the entire gut were analysed (17), and the point method (18) was followed.

RESULTS

Compositon of The Diet:

The contents of the alimentry canals of *G. affinis* and *A. dispar* are presented in tables (1) and (2). The main components of the diet in both species were the aquatic insects (Odonata, Hemiptera, Coleptera, Trichoptera and Mosquito larvae), filamenteous algae (chlorophycea), crustaceans, copepods, ostracods and detritius. There were come variation in the quantity and quality of these components in both species. *G. affinis* consumed large quantities of aquatic insects (57, 15) than *A. dispar* (19, 20), while deteritus and crustaceans were more frequently consumed by *A. dispar* than by *G. affinis* as their total diet, respectively. Fish eggs, aquatic plants and unidentified matters were very rare.

Diel Feeding:

There was no correlation detected between feeding intensity and tidal stage in both species.

G. affinis:

The main period of feeding was from 3.00 hr with two peak of feeding, the first at 07.00 hr and the other at 11.00 hr. This was followed by a steady decrease in feeding intensity until late evening (Figure 1). Fresh food items were found in the stomach at most time. The percentage of fish with empty stomach supported this feeding cycle.

A. dispar:

This fish appeared to have one feeding period (Figure 2) IT started from 7.00 hr. and reached its peak at 11.00 hr when their stomachs reached maximum fullness. A sharp decrease in the feeding intensity was obsereved after this time and no fresh food items were fouond in the stomach. As athe percentage of empty stomach increased ther was a similar decrease in the fulness of their stomachs.

Diel Changes in Food Items Composition:

G. affinis :

The major food items (aquatic insects) were abundantin the stomachs during hours of late night and early morning and approximatly the buld at 23.00, 01.00 and 3.00 (Table 1). The filamentous algae was the predominant food in the afternoon and at dusk 13.00-19.00 hr. Detritus and crustaceans occured at noon more than at any other time of the day.

A. dispar :

Detritus were found to be the abundant food during most of the feeding period (Table 2). Aquatic insects larvae and crustacea were the predominant items during mid-night. Filamentous algae wer consumed in large quantities during early evening.

Diel Catch :

Tge catches of *G. affinis* during the daylight were about 2.4 times greater than those at night (Figure 3). On contrast, the catches of *A. dispar* during the night were about 3.2 times greater than those at night (Figure 3). On contrast, the catches of *A. dispar* during the night were about 3.2 times greater than those at daylight.

DISCUSSION

Composition of diet revealed that *G. affinis* was mostly carnivorous, and aquatic insects were eaten more heavily than other food items, whereas *A. dispar* was mostly omnivorous. This finding is in agreement with those of (2, 16). The present study confirms that *G. affinis* and *A. dispar* have certain times of feeding during the day. The feeding activity of botn species in Al-Asafiyah river showed a dialy rhythm. This has no correlation with the tidal stage during the 24 hr period. *G. affinis* showed two peak of feeding activity, while *A. dispar* showed one peak at midday. Most food of *G. affinis* was consumed during daylight, but the feeding was very slow throughout the night, the fresh food items were present at most of the time in the stomachs. Al-Watban (16) found that *G. affinis* showed two peaks of feeding intensity at 16.00 and 24.00. The greatest filling percentage of the stomach of *A. dispar* was during the daylight and the higher percentage of empty stomach occured during the night. It means that *A. dispar* is a day feeder. A similar feeding pattern was found in herring (19) and young white crapple (20). No new food items were taken during the night, this may lead to the conclusion that *A. dispar* feed during the daylight and digest during the night. Figures (1, 2) show that the stomachs of *A. dispar* reached its maximum fullness during feeding period, while the stomachs of *G. affinis* reached nearly half its size of fullness. This result sugests that *A. dispar* is a short time activefeeder in contrast to *G. affinis*, which is a long time active

feeder. There was a shift in the major food items of *G. affinis* during the daily hours as the availability of the chosen preys depends on the zooplankton. This will affect the food composition of the stomachs. The larvae of aquatic insect were the most accessible food items for *G. affinis* for *G. affinis* at night. This coincides with the nocturnal activity of aquatic insect (1, 21), whereas filamentous algae were consumed in large quatitave at daylight. In many fishes diel periodicity of feeding coincide with the drift pattern (13, 14) and locomotry activity (12). In *G. affinis* differences in diel catch have been observed, showed that it was greater during daylight than at night. It was often correlated with the diurnal cycles of feeding. This agreed with the findings of (22, 23). On the other hand, in *A. dispar*, catches during night were 2.3 times higher than during day. He stated that the main causes seem to be daylight avoidance of the net. No correlation is observed between the diel catch and the diel feeding activity of *A. dispar*. The diel distribution of this species may be correlated with the fluctuation of temperature and light intensities. The differences in the feeding cycle of *G. affinis* and *A. dispar* suggest that the photoperiodic factor may initiate the active feeding in case of *A. dispar*, while the decrease of food level in the stomach of *G. affinis* may play as a strongstimulus to initiate feeding. It is clear from the present results that the feeding cycle of these two cohabiting species different type of food at different times of the day. Therefore a reduction of direct competition for food may occur.

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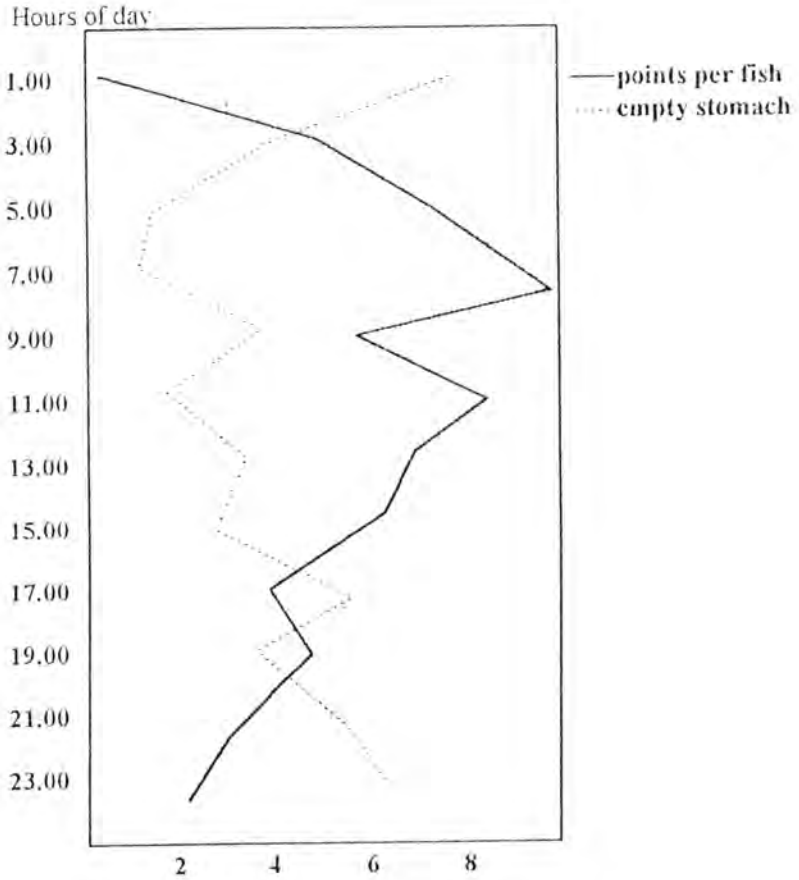
Table 1: Diet composition, a percentage of points per food items and (percentage of occurrence) of *Gambusia affinis* collected during 14-15 October 1989 from Al-Asafia River.

food items	Hours of Day (No. of Fish)											
	1.00 (40)	3.00 (50)	5.00 (38)	7.00 (50)	9.00 (64)	11.00 (51)	13.00 (49)	15.00 (51)	17.00 (52)	19.00 (50)	21.00 (50)	23.00 (50)
aqutic insects	100.0 (100.0)	95.63 (100.0)	86.69 (90.32)	68.14 (80.48)	52.99 (66.66)	35.85 (60.97)	22.12 (20.58)	11.72 (13.5)	23.55 (24.0)	16.86 (26.66)	82.19 (73.91)	88. (9
Filamentous algae	- -	- -	1.79 (3.22)	6.25 (12.19)	17.38 (35.89)	19.44 (34.14)	39.39 (58.82)	43.21 (64.86)	33.33 (56.0)	30.52 (56.6)	9.94 (30.43)	- -
Detritus	- -	2.18 (3.7)	9.71 (9.67)	16.53 (31.7)	18.52 (41.02)	26.51 (43.9)	23.03 (50.0)	26.54 (56.75)	38.66 (60.0)	29.31 (50.0)	5.23 (21.73)	- -
Crustacea	- -	2.18 (3.7)	- -	9.07 (7.31)	1.42 (5.13)	14.39 (14.63)	15.45 (14.7)	15.12 (1.91)	4.44 (4.0)	17.67 (13.33)	2.61 (4.34)	10. (11.
Fish eggs	- -	- -	- -	- -	8.26 (15.38)	- -	- -	- -	- -	- -	- -	- -
Aquatic plants	- -	- -	1.79 (3.33)	- -	- -	- -	- -	3.39 (10.81)	- -	1.60 (3.33)	- -	- -
Unidentified materials	- -	- -	- -	- -	1.42 (2.56)	3.78 (7.31)	- -	- -	- -	4.01 (3.33)	- -	- -

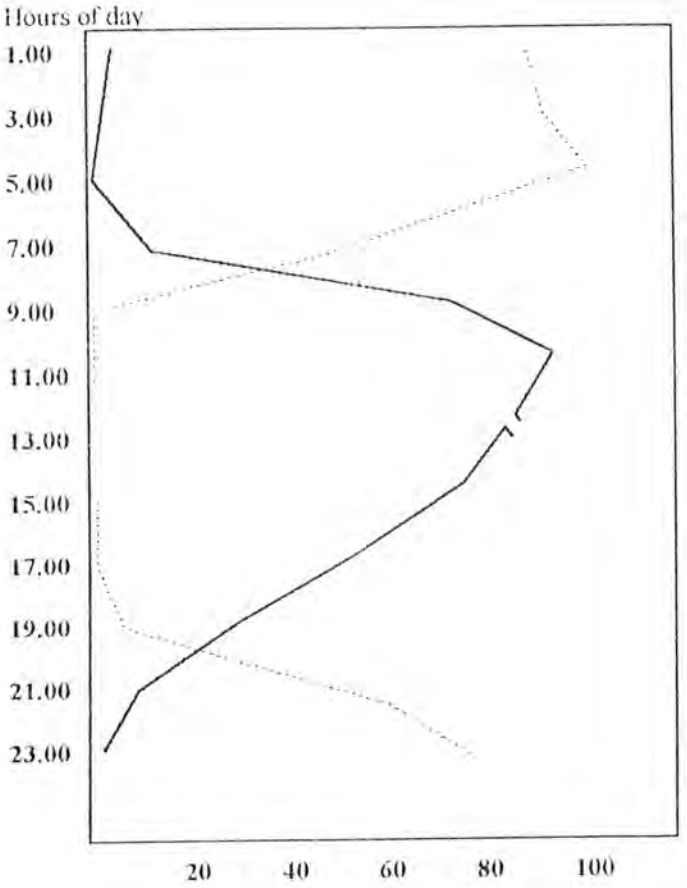
Table 2: Diet cmposition a percentage of points per food items and (percentag of occurrence) of *Aphanius dispar* collected during 14-15 October 1989 from Al-Asafia River

food items	Hours of Day (No. of Fish)											
	1.0 (50)	3.0 (50)	5.0 (27)	7.0 (50)	9.0 (5)	11.0 (3)	13.0 (0)	15.0 (6)	17.0 (48)	19.0 (31)	21.0 (50)	23.0 (50)
aquatic insects	- -	66.66 66.66	- -	24.63 (29.1)	12.19 (20.0)	- -	- -	32.72 (100)	16.93 (72.91)	11.51 (44.82)	15.38 (50.0)	29.2 (60.0)
Filamentous algae	*10.52 (25)	- -	- -	7.97 (20.4)	2.43 (20)	20.0 (100.0)	- -	18.18 (100.0)	27.59 (93.75)	27.96 (82.76)	34.18 (55)	14 (22
Detritus	10.52 (25)	33.33 (33.33)	- -	62.31 (83.3)	13.41 (60)	64.61 (100)	- -	34.54 (100)	44.37 (97.91)	55.59 (100.0)	26.49 (65)	21 (44
Crustacea	78.94 (75)	- -	- -	3.62 (11.1)	71.95 (80)	15.38 (33.33)	- -	10.0 (3.3)	2.18 (14.58)	1.64 (10.34)	11.96 (35)	3 (49
Fish eggs	- -	- -	- -	- -	- -	- -	- -	- -	3.35 (8.33)	1.64 (3.44)	11.96 (10)	- -
Aquatic plants	- -	- -	- -	- -	- -	- -	- -	4.5 (50)	5.54 (29.1)	- -	- -	- -
Unidentified materials	- -	- -	- -	1.44 (4.1)	- -	- -	- -	- -	- -	1.64 (3.44)	- -	- -



% Empty stomach (·····)

Figure 1: The daily variation in the stomach fullnes and percentage of empty stomach of *G. affinis*.



% Empty stomach (·····)

Figure 2: The daily variation in the stomach fullness and the Percentage of empty stomach of *A. dispar*.

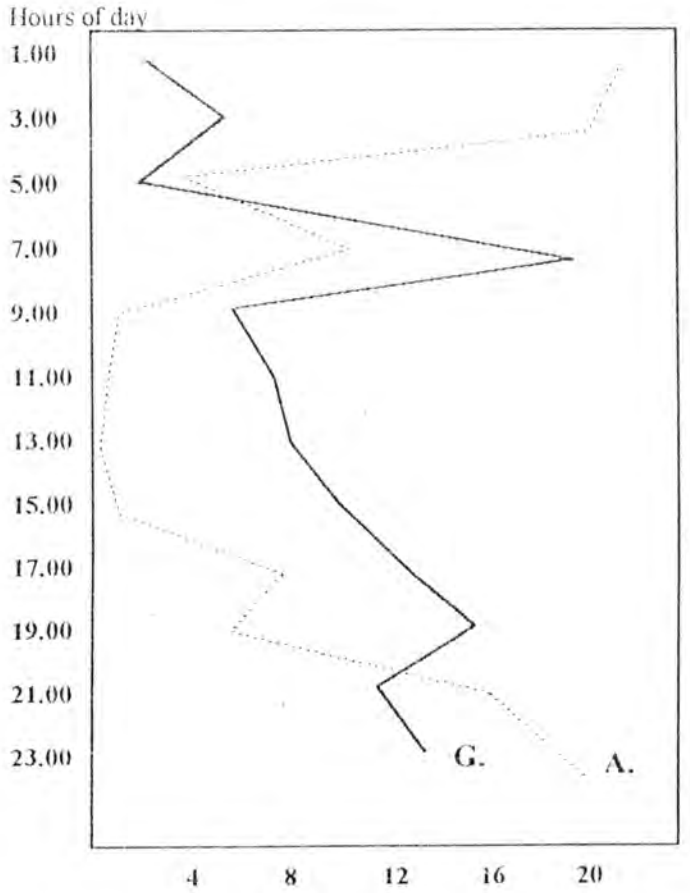


Figure 3: The daily variation in the catch percentage of *G. Affinis* and *A. dispar.*

Genetic Study on The Efficiency of Nitrosoguanidine Induced Baker's Yeast Mutants

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الخلاصة

تم عزل خمس طفرات لخميرة الخبز وعوملت هذه العزلات بالعامل المطفر (النايتروسوكواندين). اظهرت هذه العزلات مقاومة لـ٣-٤ مايكروكرام/مل (سايكلوهكسمايد) بالمقارنة مع السلالة الام . اختيرت هذه العزلات من ناحية الانتاجية والفعالية والنشاط التخميري وانتاج الايثانول حيث اظهرت النتائج ان العزلة R_2 كانت اكثر كفاءة من العزلات الاخرى فقد امتازت بنشاط تخميري قدر بـ (٦٠) سم بعد (٧٠) دقيقة من التخمير وكذلك كانت ذات حيوية قدرت بـ ١٠٠٪ واعطت كمية قليلة من الكحول وكذلك مستوى عالي من المواد الصلبة بالمقارنة مع السلالة الام .

ABSTRACT

Five nitrosogunaidine induced mutants of local baker's yeast were isolated. They were found resistant to 3-4 μg/ml cyloheximide as compared with wild isolates. These mutants were evaluated for their activity, viability, biomass and ethanol production. Results showed that R_2 strain was the most efficient one in all parameters studied. It was characterised as follows: activity of 60 cm after 70 min. of fermentation, 100% viability, low amounts of ethanol, high biomass production and high total soluble solids when compared with wild isolates.

INTRODUCTION

Strains improvement of baker's yeast through genetic technique including selection of naturally occuring variants, selection following mutagenic treatment, and isolation of new hybrids and fusants (1, 2).

In this regard, Genetics has emhasized on the hybridization (3) and protoplast fusion (2). It has been suggested that mutant screening programme of baker's yeast should be directed toward isolation of more efficient mutants in utilizing substrate during fermentation to biomass, quality (1).

Resistant mutant can arise both spontaneously and after mutagenic treatment (2). Nitrosoguanidine (NTG) has been used to induce mutation in several organisms such as *E. coli* and *Schizosaccharomyces pombe* (4). It has also been used to induce cyclohexmide resistant mutants in *Neurospora cressia* and *Saccharomyces cerevisiae* (5).

Cycloheximide, a drug produced by *Striptomyces, griseus* considered to be toxic to some fungi and *protozoa* (6). Yeast species are completely inhibited by very low concentrations (0.2 μg/ml) of cyclohexmide, whereas others are able to grow well at higher concentrations reaching 1000 μg/ml (5, 7).

Cycloheximide resistance is caused by alteration in either one of the ribosomal proteins or permeases of cell (4).

In this study, an attempt was made to search for cycloheximide resistant variants of

locally isolated *S. cerefisiae* Then subjecting the variants to Nitrosoguanidine treatment in order to improve their ability for high activity and viability.

MATERIALS AND METHODS

Yeast isolates: local wild type baker's yeast strain was used through this study as a mother strain (5). It was obtained from the Biology department, Science College, Baghdad University which was identified by Centra albureau Vool Schimmelcattures-Netherland as *Saccharomyces cerevisiae.*

Propagation medium: *Saccharomyces cerevisiae* isolates were isolated and preserved on yeast extract Agar (YEA). Black strap beet molasse liquid medium was used as propagation medium. It composed of ($g.l^{-1}$) molasse 80, Urea 1.5, $MgSO_4.7h_2O$ o.38, $(NH_4)_2SO_4$ 0.66, Thyamine 0.004, Calecium Pentathonate 0.002.

Minimal medium Agar (MMA): Contained (gl^{-1}) glucose 5, $KH2PO_4$ 4, Na_2HPO_4 0.5, NH_4Cl 3.0, NaCl 0.5, $MgSO_4.7H_2O$ 0.04, $CaCl_2.2H_2O$ 0.01, Feelz $^{\wedge}H_2$) 0.008, $ZnSO_4.7H_2O$ 0.001 plus 5 ml/lt of a 16 component vitamin stock solution (8).

Cultural conditions: Yeasts were grown in 1L. flasks containing 250 ml of either (YE) broth or molasse medium. Treatments were incubated in an orbital shaking incubator with 150 rpm at 30°C for 24-28 hr. After incubation, molasse medium was inclated with 5%, of 24hr. old (YE) culture and incubated with shaking and aeriation (1 Lair/1 medium/ 1hr) for 28hr. Yeast biomass was participated by a refergerator centerfugation at 4°C with 5000 rpm for 15 minutes.

Nitrosoguanidine treatment of mutants: Nitrosoguanidine (NTG) was used to treat *S. cerevisiae* mutants to improve growth characteristics and production of baker's yeast. Mutagenesis procedure was performed according to Savchenko and Kapulsevich 1979 (9).

Cycloheximide sensitive and resistant variants: Single colony of the two day old culture of each isolate of *S cerevisiae* was separately streaked on (YEA), and minimal media agar supplemented with 1, 2, 3, 4 or 5 µg/ml Cycloheximide. The plates were incubated at 30°C for 5 days (4).

Determination of yeast viability: Viability of yeast was determined according to Parkkinen et al., 1979 (10) by counting unstained cells.

Determination of yeast activity: Yeast activity was determined according to the AACC method (10-10) (11), which depends on the rising power of the yeast after 0, 20, 30, 40, 50, 60 and 70 minutes of fermintation.

Dry weight and ethanol determination of yeast were determined according to the AOAC method (12) and ethanol was measured according to the methods of analysis of the American society of Brewing Chemists (13).

RESULTS AND DISCUSSION

Sensitivity of *S. cerevisiae* to various concentration of cycloheximide was determined. Results show that *S. cerevisiae* isolates are highly sensitive to cycloheximide (Table 1). All tested colonies were able to grow in presense of 0.5 µg/ml cycloheximde. Only four colonies were found to be sensitive to such concentration. On the other hand, most of colonies were unable to grow on (YEA) supplemented with 1.5 µg/ml cycloheximide. Only two colonies showed resistance to this concentration. These two colonies were treated with nitrosoguanidine. The number of cycloheximide resistants is cassified according to the day of colonies appearance (Table 2). The mutation frequency of cycloheximide resistant mutants was obtained as 10^5 sunvival percentage treated cells with (NTG) (Table 2).

Master plates were used to determine the cycloheximide resistant level. Replica plating on minimal medium (YEA) with/

without cycloheximide, showed that all colonies grew well at 4 μg/ml after 2 days of incubation at 30°C.

Five mutants (R_1, R_2, R_3, R_4 and R_5) were obtained. The R_2, which was resistant to 4 μg/ml of cycloheximide, was found to be auxotrophic to adenine requirement. Two colonies from R_2 mutant and one from R_4 were found to be sensitive to this concentration, but resistant to 3 μg/ml (Table 3).

Results of dough fermentation (raising power) of the cycloheximide resistant mutants are illustrated in Figure (1). Activity of R_2 mutant was higher than other isolates. It was 40 cm after 30 minutes of fermentation which was higher than those of wild type, R_3 and R_4 mutants, even after 50 minutes of fermentation. After 70 minutes of fermentation 60, 50, 48, 40, 36, 30, cm were recorded as activities of R_2, R_4, R, R_3, R_5, R_1 respectively.

Trivedi et al., (14) combined two yeast strains by protoplast fusion to obtain a high activity strain in formulas containing little or no sugar.

Viabilities of tested isolates (Table 4) were ranged between 75.7% (for R_1) to 100% (for R_2). Results show signigicant differences in the vialibity. R_2 mutant gave highest vialibity among other isolates. Table (4) shows that R_2 mutant gives lower amount of ethanol but higher biomass yield as well as more total solids when compared with the mother isolate.

The improved qualities of R_2 is associated with shold increase in cycloheximide resistance (4 μg/ml) in comparison with mother isolate (R) which is sensitive to this concentration.

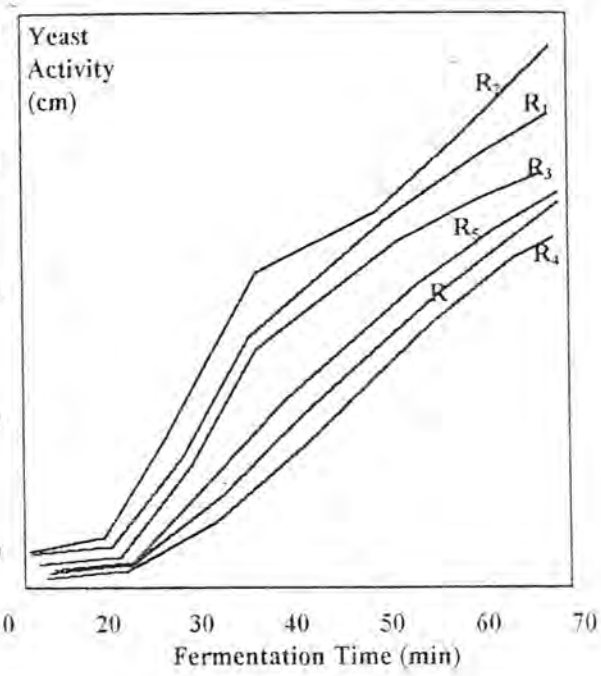

Figure 1: Activity of yeast strains

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Table 1: Cycloheximide tolerance and sensitivity of *S. cerevisiae*

Exp. No.	Cycloheximide Conc. (µg/ml)	*Sensitive Resistant (No of colonies)		Tolerance of cycloheximide %
1	0.5	2	350	0.57
2	0.5	2	310	0.64
3	1.0	250	2	0.80
4	1.0	220	2	0.91
5	1.5	103	1	0.97
6	1.5	120	1	0.84
7	2.0	95	0	0
8	2.0	70	0	0

* Cycloheximide sensitive and resistant colonies wer measured by plate count on YEA medium.

Table 2: Survival percentage of treated cells, total number of mutants appearing after the given incubation period, and mutation frquency of cycloheximide resistant mutants obtained after NTG treatment

Time of treatment with (NTG) minutes	Survival of Isolates (%)	No. of cycloheximide resistant colonies after incubation for 5 days					Mutation frequency mutants for 10^5 survival
		1	2	3	4	5	
0	100	0	0	0	0	0	0
10	55.8	0	1	2	3	1	1.06
20	33.2	0	2	4	3	2	3.3
30	20.0	0	0	1	2	1	2.0
40	0	0	0	0	0	0	0

Table 3: Random spore anallysis of crosses between cycloheximide resistant mutants and wild type

Mutant *S. cerevisiae*	No. of resistant colonies	No of senseitive colonies (4 μg/ml)	% of sensitive colonies
R_1	585	0	0
ada R_2	250	2	1.2
R_3	216	0	0
R_4	105	1	0.95
R_5	75	0	0

Table 4: Microbiological and Biochemical analysis of cycloheximide resistant mutants and auxotrophic mutant induced by NTG of *S. cerevisiae*

Strains	Biomass g/l	Total solids %	Viable cells %	Ethanol production %
Wild type R	43.5	27.8	90.8	0.623
R_1	40.0	20.7	75.7	0.925
ada R_2	48.1	30.0	100.0	0.352
R_3	43.7	26.5	91.2	0.635
R_4	45.8	28.4	94.0	0.522
R_5	42.0	25.3	90.0	0.615

Control of Khapra Beetle *Troqoderma granarium* Everts and Red Flour Beetle *Tribolium Castaneum* (Herbst) by Local Bacterial Isolates

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الخلاصة

ثلاثة انواع من البكتريا عزلت مختبرياً من يرقات البعوض والذباب المنزلي المينة . وقد شخصت هذه البكتريا *Bacillus* , *Kurthia zopfii* (D2) , *Lactobacillus larvicus* (D1) وعلمت بالترقيم الرموز التالية *thuringiensis* (D4b) . لقد تم اختيار مختلف اطوار حشرة خنفساء الخابرا وخنفساء الطحين مع هذه البكتريا وكانت النتائج بان البكتريا D4b اعطت نسبة هلاكات عالية لليرقات والحشرات الكاملة لخنفساء الطحين ونسبة هلاكات متوسطة لخنفساء الخابرا ، اما البكتريا D1 , D2 , فاعطت نسبة هلاكات قليلة على كلتا الحشرتين .

ABSTRACT

Three bacterial strains were isolated localy from dead mosquito and house fly larvae. These identified *lactobacillus larvicus* species designated (D1), *Kurthia zopfii* (D2), and *Bacillus thuringiensis* (D4b). Developmental stages of khapra Beetle, *Trogoderma qranarium* Everts and Red Flour Beetle *Triblium castaneum* (Herbst) were treated with these isolates- *B thuringiensis* (D4b) revealed a high mortality rates larval and adult stages of Red Flour Beetle. But moderate mortality on Khapra Beetle. Stages s. *Kurthia Zopfii* (D2) was slight effect. Against Red Flour Beetle, while *Lactobacillus* (D1) was slightly effective against the two insect species.

INTRODUCTIOM

Many insect pests cause economical loss of seeds stored in siloes and grain houses (1). To aviod the side effect of pesticides and other polutants, a attempts are being done to replace these chemicals gradualy by bacteria throughout the world (2). Several bacterial strains such as *Bacillus thuringiensis* Berliner and *B. Sphericus* Neide were successfuly adapted (2, 3). Applied studies were done, reportedtion improving the killing effect of different kinds of bacteria on some pests. The larvicidal potential of Bacteria is affected by insect habitats and the prevailing environmental conditions (3). Bacterial strains with high killing effect have permitted successful commercial production and use of microbial insecticides (4, 5, 6, 7) *B thuringiensis* was found very effective on stored wheat moth and for the protection of stored weaht production against lepidopterous pests *thuringiensis* H14 was found effective on rice weevil *sitophulus oryzae* (L), on flour betls *Tribolium confusum* Jacquelling and on Indian meal Moth Plodia *interpunctella* (Hubner) for two seasons in grain storage in Kanses (9). In Iraq stored dates in packing houses were protected from the infestation of fig moth insect *Ephestia cautella* (walk) by applying the fungus *Beauveria bassiana* (Vaill) and *B thuringiensis* Berliner (10). Little local information is available on the use of bacteria for the Biological control of invading insevts. The goel of this research to use the Bacteria as biological agent against store insect.

MATERIALS AND METHODS

The bacteria were isolated under aseptic conditions from dead hous fly larr of

fish market collected from Baghdad and dead mosquito larvoe from stock culture of Education college in Baghdad. The isolations were cultured and identified by physiological and biochemical characteristic [1]. These were *Lactobacillus larvicus kuthia zopfii* and *Bacillus thuringiensis*, designated D1, D2 and D4b respectively. The tested insects were obtained from grain store houses and identified according to key Hinton and Corbet key, and Al-Azawi and Mohammed key (12, 13). These were *Trogoderma granarium* Everts (Khapra beetle) and *Tribolium custaneum* (Herbst) (Red Flour beetle). The two beetls were rared in 500 I glass jars size, filled with rice and kept under 27-30 °C and relative humidity of 70-75%. Twenty grams of rice were placed in 100 mm diameter petridishes actively grown culture of each bacterial isolate was diluted to read a count of 10^4 and 10^9 cells/ml using plate method. One ml of each dilution was spread on rice in plates in triplicates, with 30 adults were transfered in each, and the process repated and incubated at 27 °C. The effect of bacteria has followed through developmental stages of these survived adults. Observations were recorded every 24 hours. Negative controls (no bacteria) were designed for treatments of both insects. Number of egg, larrae and adults mortality were recorded.

RESULTS

The use of *Bacillus thuringiensis* (D4b) revealed a moderate mortality rate on larvae and adults of khapra beetles, (3.2, 5.2, and 5.0, 6.3) espectively. But no significant difference observed between mortalities with *Lactobacillus larvicus* of *kurhia zophii* were applied (Table -1). The Red Flour Beetels was also affected by the local isolate of B *thuringiensis* (D4b) and at high concentration (10^4 cells/ml). The effect was found throughout the larvat, pupal and adult stages.

Table 2 presents different bacterial isolates showing different effect on the tested pests. *Lactobacillus* gave a slight mortality rate on khapra beetle and Red Flour beetle (Table 3). *Kurthia zopffii* resulted in a moderate lethal effect on Red Flour betle but not on khapra beetle. The local isolate of B *thuringiensis* (D4b) was highly on Flour beetle, mainly on the larval stage, and was moderately effective on khapra beetle. The used bacterial isolates do not seem to invade egg shell of the two beetles tested.

DISCUSSION

All stages of the two beetles tested were exposed to three bacterial isolates at modified laboratory conditions similar to those exist in silos and grain storage houses. Results clearly demanstrated the effectiveness of *B thuringiensis* (D4b) on Red Flour beetels mainly on the tarval stage and to less extent on khapra beetls.

Similar effected was shown for *B thuringiensis* on the moth fly (Lepidoptera) in wheat stores (8). We recommend the use of our local bacterial isolate *B thuringensis* (D4b) might be developmental dstarivcides against the grain insect *Trogoderma granarium* Everts and *Tribiolium castaneum* (Herbst). The same bacterium has been found effective against fungus gnat insect *Bradysia caprophila* (Diptera, Sciaridae), (14). *B thuringiensis* H 14 was also found to be larvicidal against Mosquito Diptera (3) and against black flies simuliidae (2).

Table 1 Average mortality (%) of khapra Beetle caused by bacterial isolates.

Bacteria	Instar	10^{-9}	10^{-4}	Control
D1	egg	0.0a*	0.0a	0.0a
	larva	2.2a	2.5a	0.0a
	Pupa	0.0a	0.0a	0.0a
	Adult	2.0a	2.3a	0.0a
D2	egg	0.0a	0.0a	0.0a
	larva	1.0a	1.0a	0.0a
	Pupa	0.0a	0.0a	0.0a
	Adult	1.0a	1.0a	0.0a
D4b	egg	0.0	0.0a	0.0a
	larva	5.2b	3.2a	3.2a
	Pupa	0.0a	0.0a	0.0a
	Adult	5.0a	6.3b	0.0a

* Mortality follow with different letter in the same column indicate significant difference in the 0.05 level of confidence.. D1 = *Lactobacillus larvicus*., d2 = *kurthia zophii*., D4b *Bacillus thuringiensis*

Table 2 : Average mortality (%) of Red Flour Beetle caused by bacterial isolates

Bacteria	Instar	10^{-3}	10^{-4}	Control
D1	egg	0.0a*	0.0a	0.0a
	larva	2.5a	3.3a	0.0a
	Pupa	0.0a	0.0a	0.0a
	Adult	3.0a	3.0a	0.0a
D2	egg	0.0a	0.0a	0.0a
	larva	3.9a	4.4a	0.0a
	Pupa	0.0a	0.0a	0.0a
	Adult	3.0a	4.2a	0.0a
D4b	egg	0.0a	0.0a	0.0a
	larva	35.7b	28.6b	0.0a
	Pupa	10 c	12.0c	0.0a
	Adult	18.5d	22.3d	0.0a

* Mortality follow with different letter in the same column indicate significant differenc in the 0.05 level of confidence., D1 = *Lactobacillus larcicus.*, d2 = *kurthia zophii.*, D4b *Bacillus thuringiensis*

Table 3 : Accumallation mortality of khapra beetle and Red Flour Beetle caused by three bacterial isolates

Mortality (%) of khapra Beetle

Bacteria	egg	larva	Pupa	Adult	Total
D1	0.0	2.3	0.0	2.2	4.5
D2	0.0	1.0	0.0	1.0	2.0
D4b	0.0	4.2	0.0	5.1	10.0
Control	0.0	0.0	0.0	1.0	2.0

Mortality (%) of Red Flour Beetle

Bacteria	egg	larva	Pupa	Adult	Total
D1	0.0	2.4	0.0	3.0	5.9
D2	0.0	4.2	0.0	3.6	7.9
D4b	0.0	37.1	11.0	20.4	68.5
Control	0.0	1.0	0.0	1.0	2.0

D1 = Lactobacillus larvicus.
D2 = kurthia zopfii
D4b = Bacillus thuringiensis.

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Effect of Seed Sizes and Planting Menthods on Growth and Yield of Broad Bean *Vicia Faba*

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الخلاصة

أجريت تجربة حقلية في عام ١٩٩٢ في مزرعة خاصة في مدينة الكوفة ، الهدف من التجربة هو لأيجاد تأثير حجم البذور (كبيرة ، متوسطة ، صغيرة) وطريقة لزراعة (مروز ، الواح ، نثراً في خطوط داخل الواح) على نمو وحاصل نبات الباقلاء . الصفات تحت الدراسة شملت طول الساق ، وزن اجزاء المجموع الخضري والجذري الرطب والجاف ونسبة الجذر على المجموع الخضري الجاف في مرحلتي النمو الخضري ومرحلة ١٠٪ تزهير . اشارت النتائج الى اهمية حجم البذور وطريقة الزراعة معا في تقدير الصفات السابقة وكذلك لحاصل النبات الواحد . اعلى حاصل كان مقترناً مع استخدام بذور كبيرة الحجم كما اشارت النتائج الى امكانية استخدام حجم البذور وطريقة الزراعة كوسيلة من الوسائل الحقلية لضمان انتاج نباتات قوية النمو .

ABSTRACT

A field experiment was conducted during 1992 in a private farm in Kufa . The experiment goal is to determine the effect of different size seeds and planting methods on growth criterion of broad bean. Three seed size; small, medium and large and three planting methods; row-drilled, plot-drilled and line-hilled were included in the experiment. Criterion under study include; plant height, fresh and dry vegetative growth portions, root/shoot dry weight ratio during early growing season and at 10% flowering as well as yield per plant. Results indicated that not only seed size is of importance in determining growth criterion but also planting method. Higher yield per plant was associated with larger seed size. Moreover smaller seed size with proper method of planting can be used as a method of improving managment practice in the field for better yield.

INTRODUCTION

Plant growth is a very complex phenomenon. It can be affected by a wide range of enviromental and genetic factors. Factors that have a short term effect especially during the period of seed germination and seedlings development may also have an effect on the subseqent plant growth.

Seed size or weight in small seed crops such as; wheat, barely, sorghum and lettuce has been considred as an important factor in plant growth and development. Several investigators have reported that sowing large seeds had a beneficial effects on growth and total dry matter or yield. Kaufmann and Mcfadden (1) in their study on four barley cultivars found that large seeds had more tillers and higher yield than both medium and small seeds. Kaufmann and

Guitard (2) in a green house study found that large seeded barley produced superior seedling growth, and the size of the first two leaves were larger compared to small seeded ones. Austenson and Walton (3) concluded that sowing large seeds accounted for 2.5 to 4.5 percent increase in wheat yield. Martin and Yarnell (4) reported that seedling size is highly affected by seed size. They have also reported a direct logarithmic relation between seed weight and the dry weight of the seedling. Maranville and Clegg (5) reported no increase in grain yield of sorghum resulted from sowing different size seeds, however kernales weight per 1000 seeds was higher with large compared to small seeds. Large and uniform seedlings were obtained in lettuce by sowing large seeds, WuRRet. al (6). Faba bean has a relatively larger seed size than the previously studied crops. The purpose of this study is to determine the effects of seed sizes and planting methods on growth and development of large seed crop *Vicia faba* .

MATERIALS AND METHODS

An experiment was conducted in 1992 in a private farm in Kufa, to determine the effect of seed size and planting method on growth characteristics of broad bean. Soil texture was sandy.

The experimental block was 99 M long and 33 M wide. Three planting methods; drilling in rows, drilling in plots, hilling in plots and three seed sizes; small, medium and large. The weight per 100 seeds for each size was 75, 107 and 150 grams, respectively. The design of the experiment was split plot in which planting methods were the main plots and seed size was the sub-plots all were randomdly assigned in three replications. The seeds were a local variety of a last year product sown in 29 of November. After sowing, the field was irrigated at four-day intervals for three weeks for better plant estabishment and then at weekley bases. The Duncun Multiple Range test was used (at 0.01level of significance) as a methods of mean sepration whenever treatment effect was evident. Criterion under study were as follow :

Plant height :

Plant height was measured for the soil surface to the top of the plant by using a reguler ruler at weekley intervals and was continued for five weeks.

Plant dry weights :

It included root, shoot and whole plant dry weights. Ten plants were randomly chosen from each treatment and carefully removed from the soil to minimize root losses due to pulling. The samples were brought to the laboroatory for measurment. In the laboratory plant samples were cleaned from soil particales and their shoots and roots were seperated by knife and cut individually into a small pices and placed in a drying pots and put into an oven at 105 C^{o} for 48 hours. The dry weight was recorded by using Mattler 1600 ectrical balance. This was done twice during the growing season; one month after planting and when flowreing were visually possible (at approximately 10% flowering).

Root to shoot ratio :

This criteria was measured by dividing root dry weight over shoot dry weight at each sampling time.

Number of shoots per plant :

Number of shoots per plant where also counted twice during the growing season; one mounth after planting and at 10% flowering .

Yield per plant :

Only yield of green pods of four harvesting periods were considred. Pods less than 5cm in lenght were not considred as a portion of the total yield.

Table 1. Effect of Seed size and Planting method interaction on Plant height (cm) during the vegetative growth period.

	Planting Method			
Sampling Date	Seed size	Row-drilled	Plot-drilled	Line-hilled
29/1/1993	Small	13.21 ABa	11.11 Aa	8.66 Ab
	Medium	11.10 Ba	11.88 Aa	8.88 Aa
	Large	14.99 Aa	12.11 Aa	9.88 Ab
5/2/1993	Small	14.55 Ba	13.20 Ba	10.99 Bc
	Medium	12.66 Cb	14.22 Aa	12.44 Abc
	Large	16.66 Aa	14.77 Ab	12.79 Ac
12/2/1993	Small	15.00 ABa	13.44 Aa	11.55 Ab
	Medium	13.44 Ba	14.88 Aa	12.75 Aa
	Large	16.66 Aa	15.77 Aa	12.99 Ab
19/2/1993	Small	15.00 Ba	16.66 Ba	14.77 Aa
	Medium	15.55 Ba	16.05 ABa	14.44 Aa
	Large	19.10 Aa	17.22 Aa	14.99 Ab
26/2/1993	Small	16.99 Ba	19.88 Aa	16.99 Aa
	Medium	22.22 Aa	17.99 Aa	16.11 Ab
	Large	22.77 Aa	18.77 Aa	17.33 Ab

Z, Capital alphabet for vertical means and small alphabet for horizantal means

Y, Mean seperation in rows and columns is by Duncun multiple range test, 1% level.

Table 2. Effect of seed size and planting method interaction on the vegetative growth dry weights (g); shoot, root and whole plant of broad bean one month after planting.

	Planting Method			
Vegetative growth part	Seed size	Row-drilled	Plot-drilled	Line-hilled
Shoot	Small	1.40 Ba	1.30 Ba	1.49 Ba
	Medium	2.09 Ba	1.12 Ba	1.25 Ba
	Large	2.40 Ab	3.08 Aa	2.98 Aa
Root	Small	0.63 Ba	0.62 Bb	1.15 Ba
	Medium	1.18 Aa	0.51 Bb	1.03 Ba
	Large	1.78 Aa	1.80 Aa	1.88 Aa
Whole Plant	Small	2.03 Ba	1.92 Ba	2.64 Ba
	Medium	3.27 Aa	1.63 Bb	2.28 Bb
	Large	4.18 Aa	4.88 Aa	4.86 Aa

Z; Capital alphabet for vertical means and small alphabet for horizantal means.

Y; Mean seperation in rows and columns is by Duncun multiple range test, 1% level.

Table 3. Effect of seed size and planting method interaction on shoot numbers and root/shoot dry weight ratio of broad bean one month after planting.

	Planting Method			
Criterion	Seed size	Row-drilled	Plot-drilled	Line-hilled
Shoots/ plant	Small	2.33 Ba	2.32 Ba	2.00 Ba
	Medium	4.00 Aa	1.67 Cc	2.64 Ab
	Large	2.75 Cab	3.03 Aa	2.66 Ab
Root/ Shoot Dry weight	Small	0.45 Cb	0.46 Bb	0.76 Ba
	Medium	0.56 Bb	0.45 Bc	0.82 Aa
	Large	0.74 Aa	0.58 Ab	0.63 Cb

Z; Captical alphabet for vertical means and small alphabet for horizantal means.

Y; Mean seperation in rows and columns is by Duncun multiple range test, 1% level.

Table 4. Effect of seed size and planting method interaction on vegetative growth dry weight (g); shoot, root and whole plant of broad bean at 10% flowering

	Planting Method			
Vegetative Growth part	Sed size	Row-drilled	Plot-drilled	Line-hilled
Shoot	Small	1.55 Ba	1.56 Ba	1.80 Ba
	Medium	1.47 Bb	1.90 Bb	3.43 Aa
	Large	3.26 Ab	3.72 Ab	4.40 Aa
Root	Small	0.45 Cab	0.88 Ba	0.64 Ba
	Medium	0.84 Bb	0.91 ABab	1.14 Aa
	Large	1.35 Aa	1.18 Aa	1.22 Aa
Whole plant	Small	1.95 Cb	2.44 Ca	2.44 Ca
	Medium	2.31 Bc	2.81 Bc	4.57 Ba
	Large	4.61 Ac	4.90 Ab	5.62 Aa

Z; Capital alphabet for vertical means and small alphabet for horizantal means.

Y; Seperation in rows and column is by Duncun multiple range test, 1% level.

Table 5. Effect of Seed size and planting method interaction on shoot / plant and root /shoot ratio of broad bean at 10% flowering.

	Planting Method			
Character	Seed size	Row-drilled	Plot-drilled	Line-hilled
Shoot/ plant	Small	4.00 Aa	1.75 Bb	2.00 Ab
	Medium	2.30 Bb	4.55 Aa	2.00 Ab
	Large	4.36 Aa	4.25 Aa	2.60 Ab
Root/ shoot	Small	0.29 Bb	0.56 Aa	0.35 Ab
	Medium	0.75 Aa	0.48 ABab	0.33 Ab
	Large	0.41 Aa	0.32 Bab	0.27 Ab

Z; Captical alphabet for vertical means and small alphabet for horizantal means.

Y; Mean seperation in rows and columns is by Duncun multiple range test, 1% level.

Table 6. Effect of seed size on, dry weight of shoot and totals one month after planting and root dry weight at 10% flowerind as well as green pod yield per plant of broad bean.

	Dry weight (g) one month after planting		Root dry weight at 10% flowering	Green pod yield / plant
Seed size	Total	Shoot	(g)	(g)
Small	2.26 B	1.39 C	0.66 C	440.11 C
Medium	2.62 B	1.54 B	0.96 B	556.22 B
Large	4.61 A	2.82 A	1.24 A	759.55 A

Z; Means did not share the same letter are different at 1% probability level (Duncun multiple range test).

Table 7. Effect of seed size and planting method interaction on green pod/plant (g) yield of broad bean.

	Planting Method		
Seed size	Row-drilled	Plot-drilled	Line-hilled
Small	526.00 Ca	436.33 Bb	355.00 CC
Medium	665.66 Ba	415.00 Bc	588.00 Bb
Large	744.00 Ab	850.00 Aa	684.66 Ac

Z; capital alphabet for vertical means and small alphabet for horizantal means.
Y; Mean seperation in rows and columns is by Duncun multiple range test, 1% level.

RESULTS AND DISCUSSION

Plant Height

The design of the experiment revealed a significant interaction (at 0.01 level of significance) between seed size and planting method for testing plant height. This interaction continued to appear whenever data of plant height were analyzed from the first week of sampling untill last (Table 1). It can be seen from this table that plant heights were supperior when large seed size was used at the row-drilled and polt-drilled planting methods when compared to that of line-hilled planting method. Moreover although no significant differences in heights of plant from the polt-drilled and row-drilled planting methods, except for the second week of sampling at all seed sizes, plants at the row-drilled planting method tended to be taller than those of the plot-drilled planting (same table). This indicates that not only seed size is of important in determining plant height, but also planting method. The differences in the response when both factors were considred may be related to differing planting method which can cause a change in the microenviroment surrounding the resulted seedlings. And as a result planting method which allows favorable enviroment in plant vacinity along with the proper seed had a good size plants. This results is in agreement with work done by Hunter abd Kannenberg (7), Wurr et al (8), and Al-Haddi and Kasrawi (9).

Plant dry weights

There was a significant interaction (at 0.01 level of significance) between seed size and planting method for testing dry weights of;, root, shoot and the whole plant one month after planting and at 10% flowering (Table 2 and 4), respectively. It can be seen from table 2 that large seed size had consistantly heavier shoot dry weight than both medium and small sized seed at all planting methods included in the experiment. However medium and large seed size treatments produced heavier root dry weight at the row-drilled planting method, while heavier dry weight of roots were produced only at the larger seed sized for the polt-drilled and line-hilled planting methods. Whols plant dry weight was heavier at medium and large seed size at the row-drilled planting method. While at the plot-drilled and line-hilled planting methods, whole plant dry weight was only heavier at the large seed size. Table (6) shows that seed size has a great influence on whole plant and shoot dry weights, one month after planting and on root dry weight at 10% flowering. Generally all cases dry weight resulted from large seeds was supperior to that of medium and small sized seed, respectively. At 10% flowering (Table 4) shows that larger seed size had consistantly higher; root shoot and whole plant dry weights at the row-drilled planting method. Althought; root, shoot and total dry weights were heaviest for the larger seed size treatment at all planting methods it was found that dry weights of roots and shoots resulted

from medium and larger seed treatments at the plot-drilled and line hilled planting methods were inconsistant if compared to results obtained from small sized seed treatment (same table).

The heavy root, shoot and whole plant dry weights obtained from the use of larger seeds at different planting methods one month after planting and at 10% flowering suggests that large seeds can be used as a useful practice to produce strong and efficent plant. This is possibly due to the fact that large seed size has more reserve foods in their cotyleadons compared to small seeds. And for this reason larger seeds are able to nourish, maintain and develop a well and healthy seedlings that are able to endure the erratic nature of the enviromental factors. This results agreed with results obtained by Haskins and Gross (10) on sorghum and sweet clover and with Burris et. al (1) on soybeans. The inconsistancy in root and shoot dry weights at 10% flowering (Table 4) may be related to differing planting method or to the stage of plant development in which charbohydrates was involving in flower formation (Edmond et. al (12) and Tayler and Weber (13).

Number of Shoots Per Plant and Root / Shoot Dry Weight Ratio

Results of the interaction between seed size and planting method for testing number of shoot per plant and root / shoot dry weight ratio for one month after planting and at 10% flowering are presented in table 3 and 5, respectively. Table 3 shows that shoots per plant were higher with medium sized seeds at row-drilled planting method. And for the larger and or medium sized seeds at plot-drilled and line-hilled planting method, respectively. At 10% flowering, however, larger seeds produced more shoots per plant even at the row-drilled planting method.

This indicates the necessisty for the use of larger seeds in planting. The lower number of shoots per plant obtained at the medium sized seeds of the row-drilled, at 10% flowering (Table 5), is not odd. This is because number of shoots per plant is genetically fixed, Stoskopf (14). And potential branching can be affected or supperssed by unfavorable weather conditions it is possible thus shoots developed at the early stages of plant development may die under unfavorable weather condition.

Root / shoot dry weight ratio was highest at the larger seed size of row-drilled and plot-drilled planting methods (Table 3) and for the medium sized seeds at line-hilled planting method. At 10% flowering (Table 5) higher root / shoot dry weight ratios were obtained at the large and small seed sized of the row-drilled planting. And at the large and medium sized seeds at the plot-drilled planting method. Since root / shoot dry weight is a measure to evaluate the capacity of root system to sustain shoot growth, Stoskopf (15). And roots play a major role in securing sufficient water and nutrients to establish a strong plant. This suggests the possibility of using different seed sized for different planting method. And to the importance of the use of larger seeds in planting. This result is in greement with WuRR et. al (6, 16).

Yield Per Plant

Yield per plant was increased significantly (at 0.01 level of significance) with the increase in seed size (table 6). The interaction between seed size and planting method (table 7) indicated that large seed planted at plot-drilled produced significantly higher yield per plant compared to row-drilled or to line-hilled planting method. Small and medium sized seds were lower in yield per plant at the plot-drilled or line-hilled planting method compared to row-drilled planting. These results suggest that selection for seed size and planting method can optimize yield per plant. For example large sized seeds at all planting method were superior in yield per plant, but small and medium sized seeds had its best pereformnace at the row-drilled planting method.

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Phylloplane Fungal Populations and Their Celluiase Activity on Three Plant Species of Myritaceae.

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الخلاصة

تم عزل عشرة انواع من الفطريات ومن ثلاثة انواع نباتية تابعة للعائلة الآسية وشملت فرشاة البطل – اليوكالبتوس والآس . وتم مقارنة الجماعة السكانية للفطريات على النباتات المدروسة باستخدام معامل التشابهة والاختلاف ، واعزيت الاختلافات الى طبيعة كل نبات وقد حدد النشاط الانزيمي السيليوزي لكل من الفطريات المعزولة حيث اتضح ان هذه الفطريات تمتلك نشاط انزيمي يختلف من نوع الى اخر وقد اظهر الفطر *Penicillium* نشاطاً انزيمياً عالياً B-glucosidase . بينما ابدى الفطر *Oryzae Nigrospora* فعالية عالية للأنزيم *Exoglucanase* بينما الفطر *Trichoderma Viridi* اظهر نشاطاً عالياً للأنزيم *Endoglucanase* .

ABSTRACT

Ten fungal species were recovered from three plants of the family Myritaceae including, *Calistimon viminalis, Eucalyptus camaldulensis* and *Myritus communis.* Similarity index and coefficient of variation were used to compare between the fungal populations of the studied plants. Variations in fungi was related to the differences in plant nature. Enzymatic activity of cellulase was determined for each fungal species. It appeared that fungi on these plants were varied in their cellulase activity and type. Highest activity of B-glucosidase was found in *Penicillium* sp. while exoelucanase in *Nigrospora oryza* and endoglucanase in the fungus *Trichoderma viridi.*

INTRODUCTION

Several studies have been carried out on the mycota of higher plants (4). Despite of the role of fungi on living plants has not been verified, however, a mutual relationship between fungi and plants was suggested (13). Recently an increased attention has been paid to fungi of certain plant families (9, 12). It has been also documented that fungi produce different enzymes particularly those live on plant leaves under tress conditions (5) .

The present study attempte to examine the fungal populations on three plant species of Myritaceae in southern Iraq. Meantime the cellulolytic enzyme activity of the recovered fungi was tested.

MATERIALS AND METHODES

Three plant species including *Calistimon viminalis* (Sol. Ex Gaerth.) Don., *Eucalyptus camaldulensis* Dehnb. And *Myritus communis* L. were choosen for this study. These palnts are commonly growing in southern region of Iraq. Three plants of each species were marked for a regular sampling on intervals, ten leaves were randomly gathered from each plant, placed in plastic bags and processed in the laboratory. Leaves

samples were cut into small pieces (0.5 cm long) and washed several times in a sartorious containers with sterilized distilled water as desicribed by (11). Five pieces of leaves were plated on a petri dish containing czapex agar medium. Three replicates were made and incubated at 20 ± 2 C then surveyed for any fungal growth. Fungal data analysis was done by counting the total colonies on media.

A comparison of the fungal populations of the three plant species was determined using the simillarity index and coefficient of variation of fungal populations according to (7). Frequency and occurrence percentages of fungal species were calculated according to (10). The cellulolytic enzyme activity of the isolated fungal taxa was tested including ; B-glucosidase, endoglucanase and exoglucanase following (8) :

1- B-glucosidase : Fungal culturees were made in conical flasks containing mandles medium (8) to which filter papers of Whatman No.1 (0.62 g) was added as cellulose source. One ml of P-Nitrophenyl-B-glucopyranoside was prepared (14) then 1 ml of the enzyme was added to culture in test tube, places in water bath at 45 C for 30 min, 2ml of 1M Na_2CO_3 was added. The enzymatic activity was measured in spectrophotometer at 410 nm.

2- Endoglucanase : 4.5 ml of soluble cellulose (CMC-Na) was added to the fungal culture filterate in test tube, placed in water bath at 45 C for 1 hr, then an indicator DNS (Dinitrosalicylic acid) was added. Enzyme activity was measured by spectrophotometer at 550 nm.

3- Exoglucanase : 2 ml of Na-citrate buffer solution at pH 5 with 6 cm filter paper, then 2 ml of the fungal enzyme filterate was added in a test tube, placed in water bath at 45 C, for 24 hr, 3 ml of DNS was added. Enzyme activity was determined by spectrophotometer at 550 nm. In all cases standerd curve was made. B-glucosidase and endoglucanase were expressed as 1 umol P-nitrophenol (min) $(ml)^{-1}$ enzyme activity while exoglucanase was expressed as 1 umol reducing sugar (hr) $(ml)^{-1}$.

RESULTS

Ten fungal species among 637 isolates were recovered from leaves of the three plants (Table 1). The recovery of fungi from each plant was varied according to the time of collection. Amongst the fungi, *Alternaria alternate* and *Penicillium* sp. Were the most species common on leaves over all the collections. Along with these two fungi, *Aspergillus niger, Cladosporium cladosporoides* and *Ulocladium artum* were found on the three plants. While *Nigrospora oryza* was the only fungus isolated from *M. Communis* and *Trichderma viridi* from *C. Viminalis*. The frequency percentages of fungal isolated were found to be as ; 38% on *M. Communis*, 33% on *C. Viminalis* and 29% on *E. Camaldulensis* (Table 2). *Alternaria alternata* represents the highest frequency (36, 28 and 28%) on *M.communis, E. Camaldulensis* and *C. Viminalis* respectivly. The occurrence percentages of fungi are given in Table 2. Highest occurrence (100%) was accounted for *A. Alternata* and *Penicillium* sp. And for *C. Sladosporoides* on *C. Viminalis*.

A comparison of the fungal populations on the three plants, using similarity index, showed that similarity was higher (80%0 for fungi on *M. Communis* with *C. Viminalis* and lower (40%) for fungi on *E. Camaldulensis* with the two plants (Fig. 1) . Coefficient of variation values indicated that fungal populations were highly variable between *M. communis* and *C. Viminalis* (V = 2.7) and less variable (V = 1.3) between other plants combinations.

Table 3. Shows the activity of the three enzymes of the cellulase complex produced by the isolated ten fungal species. It appeared that the activity of B-glucosidase was higher (30 IU per min) fpr *Penicillium* sp. And lower (2.2 IU) for *Ulocladium artum*. While the activity of endoglucanase was higher (360 IU per min) for *T. Viridi*.

Among the these fungi *N. Oryza* revealed the highest activity of exoglucanase (287 IU per hr), where as two species (*Phoma* sp. And *U. Artum*) showed no enzymatic activity of exoglucanase.

DISCUSSION

Although the isolated fungi from the three plant species, in this study, have been commonly isolated from various living and dead plants in different habitats (4), the most common dpecies however, are *A. Alternata, C. Cladosporoides, Penicillium* sp. and *U. artum*. These fungi were frequently reported from desert ecosystems (1). These fungi seem to be adapted to high temperature habitats. It has been observed that the fungal populations of the studied plants are somehow similar. However, fungi on *Calistimon vimivalis* and *Myritus communis* are less varied. While the fungi of *Eucalyptus camaldulensis* slightly differ from the other two plant species. Such variation is mainly based on the total fungal isolates using coefficient of variation index. The similarity and dissimilarity in fungal populations among these plants can be related to the physical or chemical nature. Leaves surfaces at higher plants are varied in their morphology and anatomy (2). It has also been documented that leachates produced by leaves have an effects on the mycota associated with plants in general (6). Morover, the low fungal isolates number recovered from *E. Camaldulensis* might be related to the production of specific chemical compounds that would act as an antifungal agents. This assumption needs to be verified by a further experimentation.

Recently, Petrini (12) has pointed out that the fungal populations present on different species of plants of the family Ericaceae are similar. Other studies (3, 9, 10, 11) were also showed that the fungal populations are similar on plant species belonging to the same families (egs. Chenopodiaceae, Gramineae and Zygophylaceae). The present data are in agreement with the findings of other works (9, 12).

The enzymatic assay revealed that these fungi produce different levels and types of enzymes cellulase. This nature of enzymatic productivity perhaps allowed these fungi to invade and dominate the leaves surfaces of plants. The most predominant fungus was *A. alternate*. Gessner (5) has found that this fungus produces a variety of enzymes besides to the cellulase. This fungal species has been also reported from other plants as a dominant fungus (3, 10). High activity of B-glucosidase in *Penicillium* sp. may enable this fungus to be highly occurred (100%) on these plants. The high activity of exoglucanase in the species *N. Oryza, A. Niger* and *D. Hawiansis* as well as the high activity of endoglucanase in other specie s namely *T. Viridi* and *Penicillium* may allow these fungi to occur on plant leaves.

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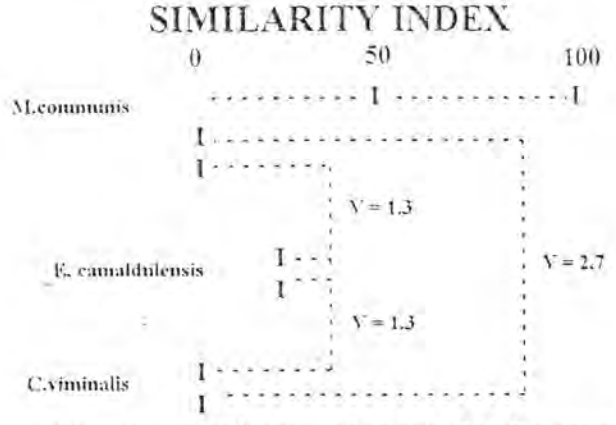

Figure1, Similarity index and Coefficient of variation (V) of fungal populations on three plants.

Table 1. Recovery of ten fungal taxa from three plants according to four collecting times.

Species	M.communis				E.camaldulensis				C.viminalis			
	1	2	3	4	1	2	3	4	1	2	3	4
Alternaria alternata(Fr.) Keissler	+	+	+	+	+	+	+	+	+	+	+	-
A.citri Ellis et Pierce	+	+	-	+	-	-	-	-	-	-	-	+
Aspergillus niger van Tieghem	+	-	-	-	+	-	-	-	-	-	-	-
Cladosporium cladosporoides (Fr.) Vries	-	-	-	-	+	-	+	+	-	-	+	-
Drechslera hawiansis	-	-	-	-	-	-	-	-	-	-	-	-
Nigrospora oryzae	-	-	-	-	-	-	-	-	-	+	+	-
Penicillium sp.	-	+	+	-	-		+	+	-	-	-	-
Phoma sp.	-	-	-	-	-	-	-	-	-	-	+	-
Ulocladium atrum	-	-	-	-	-	-	-	+	-	-	-	-
Trichoderma viridi Pers. & Gary	-	-	-	-	-	-	-	-	-	-	-	-

* Number represents time of collection : 1= November, 2 = January, 3= March, 4 = May.
** += present, - = absent of fungi

Table 2. Total isolates, frequency and occurrence percentages of ten fungi on leaves of three plants.

Species	M.communis			E.camaldulensis			C.viminalis		
	T	F	O	T	F	O	T	F	O
A. Alternata	86	36	100	50	28	100	51	28	100
A. Citri	18	5	25	8	4	50	0	0	0
Asp. Niger	28	12	25	11	6	25	47	22	25
C.cladosporo-ides	36	15	75	22	12	50	56	27	100
D.hawiansis	3	2	25	3	1	25	0	0	0
N. Oryzae	0	0	0	5	3	25	0	0	0
Penicillium sp.	33	13	100	31	16	100	50	24	100
Phoma sp.	5	21	25	10	6	25	0	0	0
U. Atrun	32	13	100	44	24	75	7	3	50
T. Viridi	6	3	25	0	0	0	0	0	0
Total	242	38	-	184	29	-	211	33	-

T = Total isolates, F= frequency percentages, O=occurrence porcentages of isolates.

Table 3. Enzymatic activity of cellules in culture filterate of ten fungi isolated from leaves of three plants species (Myritaceae).

Species	Activity (IU)		
	B-glucosidase	Endoglucanase	Exoglucanase
A. Alternata	18.5	45.9	36.7
A. Citri	5.6	43.5	40.5
Asp. Niger	8.1	58.3	199.0
C. Cladosporoides	2.6	15.3	50.4
N. Oryzae	4.8	90.0	287.0
D. Hawlansis	8.7	103.8	195.0
Penicillium sp.	30.0	133.0	7.0
Phoma sp.	3.5	76.6	0.0
U. Atrum	2.2	25.0	0.0
T. viridi	3.4	360.0	1.0

IU = International unit (for exoglucanase activity measured as umol per ml/hr , while other two enzymes activity as umol per ml/min.).

Alteration in Enzyme Activities and Protein Profiles in the Tissues of Mosquitofish *Gambusia affinis* (Baird and Girard) Exposed to Three Concentrations of Cadmium

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الخلاصة

تم تعريض سمكة البعوض Gambusia affinis الى ٢ ، ٤ ، ٨ ملغم كادميوم /لتر لفترة ثمانية ايام ، ودرس تأثير الكادميوم على نشاط الانزيمات وطرز البروتينات في مستخلص كل من القناة الهضمية والفضلات ، إنخفض نشاط الفوسفاتيز القاعدي عند التركيز الأوطأ ثم ازداد تدريجيا عند التعريض الى التراكيز الاعلى ، بينما إنخفض نشاط الفوسفاتيز الحامضي عند جميع التراكيز . كما أظهر الانزيم الناقل للأمين GPT زيادة في نشاطه عند التعريض الى التراكيز العالية من الكادميوم في القناة الهضمية دون الفضلات . أما الأنزيم الناقل للأمين GOT فلم يظهر تغيرا يذكر . سبب الكادميوم تغيرات مهمة في المكونات البروتينية لمستخلص الأنسجة بأستعمال تقنية الترحيل الكهربائي .

ABSTRACT

Gambusia affinis fish were exposed to concentrations of 2,4,8 mg. Cd/l for 8 days . Enzyme activities and protein components of alimentary canal and muscles were studied for the effect of cadmium . Alkaline phosphatase activity decreased at lowest concentration of cadmium followed by gradual increase at other higher concentrations . Acid phosphatase activity increased at the higher concentration in alimentary canal but not in muscle , whereas GOT activity did not reveal significant changes . Cadmium caused significant changes in the protein components of tissues extracts by electrophoresis .

INTRODUCTION

Cadmium is one of the toxic metals besides other metals , which interact with metabolism of divalent essential metals at different subcellular levels , and accumulated in animal tissues (1,2,3,4).

Biochemical parameters studied so far for the effects of cadmium in different species of fish are mainley focused on the synthesis and properties of cadmium-binding proteins (5,6,7) , with little information on other soluble protein components (1,8).

Gambusia affinis is an ecologically important and widely distributed fish. Althought it is not used as edible food, it is important for point of view of controlling diseases such as Malaria and Bilharzia (9) . Several studies have been carried out on the toxicity of cadmium on G. affinis (9, 10), but little about the effect of the metal at molecular levels .

The aim of the present study is to investigate the effects of different concentrations of cadmium on some enzymes activities and electrophoretic patterns of protein of alimentary canal and muscles of G. affinis .

Materials and Methods

The specimens of mosquitofish *Gambusia affinis* were collected during October 1993 from sweage disposal canal in Altaamem area (popular area) at Al-Ramadi province. The fishes were transferred to the laboratory (25° C ±2) and kept in a plastic containers containing 5 litters of declorinated tap water. Thirty individuals (medium size) were kept in each container. The water of containers was replaced every second day. No food was supplied.

Cadmium was added in the form of $CdCl_2$, one week after sample collection. Contron animals were kept under the same conditions but excluded the addition of metal.

Alimentary canal and muscles from the control and exposed animals were removed, weighted and homogenized in 10 and 5 volumes respectively of child 0.1M Tris-Hcl buffer, pH, 7.2, using teflon pestle homogenizer. Pools of at least 20 organs were used for each homogenation.

Electrophoresis was carried out in HSI slab gel electrophoresis unit model SE 500 connected to 2103 LKB power supply. The slab of polyacrylamide gel (7.5%) were prepared in the laboratory (11). The omongenate was centrifuge at 2.000 xg for 10 minutes using Eppendorf centrifuge model 5412. To a small volume of supernatant an equal volume of sample buffer was added and 50µl applied in each well. The other electrophoretic steps and staining of the gel were carried out as described by Thaker and Haritos (11). Assays of phosphatases and transaminases were carried out according to King and Armstrong (12) and Reitman and Frankel (13) respectively.

RESULTS

The results of the experiments on enzyme activities in alimentary canal and muscles of *G. affinis* exposed to 2,4 and 8 mg Cd/l of water for 8 days are shown in Figure 1 and 2. Alkaline phosphatase activity was found to decrease to 85% at 2 mg Cd/l as compared to 100% of control activity in both tissues, followed by a gradual increase at higher concentrations to reach 128% and 142% in alimentary canal and muscles respectively. In contrast, acid phosphatase activity was found to decrease to 47% at different concentrations of cadmium in alimentary canal, while flactuated profile was obtained in muscles wxtracts.

Glutamate purovate transaminase (GPT) activity was found to increase to 125% (approximately) at 4 and 8 mg Cd/l in alimentary canal and decrease to 84% at 4 mg Cd/l in muscles. Glutmate oxaloacetate transaminaes (GOT) activity does not revealed significant changes at differen concentrations in both tissres except at 2mg Cd/l the activity was found to decrease to 90% in alimentary canal extracts.

By comparison of the electrophoretic profiles of animals exposed to cadmium (lanes 2,3 and 4 in Figures 3 and 4) and control animals (lanes 1 in Figures 3 and 4), several changes wer observed at different molecular weights. First, in alimentary canal (Figure 3). One band (indicated by a) was induced at different concentration of cadmium (lanes 2, 3 and 4). Two more bands (indicated by b1 and b2) were induced by cadmiumat concentrations of 4 and 8 mg Cd/l (lanes 3 and 4 respectively). One more specific band (indicated by Ca) appeared and other one (indicated by e) disappeared at 8 mg Cd/l (lane 4). Second, in muscles (Figure 4), cadmium was also found to cause chage(s) in the electrophoretic profile of protein. Two bands (indicated by a1 and a2) appeared at all concentrations of exposure (lanes 2, 3 and 4). One more band (indicated by b) at the concentrations of 4 and 8 mg Cd/l (lanes 3 and 4). On the other hand two bands (indicated by C1 and C2) disappeared at the concentrations of 4 and 8 mg Cd/l (lanes 3 and 4 respectively) and one band (indicated by d) at 8 mg Cd/l.

DISCUSSION

In the present investigation, cadmium was provided through the surrounding medium as this has been reported to be an effective way of cadmiumuptake for other species of fishes *Mugil cephalus* (1) and *Fundulus heteroclitus* (8).

Alkaline phosphatase is a metalloenzyme with an active center containig zinc (14). This metal might be able to be replaced by cadmium causing enzyme inhibition as in E. Coli (15, 16). It has reported that, in scallop *Mizuhopecten yessonsis* exposed to cadmium (0.5 mg Cd/l) an initial inhibition at 14 days was followed by activation at 30 days and return to normal level at 60 days (17), while it has shown that, alkaline phosphatase activity in rat kidney brush border increases two folds after injection with high load of cadmium (18). Our results have shown that the activity in the alimentary canal and muscles revealed an initial decrease followed by an increase upon increasing in cadmium concentration in the ambient environment.

Acid phosphatase contains Mn (19). This metal may be replaced by cadmium that results in the inhibition of enzyme activity. This explanation is supported by Evtushenko et al. (17) when the scallop *Mizuhopecten yessoensis* was used, and Hilmy et al. (1) has shown that, the activity of this enzyme decreased in heart, liver and gill but increased in the serum of the fish *Mugil cephalu* during exposure to cadmium. The results obtained in the present study suggests that cadmium exposure exerts its inhibiting effect on acid phosphatase activity of the alimentary canal and muscles extracts.

Transaminases (GOT and GPT) have been recognised to be amongest the enxymes of dignostic importance (beside phophatases) in mammals and the attention has been focused on the changes in activities in serum in case of liver damage which shows a marked increase (20). Hilmy et al. (1) has reported that, the activity of the two enxymes (GOT and GPT) were increased in heart, gill and serum and decreased in the liver of fish *Mugil cephalus* during exposure to 28 md Cd/l for four days. In *Gambusia affinis* (present study), cadmium was found to cause changes in the activity of transaminases in the alimentary and not of the muscles. This results suggested that the changes in the activity observed might caused by damage in the alimentary canal tissues and resistance of the muscles tissues to the metal.

We may conclude that, cadmium has a significant effect on the expression of protein resolved by polacrylamide gel electrophoresis in extracts of *G. affinis*. Some of the electrophoretic bands appeared at different concentrations of cadmium, which are likely indicate the induction of cadmium-binding protein(s), such protein(s) have been induced in fish *Pimephales promelas* (5) and *Salmo gairdneri* (6) after exposur to cadmium. The differential responses (appearance or disappearance) of other bands also were observed upon the exposure to a specific concentrations of metal, also were reported by Thaker and Haritos (11, 12) in shrimp *Callianassa tyrrhena* after exposure to casmium or mercury, and in the snails *Melanopsis nodusa* (unpublished results).

By evaluation of the results obtained in the present study it appears that, induction or degredation took place in all protein componenets of alimentary canal and muscles of *G. affinis*. Further studies in this field is required that might help in the development of much needed sensitive and reliable bioassays for metal toxicity based on protein expression.

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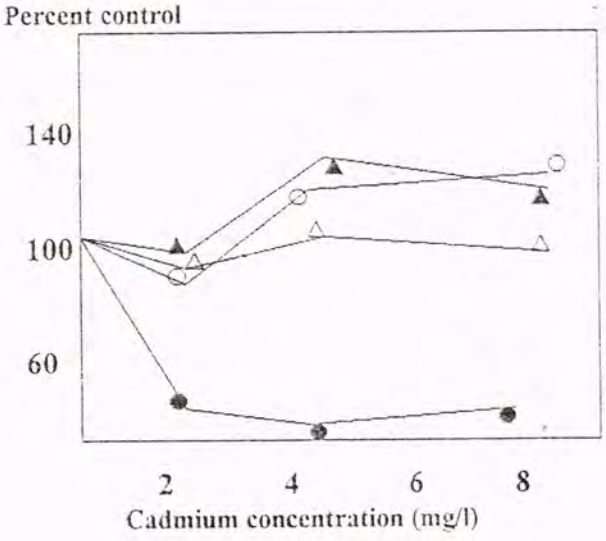

Figure 1: Effects of cadmium on the activity of alkaline phosphatase ○——○, acid phosphatase ●——●, GOT △——△ and Gpt ▲——▲ in alimentary canal extracts of fish were exposed to 2, 4 and 8mg Cd/l of water for 8 days.

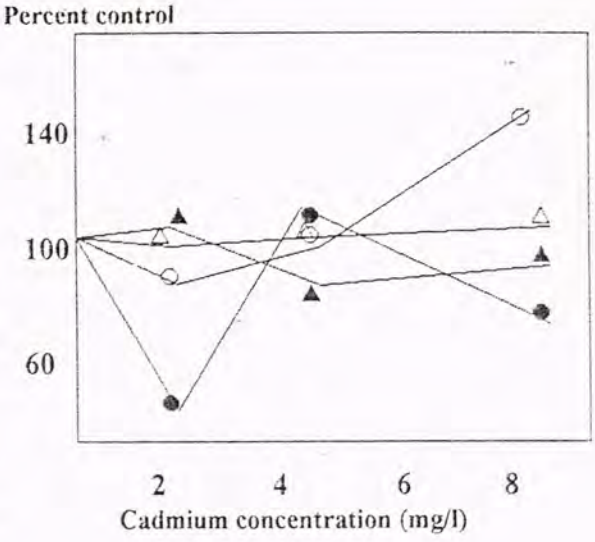

Figure 2: Effects of cadmium on the activity of alkaline phosphatase ○——○, acid phosphatase ●——●, GOT △——△ and Gpt ▲——▲ in muscles extracts of fish were exposed to 2, 4 and 8 mg Cd/l of water for 8 days.

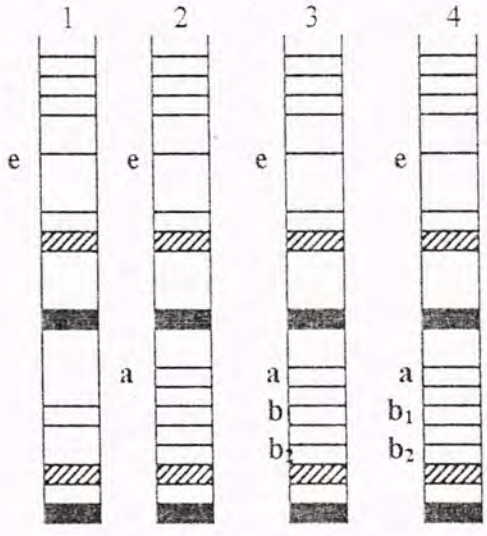

Figure 3: Effects of cadmium on electrophoretic profiles of protein of alimentary canal extracts of fish exposed to 2(lane 2), 4 (lane 3) and 8 mg Cd/l of water for 8 days. Control animals were kept in clean water (lane 1).

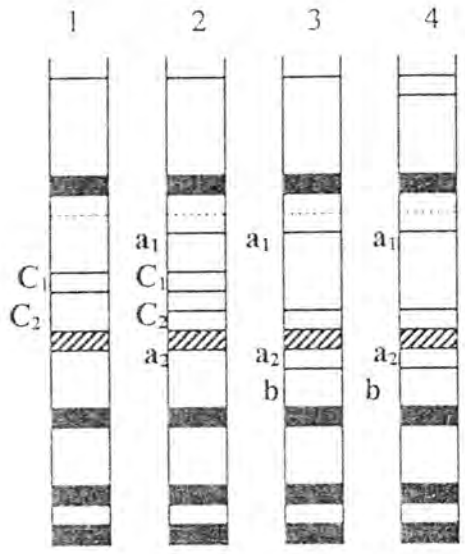

Figure 4: Effects of cadmium on electrophoretic profiles of protein of muscles extracts of fish exposed to 2 (lane 2), 4 (lane 3) and 8 mg Cd/l of water for 8 days. Control animals were kept in a clean water (lane 1).

Isolation and Identification of Bacteria Causing Chronic Suppurative Otitis Media and Thier Response to Antimicrobial Agents

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الخلاصة

خلال فترة ٣ اشهر من عام ١٩٩٣ تم جمع ١٥٠ مسحة لـلاذن من مستشفى اليرموك التعليمي ومستشفى الرشيد العسكري لمرضى يعانون من التهاب الاذن الوسطى المزمن ، وذلك لغرض دراسة انواع الجراثيم المسببة لهذا النوع من الالتهاب . وقد وجد ان نسبة عزل الجراثيم السالبة لصبغة كرام اكثر من تلك للجراثيم الموجبة لصبغة كرام . وكانت اكثر الجراثيم شيوعا هي :

1. *Ps. aeryginosa* 2. *Proteus spp.* 3. *Staph. aureus* 4. *Klebsiella spp.* 5. *E. Coli.*

ومن اختبار حساسية هذه الجراثيم لبعض المضادات الحيوية ، وجد ان الجنتامايسين هو المضاد الحيوي الاكثر فعالية ضد معظم العزلات .

ABSTRACT

From 150 patients suffering from chronic suppurative otitis media, ear swabs were collected to determine the most prevalent causative micro-organisms Gram-negative organisms were found to be more frequent than Gram-psoitive bacteria. The most commonly occuring organisms were: *Ps. aeruginosa, Proteus spp, Staph. aureus, Klebsiella* species and *E. Coli*. The sensitivity pattern of these micro-organisms to selected systemic antibiotics revealed the superiority of gentamicine to other antibiotics against the tested isolates.

INTRODUCTION

Chronic suppurative otitis media (CSOM) is one of the most common illnesses requiring medical attention among infants and Children (1).

The prevvalence of (CSOM) differ in population groups and the identified factors influencing theis variation include geographical location, environmental condition, socioeconomic status, racial and ethenic susceptibilities (2,3).

The term (CSOM) is used to embrace several quite distinct pathological processes which have in common no more than shared symptoms of long standing painless aural discharge, and some degree of deafness. The course is slow, insidious, and very often tends to be destructive (4).

CSOM with a continuously draining middle ear, indicating a tympanic membrane perforation, quite different bacteria are found as compared with acute otitis media and secretory otitis media. In such cases, *Ps. aeruginosa, Proteus* species, *Staph aureus* and Gram-negative rods are the predominant micro organisms (5). The malfuntioning Eustachian tube has been considered as a major contributing factor in the development of middle ear effusions. Other predesposing factors in the development of middle ear effusions. Other predesposing factors include: Upper respiratory tract infection causing loss of respiratory epithelium of the nose, sneezing, purulent nasal discharge and a

mechanism for spread of bacteria to the ear, age, children being more prone to obstruction of the Eusnachian tube as it is shorter, wider and more horizental than in adults, inadequately treated acute cases of otitis media, socioeconomic status, plus many other factors.

In this study, the predominant aetiological microorganisms causing CSOM and their sensitivity to specific antibiotics were assessed.

MATERIALS AND METHODS

Ear swabs from (150) patients attending the ENT unit of the outpatient clinic in AL-Yarmook teaching hospital and Al-Rashid Military hospital were studied. They were examined with otoscope, and if a patient had an active (CSOM), ear swab was taken and special notes such as age, sex, duration and residence were recorded. In addition (100) ear swabs were taken from healthy ear of students from apparent healthy ear of normal people.

Each specimen was inoculated on; Blood agar (aerobic and anaerobic), Chocolate agar, MacConkey's agar and Sabouraud's agar. The growing organisms were identified morphologically and biochemically using the API 20E system for Gram-negative bacteria, and the routive conventional procedures for the rest of micro-organisms (6).

Sensitivity testing for the (4) most common pathogens was performed using the disc diffusion method, and the atibiotic discs used were: Gentamicin (GM), polymixine (PB), Streptomysin (S), Ampicillin (AMP) Tetracycline (TE), Chloramphenicol (C), Penicillin (P), Erythromycin (E), Carbenicillin (PY), Cephalexin (CL), Piperacillin (PRL), and Refadin (RD).

RESULTS

During the period from March to June, 1993, 150 ear swabs were obtainded from patients with active CSOM. These cases included children, adolescents and adults of both sexes. The specimens were used for direct smear, inoculation into suitable media for the isolation of the aetiologic agents and for sensitivity test.

134 swabs were positive for bacterial growth, 56 patients revealed the growth of single organism and 78 patients showed mixed infections caused by 2 or more organisms.

The bacterial and fungal isolates of different species with their frequency in patients with active CSOM are presented in Table-1 of which *Ps. aeruginosa*, *Proteus* species and *SStaph. aureus* sere the most frequent isolates (27%, 18.1%, 13.1% respectively). The remaining Gram-negative bacilli showed low percentages among the isolates like *Klebsiella* species (8.4%) *E. coli* (5.4%) *Enterobacter* (1.2%), and *Citrobacter* (0.84%).

Yeast and Fungus were also isolated, *Candida* and *Aspergillus* species detected in 2.1% and 2.9%, respectively. However, 16 cultures were found negative out of the 150 specimens (10.6%).

The Table also showed a comparison between the normal flora found in normal and diseased ears. All the micro organisms, except *Pseudomonas*, *Enterobacter*, *Citrobacter*, *Pneumococcus*, *Strept. pyogens* and *H. influnzae* were found in normal ears.

Gram-negative bacteria (62.44%) were far more frequent than Gram-positive bacteria (32.49%). (Table-2).

Figures (1-5)show the sensitivity patterns of *Ps. aeruginosa*, *Proteus* species, *Staph. aureus*, *Klebsiella* species and *E. coli*, respectively.

Ps. aeruginosa was mostly sensitve to polymixin (95.3%), Gentamicin (92.1% and to a lesser extent to Refadin (76.5%). Regarding *Staph. aureus*, the majorityof isolates were sensitive to Cephalexin (80.6%) and Gentamicin (67.7%).

Table 1: Bacterial Profiles of 237 Isolates Recovered From Patients with CS

Bacterial species	Normal % individuals		Patients Normal ear%		Infected ear%	
Ps. aeruginosa	0	0	0	0	64	27
Proteus Species	2	1.7	1	0.9	43	18.1
Staph. aureus	4	3.5	6	5.6	31	13.1
Klebsiella species	4	1.7	6	5.6	20	8.4
E. coli	2	0	1	0.9	13	
Enterobacter	0	0	0	0	4	1.2
Citrobacter	0	0	0	0	2	0.84
Strept. pneumoniae	0	0	0	0	10	4.2
Strept. pyogens	0	0	0	0	7	2.9
H. influnzae	0	0	0	0	2	0.84
Staph. epidermidis	45	39.	44	41.1	13	5.4
Diphtheroids	21	18.	18	16.8	16	6.7
Candida species	2	1.	1	0.9	5	2.1
Aspergillus species	0	0	2	1.8	7	2.9
Strept. fecalis	2	1.	1	0.9	0	0
B. catarrhalis	6	5.	4	3.7	0	0
Staph. citrus	2	1.	3	2.8	0	0
B. subtilis	23	20.	20	18.6	0	0
Total	113		7		237	

Table 2: Frequency of Isolated Microorganisms From patients with CSOM

No.	Micro oganisms	No. of Isolates	%
1.	Gram-negative bacteria	148	62.4
2.	Gram-positive bacteria	77	32.49
3.	*Spergillus* species	7	2.95
4.	*Candida* species	5	2.1
	Total	237	99.98

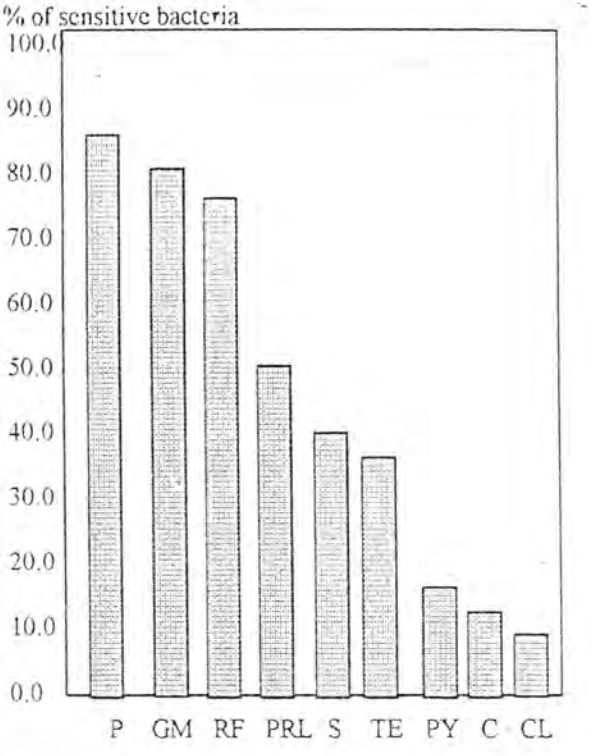

Figure 1: Antibiotic sensitivity pattern of (*Ps. aeruginosa*) isolated from patients with CSOM

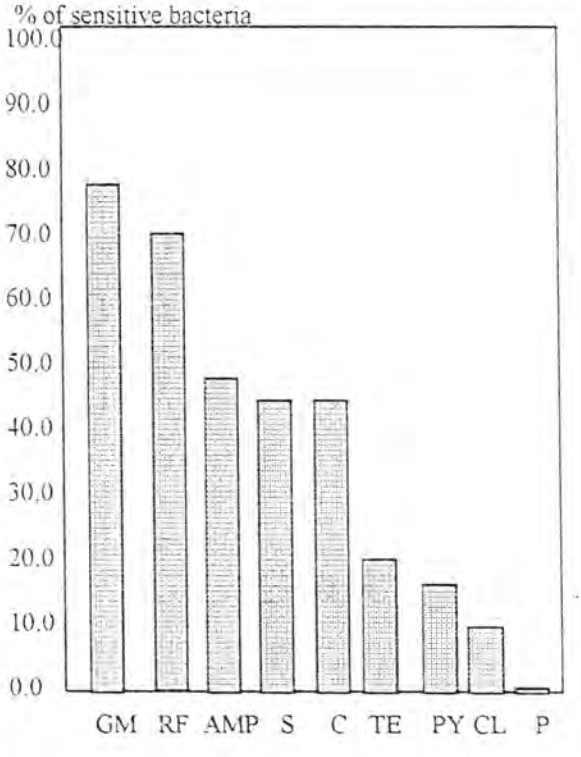

Figure 2: Antibiotic sensitivity pattern of (*Proteus spp.*) isolated from patients with CSOM

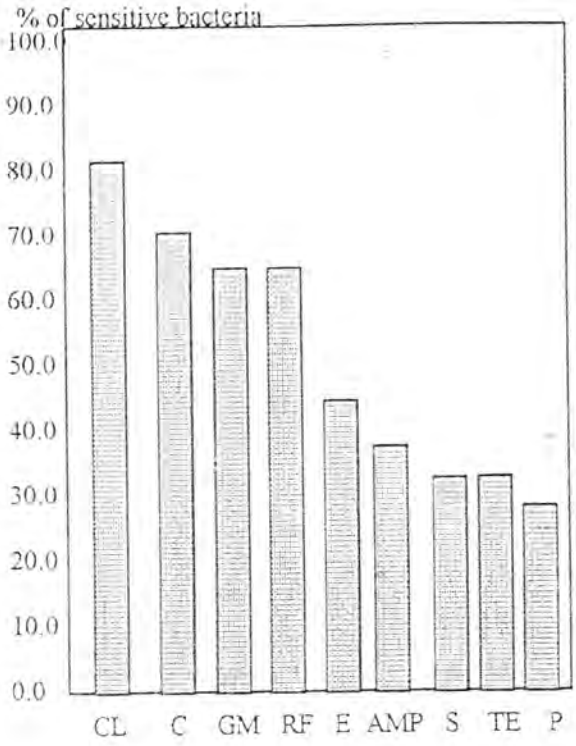

Figure 3: Antibiotic sensitivity pattern of (*Staph. aureus*) isolated from patients with CSOM

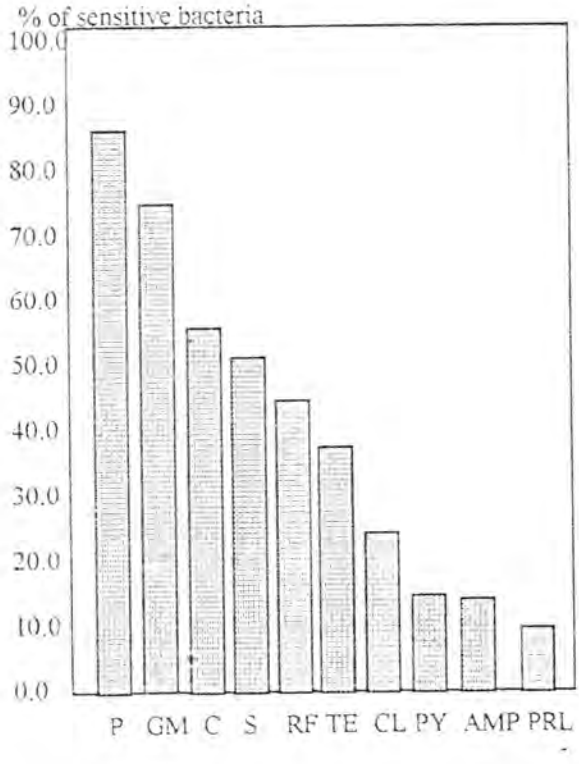

Figure 4: Antibiotic sensitivity pattern of (*Klebsiella spp*) isolated from patients with CSOM

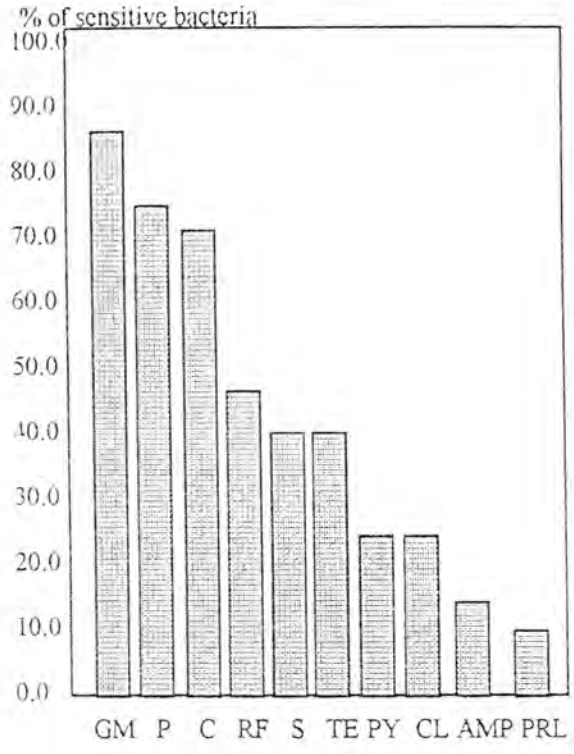

Figure 5: Antibiotic sensitivity pattern of (*E. coli*) isolate from patients with CSOM

DISCUSSION

Chronic suppurative otitis media is still a commonly encountered disease, and although antibiotics seem to be effective in controlling suppurative comflications, otitis is still a significant cause of morbidity and hearing loss (7). Infection is usually polymicrobial auditory canal or commonsal flora of nasopharynx (8).

Various microbiological studies (9, 10, 11) showed in agreement with our results, that *Staph. epidermidis, Bacillus subtilis, Staph. aureus* and *diphtheroids* were the predominant microflora isolated from apparently healthy ears.

Ps. aeruginosa was found to be the commonest isolated micro-organisms from cases of CSOM (27%), such finding was agreed upon by many workers (12, 13). This may be due to, first, *Ps. aeruginosa* are the most secondary invaders when the resustance of the middle ear is lowered (14), second, the

high incidence of *Ps. aeruginosa* indicates more general antibiotic resistance than is the case with Gram-positive strains (15), and third, it often causes otitis with an exuberent tissue reaction, so difficult to eradicate (16).

Staph. aureus was the predominant Gram-positive organism isolated from patients with CSOM (13.1%). This frequency may be due to, *Staph. aureus* has ingerent nature of developing resistant strains, secondly when the tympanic membrane was non intact, it may enter the middle ear by two routes, from the external canal (as normal flora), and by reflex otitis media (17).

The misuse of antibiotics in our area, encouraged the development of bacterial resistant strains, howecer, this study showed the superiority of gentamicin to other antibiotics againts *Ps. aeruginosa*, the most stubborn one, and against other Gram-negetive bacteria.

Staph. aureus, proteus species, *Klebsiella* species and *E. coli* were found to be sensitive to the bacteriostatic effect of chioramphenicol, and this may be explained by the fact that this drug is not widely used, and has a broad spectrum of activity.

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Effect of Aliphatic Alcohol's on Lincomycin Resistance of *Pseudomonas Aeruginosa* PAC 143

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الخلاصة

درس تأثير الكحولات الاليفية واللنكومايسين ضد بكتريا السودوموناس أيروجينوزا باستعمال تقنية قياس سرعة النمو وتقنية تحديد التركيز الأدنى المثبط للنمو ، وأظهرت النتائج أن اللينكومايسين بتركيز ٢٠٠٠ مايكروغرام/مل لا يوقف نمو البكتريا . ويتوقف نمو المزرعة البكترية عند استخدام ٥٠٠ و ١٠٠٠ مايكروغرام/مل لينكومايسين بوجود تراكيز اقل من التركيز الأدنى المثبط للنمو لكل من الكحولات المستعملة مما يدل على وجود علاقة تعاونية بين كل من الكحولات واللنكومايسين وأظهرت النتائج أيضا بان الكحولات تترتب فيما بينها حسب فعاليتها كالآتي : أميل > بيوتيل > بروبيل > أثيل > ميثيل فيما إذا استخدمت لوحدها أو مع اللنكومايسين .

ABSTRACT

The effect of aliphatic alcohol's and lincomycin alone and in combination with each other on *Pseudomonas aeruginosa* was assessed by determination of minimal inhibitory concentration and growth rate . Lincomycin in a concentration as high as 2000 µg/ml. could not stop the growth of bacteria. The results obtained ranked the effect of alcohol's in order as : amyl > butyl > propyl > ethyl > methyl . Combination of 500 and 1000 µg/ml. Lincomycin with subinhibitory concentration of the alcohol's inhibited the growth of bacteria , indicating that the combination had a synergistic effect . This synergistic effect ranked the alcohol's in the same order as when used alone .

INTRODUCTION

Lincomycin is a basic antibiotic produced by *Streptomyces lincolnensis* [1] . This antibiotic has been shown to be effective mainly against susceptible strains of Gram positive bacteria (1,2,3) . It exerts its action by interference with the protein synthesis at the ribosomal level (4).

Pseudomonas aeruginosa is characterized by its resistance to most usual antibiotics , including lincomycin , much of its resistance is due to permeability barrier which prevent antibiotic penetration (5) . It had been reported that the enhancement of the antibacterial activity of tobramycin against *P. aeruginosa* by tween 80 may be related to its effect on the permeability barrier and thus

renders the bacterium more permeable to the drug (6).

In this regard aliphatic alcohol's had been shown to increase erythromycin susceptibility of *P. aeruginosa* (7) . The cell walls of Gram negative bacteria contain up to 25 percent of their weight of lipoprotein and lipopolysaccharides . Aliphatic alcohol's , as organic solvents , can solubilize out the lipid from the cell envelope (8) . Other studies showed that alcohol's increased the fluidity of lipid and alter fatty acid composition of cell envelope (9.10) . While other observations suggested that alcohol's alter the cell permeability even at subinhbitory concentrations (7,11).

In this study , data are given about the effect of aliphatic alcohol's on lincomycin resistance of *P. aeruginosa* .

MATERIALS AND METHODS

Strain :

A local strain of *P. aeruginosa* PAC 143 from the culture collection of Department of Biology , College of Science , University of Baghdad was used in this study .

Media : Nut rient broth and agar were supplied by oxide .

Chemicals :

Lincomycin was kindly supplied by Upjohn Co. , Aliphatic alcohol's (methyl , ethyl , iso-propyl , n-butyl , iso butyl and amyl alcohol were all analar reagents and supplied by BDH.

Growth rate experiments :

The method was described by AL-Dugaily et al (8) . An overnight culture was diluted (1:100) into fresh nutrient broth and incubated at 37°C. with shaking . The growth rate was followed by means of spectronic 20 at 420 nm. At a predetermined absorbency , the drug at required concentration was added . Absorbency of samples of broth cultures after the addition of the drug was taken at fixed time intervals .

Determination of minimal inhibitory concentration

The method was as described by AL-Zaidy et al (7) . The MICS were determined in nutrient broth medium . The incubation of inoculated tubes was for 5 days at 37°C The number of viable cells per inoculated tube was $1x10^7$ viable cells per ml. .

RESULTS

Minimal inhibitory concentration experiment showed that Lincomycin in a concentration as high as 2000 µg/ml. could not stop the growth of *P. aeruginosa* PAC 143 in nutrient broth after five days of incubation at 37°C . On the other hand , growth rate experiment showed that cells of *P. aeruginosa* PAC 143 continued to grow exponentially , but at a reduced rate in presence of predefined Lincomycin concentrations (100-500 µg/ml.)(Figure 1).

The minimal inhibitory concentrations (MICs) of aliphatic alcohol's against P aeruginosa PAC 143 are shown in table (1) . The MICs were : 8% methanol , 5% ethanol , 3.5% iso-propanol , 2.5% amyl alcohol. the MICs of these alcohol's when combined with 500 and 1000 mg/ml. Lincomycin were 19-50% less than MICs obtained for alcohol alone (table 1) . Amyl alcohol showed the lowest MIC value in combination with Lincomycin . The results of effect of Lincomycin and amyl alcohol alone and in combination on the growth of *P. aeruginosa* PAC 143 are shown in Figure 2 . The presented results indicated that 0.1% amyl alcohol had enhanced effect on the activity of 150 µg/ml. Lincomycin against *P. aeruginosa* .

DISCUSSION

In this work , it was intended to study the effect of aliphatic alcohol's on the activity

of Lincomycin against *P. aeruginosa* PAC 143 , an organism whose resistance to most known antibiotics is well known (12).

Upon addition of graded Lincomycin concentrations to cultures of *P. aeruginosa* PAC 143 which maintained in the logarithmic growth phase , the growth rate is decreased , and a new Lincomycin affected steady phase is established (Figure 1) , indicating that the drug is absorbed at a regular rate by *P. aeruginosa* PAC 143 This absorption is dependent on the concentration of free Lincomycin in the medium . Aliphatic alcohol on the other hand gave the same patterns of growth as that presented in figure 1 , indicating the similarity of kinetic of action of these alcohol's on this organism at this level of concentration .

Amyl alcohol enhanced the activity of Lincomycin by inhibiting the exponentially growing cultures of *P. aeruginosa* PAC 143 , and this inhibition is much greater than that caused by each alcohol when used alone . This may be explained on the basis that alcohol's increases the permeability of this bacterium leaving more access for Lincomycin to enter the cell (7,11).

On the other hand , our results may throw the light on the hypothesis drown by many investigators who attributed the resistance of some Gram-negative cells to the high lipoid content of these cells envelopes as well as the low permeability of their membrane (9,10,13). Although the other alcohol's , when used with Lincomycin gave graphs similar to those shown in figure 2 , except that the extent of inhibition of the growth was dependent on the molecular size and concentration of the alcohol used in the combination .

The results of this experimental work may be interpreted on the basis that these alcohol's exert their antibacterial effect by modifying the permeability properties of *P. aeruginosa* PAC 143 and may be , by dissolving away the lipoid materials from the cell envelope , so enabling more of the antibiotic to be absorbed by the bacterial cell than would be taken up from the same concentration of the antibacterial component alone.

Table /1 : Minimum inhibitory concentrations (MICs) of the alcohol's alone and combination with Lincomycin against *P. aeruginosa* PAC 143 (1 x 107 cells/ml.)

Alcohol	MIC (%V/V)	MIC (%V/V in the presence of Lincomycin	
		500(µg/ml.)	1000(µg/ml.)
Methanol	8	6.5 (18.8%)	6 (25 %)
Ethanol	5	4 (20 %)	3 (40 %)
Isopropanol	2.5	1.75 (30 %)	1.2 (40 %)
N-propanol	3.5	2.5 (28.6%)	2.5 (28.6%)
Iso-butanol	1	0.6 (40 %)	0.5 (50 %)
N-butanol	1.5	0.9 (40 %)	0.8 (46.7%)
Amyl	0.5	0.3 (40 %)	0.2 (40 %)

* Numbers between brackets indicate the percent decrease in MICs of alcohol's in presence of Lincomycin.

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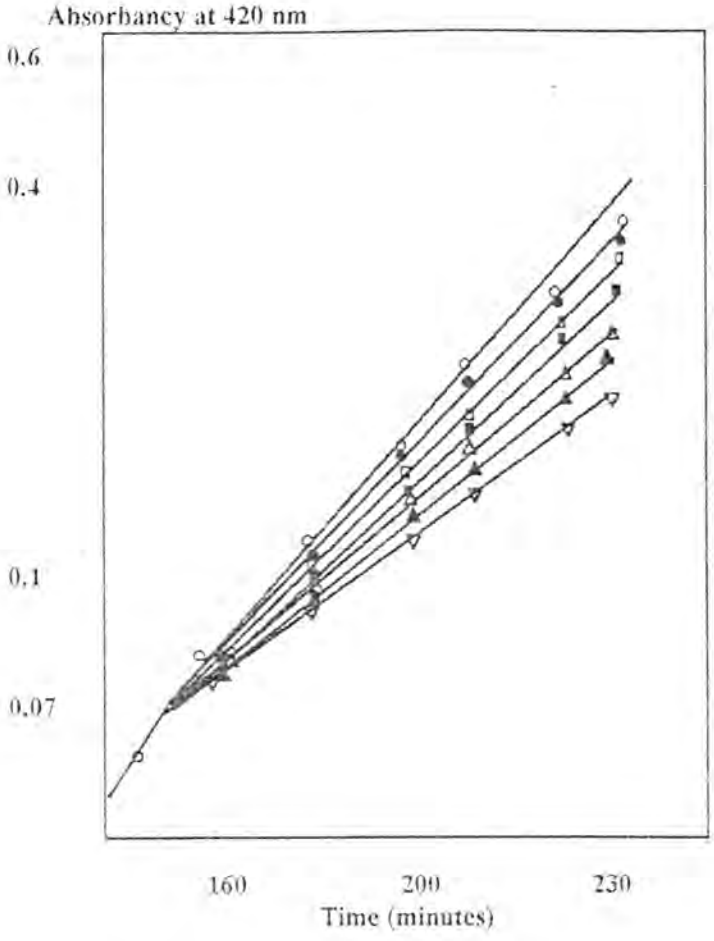

Figure 1: Effect of lincomycin on the growth of logarithmic phase cultures of *P. aerouginosa* PAC 143 Lincomycin was added at the time indicated by the arrow to give the following concentrations:
○ Zero (Control culture), • 100µg/ml. ▫ 150 µg/ml, ▪ 200 µg/ml, △ 300 µg/ml, ▲ 400 µg/ml, ▽ 500 µg/ml.

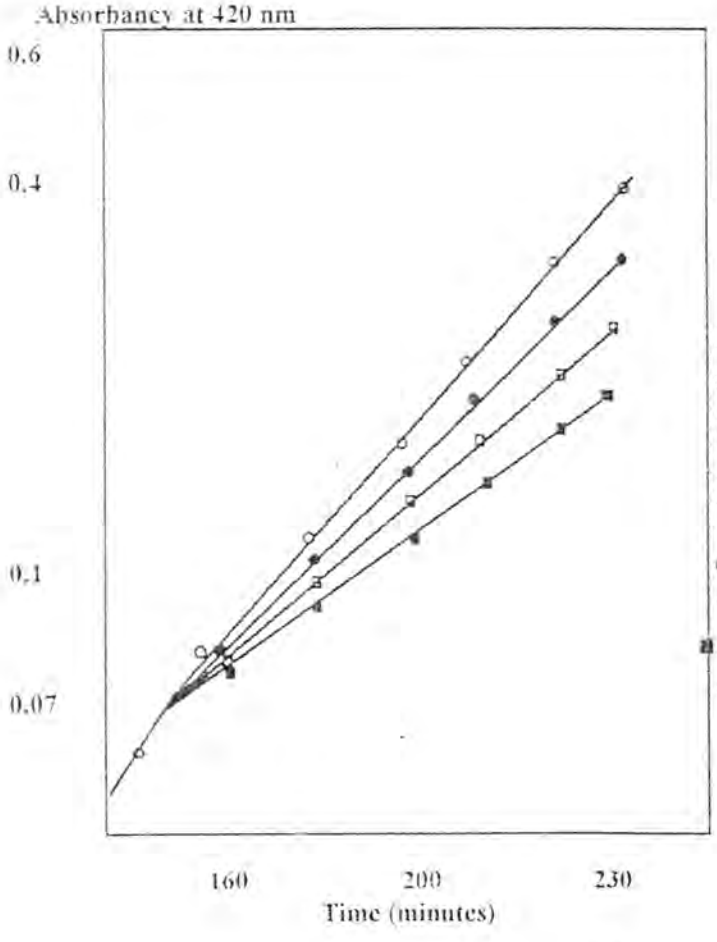

Figure 2: Effect of Lincomycin and amyl alcohol, alone and combination on the growth of *P. aeruginosa* PAC 143 Chemicals were added at the time indicated by the arrow to give the following concentrations;
○ Zero (control culture), • 50 µg/ml, Lincomycin, ▫ 0.1% v/v amyl alcohol, ▪ 150 µg/ml Licomycin and 0.1 % amyl alcohol

^{137}Cs-Gamma & Ultra Violet (254 nm) Radiation Response of Hybrid Obtained from Somatic Fusion between Rodent A_{23} and Human *Xeroderma pigmentosum* Cells

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الخلاصة

تم تثبيت الظروف القياسية لتشعيع الخلايا الابويـة (A_{23} & XP) ولخلايا الهجين(Mo-1 & Mo-2) في العالق المائي وبوجود الهواء . استخدمت معايير الاستجابة الكمية المستخلصة من منحنيات الجزء الباقي في توصيف كفاءة التصليح الخلوي للضرر الاشعاعي . لقد توضح ان الهجين (Mo-1) يتبـع الاب (A_{23}) في نمط الاستجابة لكلا النوعين من الاشعة بالرغم من الاختلاف في عدد كروموسوماته . بينما اظهر الهجين (Mo-2) زيادة كبيرة في مقاومته للاشعة فوق البنفسجية قياسا باستجابة الاب . السبب في ذلك قد يعود الى استعداد الخلية هذه لتصليح ضرر الاشعة فوق البنفسجية بما يفوق امكانياتها لتصليح ضرر الاشعة المؤينة وعززت الاقتراح ان التفاعل بين مكونات الابـاء الوراثيـة لاتغير بالضرورة استجابة الهجائن . كما وقد اقترحت امكانية وجود الية لاكروموسومية تحصل بالاضافة او بدل التصليح الاستئصالي . اوضحت الدراسة قصور العدد الاستقرائي (n) في توصيف كفاءة التصليح الخلوي ويبدو انه من الضروري اعادة صياغة نظرية الهدف الكلاسيكية لكي يصبح هذا العدد ذو فائدة من وجهة النظر البيولوجية .

ABSTRACT

Standard conditions were defined for the ^{137}Cs-gamma and UV (254 nm) irradiation of parent (A_{23}&XP) cells as well as hybrid (Mo-1 & Mo-2) cells, in PBS suspension, in equilibrium with aer. Response parameters extracted from surviving fraction curves were used to infer repair efficiency. The hybrid cells follow response characteristics of A_{23} parent but show no correlation to ploidy. Mo-2 however, demonstrate an appreciable increase in UV resistance. This may be due to amenability of repair of UV damage than gamma rays. It was assumed that interaction between parent genomes doesn't necessarilyl modify hybrid response. A possibility of non chromosomal repair mechanism(s) operating in addition to or instead of excission repair was also inferred. The extrapolation number can not, alone, elucidate the capacity of repair. It seems essential to re-model the calssical "target concept" with special emphasis on small radiation doses for the shoulder to be of value in biological terms.

INTRODUCTION

Cell fusion has been a powerful technique used for variety of purposes e.g. the control of DNA and RNA synthesis (1), the control of mitosis and differentiation of cells (2), the nature of malignancy (1), tumour immunity (3), viral transformation of cells in culture and the genetic likage and mapping (2). It is known that the intefrity of genetic materials is a prime factor governing the response of mammalian cells to radiation (6). Variation of ploidy would therefore, expected to be accompanied by change in

radiation response, more likely due to alteration of repair capacity. Quantitatively, repair capacity can be inferred using shoulder size parameter of surviving fraction curves. A relationship between survival curve extrapolation number (n) and ploidy has only been reported by (7), and no such relationship has ever been reported since. This is possibly due to the nature of cell lines and restricted nature of chromosome numbers as well as sensitivity of shoulder measurement techique using the calssical terget theory concept. Somatic cell hybrids may, thus, be used to investigate this problem; and one group of workers (8), have reported the unusual X-ray resistance of a mouse-rat hybrid indicated by change in slope (k) of survival curve rather than extrapolation numver (n). On the other hand, Limboch et al., (9), working on chimese hamster hybrids reported that ploidy has no significant effect on X-ray survival parameters. It was suggested that this may be a reflection of common ancestry (9). Further studies using interspecies hybrids, in which the novel genetic environment may have led to activation or depression of repair processes not normally expressed in the parent cells, has been more expressive.

Lately, Al0Shaickly (10), had isolated an interspecific hybrid from crosses between human *Xeroderm pigmentosum* (highly UV sensitive cancer cell) and chinese hamster fibroblast A_{23} (reasonably resistant to radiation). Comparison of parameters obtained from radiation curves of hybrid and parent cells may elucidate this very subtle relationship.

MATERIALS AND METHODS

Cell Lines:

A_{23}, a fibroblast cell line derived from chinese hamster DON. It is thymidine kinase deficent and aquire a potent repair capacity to UV and gamma radiation. *Xeroderma pigmentosum* (XP) cells, a human skin cancer cells deficient of excision repair capacity. Hybrid (Mo-1 & Mo-2) cells were obtained from fusion between rodent fibroblast A_{23} and XP parent cells (10).

Preparation of Cells for Irradiation:

Parent cells were prepared as monolayers in 13 DMEM (Dulbecco's modification of Eagle's essential minimal medium supplemented with 13% foetal bovine serum, 50g/ml streptomycin sulfate and M/ml of 500 mg/ml benzol pencillin. Hybrid cells were maintained in HAT-Oua-5 (13 DMEM supplemented with 10^{-4} hypoxanthene, $4x10^{-7}$ M methotrexate and $1.6x10^{-5}$ thymidine and $1x10^{-5}$ M iyabain). Cells were trypsonized, washed and diluted immediately before irradiation procedures.

Irradiation Procedures:

Gamma irradiation:

A Canadian 220 ^{137}Cs-gamma irradiation facility was used. It delivers at the core of irradiation chamber about one rad (0.01Gy) per second. Cells were irradiated while suspended in PBS, in screw capped tubes. Following irradiation, the cells were diluted into the appropriate medium (13 DMEM for parents and HAT-Oua-5 for hybrid cells) to assay for cell viability.

Ultra Violet (UV) irradiation:

All culture were irradiated using Hanovia Bactericidal UV unit consistiing of low pressure mercury discharge tube emitting UV (254 nm) with an incident dose rate of 9 ergs /mm^2/s. Calibration of the unit & actinometry was carried out using UV dosimeter supplied with photovoltaic cell (13). Generally, cells were allowed to attach in 13 DMEM (500 to 1000 cells/plate) at 37^{0}C for 4 h. Growth medium then removed and attached cells washed twice with PBS^{--} to remove UV absorbing substances and the dry plates irradiated with lids removed. The geometry of UV setup was adjusted to maintain a constant incident dose rate of 9 ergs/mm^2/s. The viability of attached cells was estimated by replacing the growth medium and incubation whereas cells in

suspension were treated in a similar manner to that of gamma radiation.

RESULTS

Raidiation Response of Parent and Hybrid Cells

Gamma and UV radiation survival data were obtained from Mo-1 at passage 5 and Mo-2 at passage 3. Both hybrid scll lines were cultured continuously in HAT-Oua-5. Cells were recovered after irradiation on 13 DMEM medium.

Gamma Ray Resonse

Figure 1 & 2 show resonse of paren (XP= either XP_{25} or XP_{11192} and A_{23}) and hybrid (Mo-1 & Mo-2) in terms of surviving fraction curves. Quantitative surviving parameters extracted from these curves are summarized in Table 1. Hybrid cells plated at 103 cells/plate yielded a low plating efficiency (about 15%) following suspension in 13 DMEM. Response of hybrid cells (Mo-1 & Mo-2) measured by inactivation constant (k) values (6.74 & 6.84×10^{-3} rad^{-1}) though alike but significantly differ from parents. A_{23} cell appear more resistant than XP cells (5-8 times) and hybrid (0.12 & 0.11). It was evident that A_{23} surviving curves were continuously devoid of linearity. The (k) values reported in Table 1 were estimated by constructing tangent to the curve at approximately 10% survival level. The hybrid extrapolation number (n) values (10.25 & 10.43) are clearly higher than parental (n) values (4.0 & 1.0) which indicate a pronounced shoulder to the hybrid survival curves at low dose levels.

UV Radiation Response

Survivng fraction curves of parent and hybrid cells appear in Figure 3. Quantitative parameters calculated from these curves summarized in Table 2. Survival data from Mo-2 ($k=1.92 \times 10^{-2}$ /ergs/mm^2) reveal that this hybrid is twice as resistant as Mo-1 (4.11×10^{-2}/ergs/mm^2) and same as that of parent A_{23} (4.11×10^{-2}/ergs/mm^2). A_{23} UV response, which shows strict linearity over the dose range tested, was about seven times more resistant that XP cells (XCP_{25} or XP_{11192}). Alternatively, Mo-2 was almost 15 times more resistant than XP. Sizes of shoulders represented by (n) values were again varying. Mo-1 (3.25) was about two folds more than that of Mo-2 (1.75); and about 3.3 times more than that of XP_{25} & XP_{11192} and almost similar to that of parent A_{23} (3.21).

DISCUSSION

On comparison of radio-sensitivities of parent cells (XP & A_{23}) with those of hybrid (Mo-1 & Mo-2) (Table 1&2), the following points can be observed:

1. Parent response to gamma rays was different, the extent of difference amounting to (6 times) as measured by values of inactivation constant (k). Extent of difference to UV (254 nm) radiation however, was about 8 times, using the same parameter. This phenomenon which was also noticed in *E. coli* B/s-1 (14), can be explained in terms of efficiency of repair of gamma radiation damage which apparently exceeds that of UV. Also, it may be postulated that mechanism(s) other than or in addition to excision repair may operate against gamma radiation lesions(15) (16); knowing that these lesions are numerous and different than cyclobutane dimmers induced by UV (17).
2. The response of Mo-1 hybrid is very much siimilar to that of parent A_{23} using (k) value. Alternatively, Mo-2 is much more resistant to UV than parent A_{23} or hybrid Mo-1 (about twice). These result which reverse in parent cells, apparently indicate thatUV damage is more amenable to repair within the Hybrid cell than gamma ray damage, or that the site for gamma ray induced cell inactivation may not be all chromosomal in nature. The validity of these explanations and that for parent cells depends on the assumption that interaction between parent genomes does not

necessarily modify hybrid response (9). It may therefore, be of interest to extend these sstudies to hybrids of different parentage (8). And, to correlate survival data with photoproduct (pyrimidine dimmers), determined immediately after irradiation (measure of absorbed dose) and, after incubation of irradiated cells in growth medium (measure of excision repair). Since rodent cells excise dimmers poorly (11), comparative measures of post replication repair may then provide a better index of the repair capacity of hybrid cells.

3. Generally, the hybrid cells seem to follow the response characteristic of A_{23} parent but appear to show no correlation with chromosome number. This observation is in consistent with Limboch et al. (9) finding, and could strengthen the notion of non chromosomal repair factors, specially against UV damage. Mo-2 passage nuber was only 3, and responses measured with higher passages may vary in some of their phenotypic characteristics (12). So, if passage number is an operating factor in this diversity, it can thus be postulated that expression of responsible genes are unstable at earlier passages at least in this particular hybrid (i.e. Mo-2).
4. Shoulder sizes of surviving fraction curves as measured by values of n &D_q (Table 1&2) have no clear relationship with ploidy or response data measured by (k) values. This result does not coincide with Bedford & Hall (7) observation. Thus, in order to sustain more precise estimates of n &D_q, radiation experiments should be redesigned to enlarge on small doses within shoulder area using stochastic mathermatical model in stade of classical target consept model.

Table 1: Summary of gamma radiation response data of parent (A_{23} and XP) cells and hybrid (Mo-1 and Mo-2) cells

Cell Line	[a]k x 10^{-2} rad^{-1}	[b]n	[c]D_q (rad)	Plating Efficiency
A_{23}	0.769	6.50	365	5.2
	0.752	2.61	348	3.2
	0.773	2.95	350	13.7
Mean	0.764	400	354	7.36
XP[d]	4.68	1.0	0.0	6.2
	4.39	1.0	0.0	4.6
Mean	4.54	1.0	0.0	5.4
Mo-1	0.678	10.30	360	16.0
	0.670	10.20	365	14.0
Mean	0.674	10.25	363	15.0
Mo-2	0.687	10.50	355	13.0
	0.680	10.35	368	16.0
Mean	0.684	10.43	362	14.5

a: inactivation constant = slope of linear part
b: extrapolation number = intercept of linear part with y-axis
c: intercept of linear part with x-axis (quasi-threashold dose)
d: XP =XP_{25} or XP_{11192}

Table 2: Summary of UV response data for parent cells (A_{23} and XP_{25} & XP_{11192}) and hybrid(Mo-1 and Mo-2).

Cell Line	Mean kx10^{-1} $(ergs/mm^2)^{-1}$ ± S.D	n	Mean D_q $(ergs/mm^2)$
Parents			
A_{23}	0.411± 0.05	3.21	24.0
XP_{25}	2.881± 0.32	1.00	0
XP_{11192}	2.840± 0.36	1.00	0
Hybrid			
Mo-1	0.411± 0.06	3.25	26.0
Mo-2	0.192± 0.04	1.75	29.0

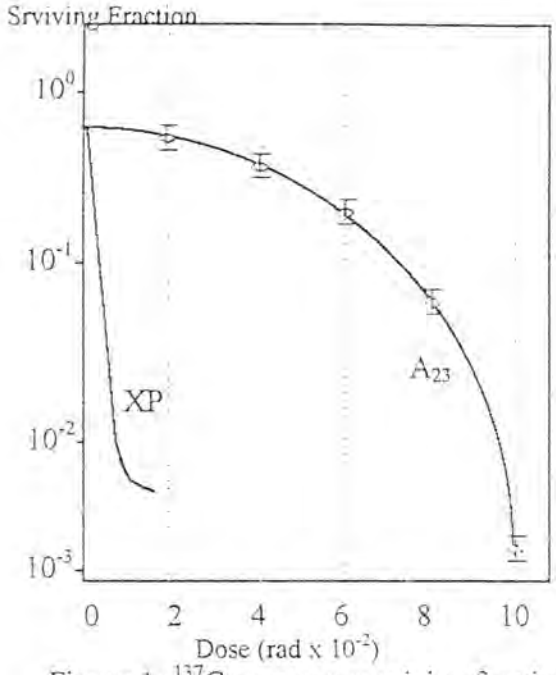

Figure 1: ^{137}Cs-gamma surviving fraction curve of XP and A_{23} cell lines irradiated in liquid suspension and in equilibrium with air. Each point on the curve represents a mean of at least two irradiation experiments performed under identical experimental conditions

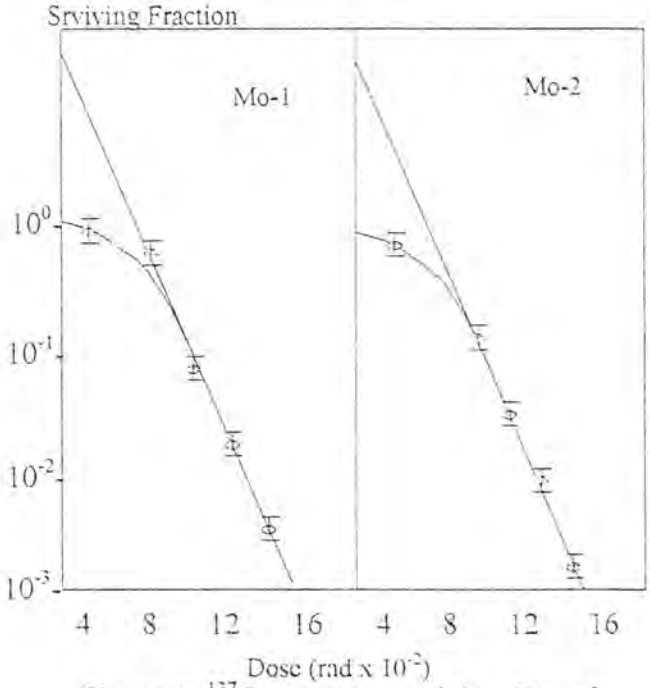

Figure 2: ^{137}Cs-gamma surviving data of hybrid cell lines Mo-1 & Mo-2 irradiated in suspension, in equilibrium with air. Each point on the graph represents a mean of at least two irradiation experiments performed under identical experimental conditions

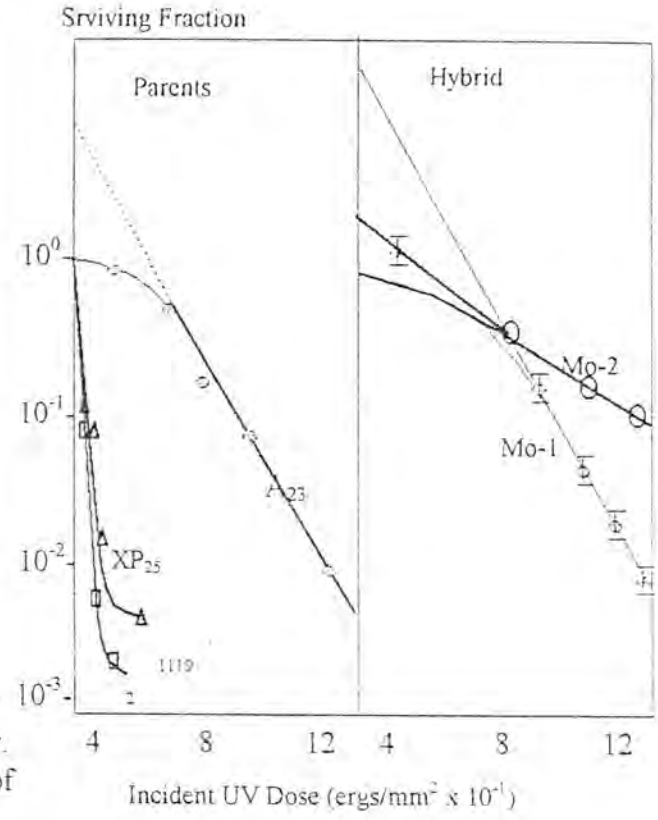

Figure 3; UV radiation survival response of parent cell lines A_{23}, XP_{25} &XP_{11192}; and hybrid Mo-1 & Mo-2 irradiated attached (dry). Each point on the curve represent at least two experiments reproduced under identical conditions.

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Comparsion Between Viable and Dead Eggs and Lrvae of *Toxocara cati* in Inducing Protection Against Infection .

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الخلاصـــــــة

بينت المقارنة بين البيوض واليرقات الحية والمقتولة بالحرارة والتجميد لدودة اسكارس القطط *Toxocara cati* و المحقونة فمويا ، داخل الجلد ، العضلات و الخلب و الوريد بأن البيوض الحية و الميتة بغض النظر عن طريقة الحقن . إن البيوض واليرقات المقتولة بالتجميد أكثر كفاءة في توفير الحماية من البيوض أو اليرقات المقتولة داخل البريتون زود الفئران البيض بأفضل مستوى من الحماية و إن طريقة الحقن بالوريد كان الأقل كفاءة .

ABSTRACT

Comparsion between viable and dead eggs or larvae of *Toxocara cati* , inoculated orally , subcutaneously , intramuscularly , intraperitonially and intraveously showed that viable eggs introduced orally provided protection value better than the viable larvae and dead eggs and larvae regardless of inoculation route . Eggs and larvae killed by freezing were found more protective than the corresponding eggs & larvae killed by heat in all routes of inoculations . It appeared that intraperitoneal injections of eggs or larvae gave the better protective value and the intravenous route gave the least protection .

INTRODUCTION

T. cati is common nematode of cat . The mature ovum is infective to a variety of paratenic hosts including man where they hatch in small intstine and the released larvae migrate to various tissus causing visceral larva migrans {1,2,3} . In addition many authers reported the adult worm in human {4,5,6}.

In the present study an attempt was tried to evaluate the viable eggs and larvae , the dead eggs & larvae (killed by heating or freezing of T. cati inoculated orally , subcutdenously , intramuscularly , intraperitoneally & intra venously in inducing protection in white mice (BALB/C) against a challenge infection .

MATERIAL AND METHODS

Cats were caught dissected & the worms collected and diagnosed . The eggs were extracted from female worms ,

according to {7} , they were washed and preserved in 0.1N H_2SO_4 .

Maturation and incubation of eggs were done in the same solution plus antibiotics (Penicillin , Nystatin) at a temperature of 26°C for 3 weeks .

Hatching of egg was done according to the {8} method , the larvae removed by centerfuging at 1500 rpm/min.

Killing of eggs & larvae by heat was done by incubation at 56°C for 24h. or by freezing (-20°C) for one week .

Mice were immunized (orally , subcutaneously , intramuscularly , intraperitorealy & intravenously) by 100 eggs or larvae in the 1st. dose and after 3 weeks they were given a booster dose of 50 eggs or larvae , then after a week they were challenged with 1000 mature eggs , killed on the 8th. day after the challenge dose . The carcass cut into small pieces and digested in 0.1 pepsin solution according to {9} method and the number of larvae were counted .

A group of mice , represent the control were given the challenge dose only .

RESULTS

The experiment showed that viable eggs provided the better protection (84.37%) in comparsion to viable larvae , dead larvae and eggs in all routes of inoculations .

Ith seem that killing of embyos inside the eggs or the released larvae by freezing gave more protection than their corrospondings that were killed by heat (though the released larvae were better than eggs).

The better route for inoculation for both eggs and larvae was intraperitoncally .

In conclusion , considering the risk of viable eggs or larvae it seem that the method of choice for immunization is by using released larvae killed by deep freezing through intrapertineal route , (see the figurs in the table below).

The antigens and routes of inoculation with the percentage of protection .

Route of inoculation	Orally		Subcut.		Intramus.		Intraper.		y	
Antigens	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Viable eggs	34	64.58%	37	61.45%	17	82.29%	15	84.37%	40	%
Dead eggs (by heat)	59	38.54%	67	30.28%	60	37.50%	52	35.41%	69	%
Dead eggs (by freezing)	54	43.75%	64	33.33%	58	39.58%	56	41.66%	67	%
Living larvae	35	63.54%	45	53.12%	25	73.95%	16	82.33%	47	%
Dead larvae (by heat)	50	47.91%	59	38.54%	55	42.70%	41	57.29%	69	%
Dead larvae (by freezing)	45	53.12%	46	52.08%	38	60.41%	25	73.95%	51	%

(1) average no. Of larvae extracted from carcase of mice.

(2) Percentage of protection in comparsion to control .

(3) Average no. Of larvae extracted from carcasses of control mice = 96 larvae.

DISCUSSION

There are many methods for expressing the development of protective acquired immunity in the host , in this study we selected the reduction in the number of larvae in the immunized host in comparsion with the corresponding controls because it is simple technique and used wildly by many authers {10,11,12,13} .

The results of inoculation of white mice with various preperations of eggs and larvae of *T. Cati* before the challenge dose of eggs showed the development of different rate of protection to the number of eggs or larvae , and route of inoculation and the viability of embryo (larvae)inside or outside the eggs .

The conclusion that viable eggs & larvae were more protective than their corresponding dead eggs & larvae have been shown in other worms {14 , 15} .

It appears that viable eggs were better than the viable larvae in inducing protection this may be due to the fact that the hatching fluid of the eggs contribute also in the development of good immunity {16 , 17 , 18 , 19} .

The higher protective values induced by the intramuscular & intraperitoneal routes of immunization may be as a result of changing the migratory behaiviour of the larvae and their wandering in the tissues .

The comparsion between heat and freezing as methods for killing embryo inside the eggs or the released larvae indicate that heat causes more distortion in the structure of eggs and larvae than the deep freezing .

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Improvement of Glycerol and Ethanol Production by Hybridization of Local *Saccharomyces Cerevisiae* Isolates

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الخلاصة

أختلفت سلالات من خميرة الخبز *Saccharomyces cerevisiae* في قابليتها على أنتاج الكليسرول والكحول عند تنميتها في وسط عصير التمر تحت الظروف متشابهة . وقد أدت عمليات تضريب السلالات الى انتخاب خمائر ذات انتاجية عالية من الكليسرول (١١ - ١٢ غم / لتر) والكحول الاثيلي (٧٥ - ٩٠ غم / لتر) بالمقارنة مع تلك غير المضربة (٢ - ٧ غم / لتر كلسرول و ٥٠ - ٧٥ غم / لتر كحول اثيلي) . وقد سجلت فعاليات انزيمية (انزيم Glyecyol 3- Phosphate dehydrogenase) واطئة وكميات عالية من الكليسرول للسلالات المضربة عند مقارنتها بغير المضربة .

ABSTRACT

Yeast strains of *Saccharomyces cerevisiae* were found to be varied in their ability to produce glycerol and ethanol from date syrup under same or different fermentation condition. Hybridization techniques resulted in selecting yeast strains with high glycerol (11-12 g/L) and ethanol (75-90 g/L) productivity, compared with those of unhybridized strains which produced (2-7 g/L0 glycerol and (50-75 g/L) ethanol. Low activities of the enzyme glycerol 3-phosphate dehydrogenase and high glycerol amounts were recorded in the hybrid strains among unhybridized ones.

INTRODUCTION

Glycerol plays an enigmatic role in wine making it is the fermentation and product after ethanol and CO_2 characteristic levels betwen 5 to 10g (1). The amounts of glycerol produced during wine fermentation is influenced by many factors including temperature. PH, sulphur oxide, grape variety and ripeness, levels of micronutrients (2).

The possibility of improving proparties of wine yeast by the application of genetic techniques has been suggested by several researchers (3, 4, 5).

Rous et al (6) isolated lucine auxotrophic mutant which produced higher levels of alcohols during fermentation. Cell fusion and back-breeding have been used to produce wine yeast strains with improved capability (7)-DNA recombinant techniques also were used as an attempt to improve malic acid utilising capability by wine yeast (8). Selection by hybridization has been made modify such wine making properties as foaming, flocculation, fermentation efficincy and sulphus dioxide tolerance (9, 10, 11).

The ability of different yeast strains to produce various amounts of glycerol under identical conditions may be related to the activity of the enzyme-glycerol-3-phosphate dehydrogenase which catalyses the conversion of dihydroxy acetone phosphate to glycerol 3-phosphate (12). The aim of the present study was to use hybridization techniques for the selection of yeast strains in order to achieve higher levels of glycerol during wine fermentation.

MATERIALS AND METHOD

*Yeast strains: Five hybridized strains of saccharomyces cervisiae developed in the laboratory of biological research center, Baghdad selected by the micromanipulator technique were used (13). They were all homothallic and given the symbols AH, AH_{10}, AH_{20}, AH_{30}, AH_{40}. The remaining 3 strains (R_{35}, R_{40}, R_{50}) were heterothallic haploid obtanied from college of Science, Univ. Of Baghdad.

* Main Substrate: Date syrup containing 250 g fermntable suger/L were used throughout the laboratory for hybridization programes.

* Fermentation conditions: Date syrup (9 ml) was inoculated with 1 ml of 2h old yeast cultures, which has been incubated in liquid media YMPG (Malt Extract 5%, yeast Extract 0.3%, peptone 0.5% Glucose 1%) at 25c. After incubation (24h) cultures were transfered to flasks containing 90 ml date syrup and reincubated semianaerobically fermentation traps without shaking at 15 c. For 20 days. Samples were taken and analysed for glycerol, ethanol, and Fermentable suger after incubation.

The above cultures were transfered to an 8L Fermentor (LKB) containing 5L sterilized date syrup medium and incubated in triplicates at 15c for 15 days.

* Chemical analysis: Glycerol, ethanol, and fermentable suger and protein were determined according to Bergmeyer et al (14), Methods of Analysis of the American Society of Brewing Chemists (15). Dubois et al. (16) and Lowry et al. (17), respectively.

* Enzyme activity determination: Activity of glycerol 3-Phosphate dehydrogense in crude extract of the fermented product was determined according to the method of Nadier et al (18), and expressed as micromoles NAD produced per minute per mg protien.

* Hybridization technique: The hybridization procedure was carried using modified Grunstein - Hangness technique (19).

RESULTS AND DISCUSSION

Eight yeast strains were screened for their ability to ferment date syrp as well as producing glycerol and ethanol. At the end of fermentation period 2-7 g/L glycerol and 60-75 g/L ethanol ranges were determined when glucose and fructose amounts were undetectable (Table 1). Homothallic diploid strains AH and AH2 were sporulated and single spores were mated with the single cells of haploid heterothallic strains R_{35},, R_{40}, R_{50}. Six dipolid starins (M_3, M_4, M_5, M_6, M_7, and M_8) were isolated from the above matings and examined for glycerol and ethanol yields (Table 2). Fourty heterothallic haploid segregants (12 from M_3, s from M_4, 5 from M_5, 3 from M_6, 8 from M_7 and 10 from M_8) were isolated and tested for glycerol and ethanol production. Yield ranges were 4 to 11.4 g/L for glycerol and 75 to 85 g/L for ethonal.

After fermenation ten high glycrol yielding segregants isolated from M_8 by micromainpulater techniques gave glycerol yields ranging from 8.5 to 12.0 g/L and ethanol from 80 to 90 g/L (Table 3). However segregants of M_{97}, M_{98} dipolid strains produced 11.5, 12.0 g/L glyceraol respectively.

These two strains M_{97}, M_{98} used for large scale fermentation when they produced 12.0, 12.8 g/L glycerol, respectively, but some amounts of ethanol (10.5% w/v) and residual suger (2.5 g/L).

Results show that the level of glycerol production during fermentation can be increased by applying selective hybridized yeast strains. Glycerol level increased from 4.5 g/L in the breeding stock strains to 11.4 g/L in the final generation diploid hydrid strains. Individual dipolid hybrids resulted in glycerol yield of 12.8 g/L compared with the most efficient glycerol producing unhybride strains (AH). Ethanol quantities on the other hand increased in the treatment of hybridized strains (83 g/L) in comparison to that of the unhydride strain (67 g/L).

Similar variations in the production of glycerol and ethanol were also observed in the previous experiments (11).

Specific activity of the enzymw, glycerol-3-phosphate dehydrongenase was determined in five yeast strains to examine the possible machanium responsible for increasing glycerol production (Table 4). From the begining fermentation unhydridized yeast strains AH_{10}, AH_{30} possesed specific activities 0.1572, 0.1035 µmole/mg protein respectively which both are higher than those for hybrid starins R_{94}, R_{97}, R_{98} even at the end of fermentation period 0.0572, 0.0221, 0.0705 µmole/mg protein respectively. (20) pointed out that *Saccharomyces cerevisiae* strains which have low specific activity produce more glycerol during fermentation. Radler et al. (12) suggested that glycerol formation is a result of competition between two enzyme, glycerol-3-phosphate dehydrogenase and alcohol dehydrogenase necessary for the reduction of NADH coenzyme.

Table 1 : Glycerol ethanol yields of hybrid *Saccharomyces cerevisiae* strains

Strains Homothallic dipolid	Glycerol g/L	Ethanol g/L
AH	4.5	60
AH_{10}	5.5	70
AH_{20}	3.3	72
AH_{30}	5.5	71
AH_{40}	2.0	66
Heterothallic hapolid		
R_{35}	4.0	75
R_{40}	3.5	70
R_{50}	7.0	73

Table 2: Glycerol and ethanol production of fourty haploid se gregants from six dipolid hybrid strains using hybridization techniques

diploid hybrid	parent strains	No. Of nts	Glycerol g/L	Ethanol g/L
M_9	$R_{50}xAH_{10}$	12	7.0	84
M_4	$R_{95}xAH_{10}$	2	5.5	82
M_5	$R_{40}xAH_{10}$	5	4.5	90
M_6	$R_{40}xAH_{90}$	3	6.8	75
M_7	$R_{95}xAH_{90}$	8	7.5	85
M_8	$R_{50}xAH_{90}$	10	11.4	83

Table 3: Glycerol and ethanol production by ten diploid hybrodizied starins isolated from M_8 yeast

Strain No.	Glycerol (g/L)	Ethanol (g/L)
30	10.1	85
30	10.2	80
32	8.5	81
33	10.7	83
34	11.0	80
35	9.8	87
36	10.0	83
37	12.0	82
38	11.5	81
39	9.0	85

Table 4: Specific activity of gycerol 3-Phosphate dehydrogenase of yeast strains

Yeast strain	Glycerol (g/L)	Specific activity (µm NAD/min/mg protein)
AH_{10}	5.5	0.1572
AH_{90}	6.5	0.1035
M_{94}	11.0	0.0705
M_{97}	12.0	0.0221
M_{98}	11.5	0.0572

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Studies of The Action of Microorganisms in Sewage Water upon Copper and Plastic Pipes

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الخلاصة

لدراسة ومعرفة تاثير الاحياء المجهرية المتواجدة في ماء المجاري على انابيب البلاستيك والنحاس تم عزل (٤٠) عزلة بكترية من كلا الانبوبين كما تم قياس درجة الحموضة (pH) والعكارة الناتجة بفعل هذه الاحياء وذلك بالاستعانة بالمجهر الالكتروني. اظهرت النتائج ان اكثر العزلات شيوعا هي *Moraxella phenylpyrouvica, Flavobacterium indologenes, Pasteurella pheumotropica, Pseudominas maltophilia.* وقد حدث تغير في نوعية ماء المجاري نتيجة لنشاط الاحياء المجهرية مما ادى الى تغير في درجة الحموضة والعكارة . اظهرت نتائج المجهر الالكتروني وجود عدد من الكسور والتشققات على سطح النحاس حفر وثغرات مختلفة الاحجام في انبوب البلاستيك .

ABSTRACT

To investigate the effect of microorganisms in sewage water on plastic and copper pipes, experiment was performed to isolate suspected microorganisms from these pipes and to measure the pH and turbidity. Of 40 bacterial isolates from both pipes, the most commonly isolated were *Moraxella phenylpyrouvica, Flavobacterium indologenes, Pasteurella pneumotropica* and *Pseudomonas maltophilia.* Quality of sewage water was studied through bacterial activity, changing the pH and turbidity. Effects of these chemical and bacteriological parameters on the pipes were examined by SEM. SEM photographs demonstrated a number of cracks and pitting on the surface of the copper pipes and a number of small and large pores on plastic pipes.

INTRODUCTION

Sewage waters being transported through the pipelines are subject to both chemical and microbial quality changes. Some organisms associated with water deterioration include Pseudomonas, Flavobacterium, Achromobacter, Proteus, Klebsiella, Bacillus Serratia, Corynebacterium, and Arthrobacter (1).

Many investigators have suggested that water deterioration in pipelines may be the result of microbiologically mediated reactions (1,12) However two types of corrosion in which micro organisms play a part can be divided (a) aerobic corrosion which is associated with the Gram negative rods "Thiobacillus"(4) and (b) anaeriobic corrosion associated with the growth of sulfur reducing bacteria (4,5).

In the past, much attension was focused on the anaerobic corrosion of Iron and steel metals. These organisms may synthesize hydrogenase and have the ability

to cause cathodic dipolarization of steel. Recently, Kirmeyer and Logsdon (9) found that the pitting corrosion of copper was cause by breakdown of the passivation film, which can be dissolved by either carbonic acid or organic acid assiociated with the microorganisms.

The objective of this study was to determine how the microorganisms in the sewage water might bring about deterioration to copper and plastic pipes with relation to the chemical quality changes.

MATERIALS AND METHODS

Sample Collection:

Samples of freasnly domestic sewage were collected from Glasgow dalmarnok sewage works in 4L sterile screw cap bottles. Stored in ice chest and transported to the laboratory within 1 h after collection. Samples were analysized no longer than 6 h. Pieces of copper and plastic pipes (1.5 cm long each) were divided horizontally into two halves. All were immersed in 70% ethanol for 2 min., and placed into the oven at 40°C for 24 h.

Samples Processing:

Four pieces of each of copper and plastic were aseptically immersed in sterile scrwo cap bottles containing 500 ml freash swage water. The bottles were incubated at 20°C and 37°C using Stuart orbital shaker at 3000 rpm for 6 weeks.

The pH, turbidity and bacteriological analysis were determined immediatly before incubation and in triweekly intervals.

Determination of pH and turbidity:

The pH of the sewage water samples was determind using Gallenkamp digital hand research pH meter (gallenkamp pH stick) after standardizations with two buffer solutions pH (4.0 and 7.0).

Turbidity was measured by a turbidimeter (Model 43900 Hach Ratio/XR). Readings were taken after 15 sec.

For all pH and turbidity assays, duuplicate readings were performed. The results are reportes as average of two replicates.

Enumeration of Cliforms and Aerobic plate count (APC):

Standard methods for the examination of water and waste water (2) were used. Total coliforms were enumerated by most probable number (MPN) techniques using lactose broth medium. Typical coliform colonies were carried through the completed test. APC bacteria were enumerated by the pour plate technique, plates which were incubated at 30°C for 2 days.

For all bacteriological assays triplicate analyses at each dilution of sample size were performed.

The results are reported as average of triplicate of a single dilution.

Identification of Microorganisms:

At the end of the incurbation period, one piece of each of copper or plastic was removed and washed with 4 ml sterile distilled water from both outer and inner surface. Using a sterile spatula, the internal surface of both copper and plastic were scratched, inoculated with nutrien broth and incubated at 30°C for 2-3 days. The broth cultures were streaked on plate count agar and Endo agar to help in detecting the Gram-negative forms. The plates were incubated at 30°C for 2-3 days.

Twenty colonies (were possible) were picked from copper and plastic plates. Isolates were purified three times by streaking onto plate count agar and incubated at 30°C for 2 days.

Pure cultures were maintained on slants of plate count agar at 5°C.

All isolates were placed into genera and species depending on colony and cell morphology, Gram stain, sporulation, capsule formation, Catalase and oxidase reactions, motility, voges proskaur, methyl red, citrate utilization, glycerol and glocose fermentation, gelatin liquifaction, arginine hydrolysis acisn

hydrolysis, starch hydrolysis, litmus milk fermentation. Tween 80, growth at 41 and 4°C. All above tests were performed according to Harrigan an McCance (8) and Collins and Lyne (6). Media and regents were prepared according to Standard Procedures Methods for the Examination of Water and Waste Water (2). Idetification of isolates were carried out according to Bergy's manual of Determinative Bacteriology (3), and confirmed using API 20 E and 20 NE system.

Scanning Electron Microscop(SEM):

Method described by Masaphy (11) was followed. At the end of the incubation peroid, samples of copper and plastic were washed twice with 5ml sterile distilled water, dried in the oven at 40°C for 1h, and examined using Philips PSEM 501 Scanning Electrom Microscope with a linked ESAX system.

Magnification x1.250. Bar, 10.0μm times were used. The composition of the correded copper and plastic pipes were analysed using Energy Dispersive Analytical X ray (EDAX) method.

RESULTS

Table 1 shows the effect of storing copper and plastic in sewage water on the microbial and chemical analysis.

It was found that with the copper at 20°C the maximum MPN $210x10^7$ and minimum pH value 7.20 were observed after 3 weeks storage. Total count was increased from 10 to $100x10^4$ and the turbidity unit was decreased from 1.6 to 0.8 NTU after 6 weeks. However with plastic at 20°C the maximum MPN and total count were observed after 6 weeks storage viz $150x10^7$. and $97x10^4$, respectively. Values of pH and the turbidity decreased during the storage time up to 6 weeks. At 37°C as incubation temperature for copper, maximum MPN $750x10^7$ was observed after 3 weeks storage. On the other hand, maximum total count ($340x10^4$) was observed after 6 wddks storage. Again this value was three times higher more than that at 20°C. However, with the plastic at 37°C, maximum total count, minimum pH and turbidity values were obtained after 3 weeks storage (Table 1). Various types of bacteria isolated from the inner surface of copper and plastic in sewage are presented in Table 2. Atotal of 40 isolates were identified, with *Moraxella phenylpyrouvica* being the most common (40%) in the copper pipe sample this, followed by *Flavobacterium indologenes* (25%) *Pseudomonas maltophilia* (20%) and *Pasteurella pneumotropica* (15%).

On the plastic pipe sample, *Pasteurella pneumotropica* and *Pseudomonas hydrophilia* (each comprising 25%) *Aeromonas hydrophilia* (20%), *Vibrio damsella* and *Vibrio fluvialis* (15%) each of the total identified bacterial population were detected (Table 2). Examination by SEM of the inside surfaces of the copper and plastic specimens that had been immersed in sewage water for up to 6 weeks showed that with copper speciment a number of cracks and pitting in the inner surface was occured at both 20 and 37°C (plate 1) and a massive build up of corrosion products as indicated from the chemical composition of the surface pipe by the X ray chemical analysis (Figure 1) to be predominatly sulfide and H_2S in the sewage facilitate to the formation of H_2SO_4 leading to subsequent corrosion of the copper specimen. On the other hand, the SEM photomicrograph of plastic speciment in plate 2 shows a number of both small and large pores inth e samples A and B compared with the control C. On contrast, a small amount of silicon and a large amount of chlorine were observed at 37°C.

Table 1 Effect of storage of copper and plastic on the microbial and chemical analysis.

storage		MPN/60 mlx10^7		Total count cfu/mlx10^4		pH		Turbidity	
period (wk)	Temp (^{0}C)	C	P	C	P	C	P	C	P
0	20	1.2	1.2	10	10	8.92	8.92	1.6	160
3		210	100	20	60	7.20	7.83	0.9	0.72
6		78	150	100	97	7.95	7.83	0.80	0.68
0	37	1.2	1.2	10	10	8.92	8.92	160	160
3		750	78	140	380	7.27	7.55	0.91	0.99
6		150	149	340	230	8.77	8.16	1.13	1.09

C= Copper , P= Plastic

Table 2: Distribution of microorganisms from copper and plastic in sewage samples.

Microorganisms	Numbers		Isolates (%)	
	C	P	C	P
Aeromonas hydrophilia		4		20
Flavobacterium indologenes	5		25	
Moraxella phenylphyrouvica	8		40	
Pasteurella pneumotropica	3	5	15	25
Pseudomonas maltophilia	4	5	20	25
Vibrio damsella		3		15
Vibrio fluvials		3		15
Total	20	20	100	100

C= Copper , P= Plastic

Figure 1: Scanning Electron Microscopy of Copper at 20°C (A), 37°C (B) and (C) control

Figure 2: Scanning Electron Microscopy of plastic at 20°C (A), 37°C (B) and (C) control

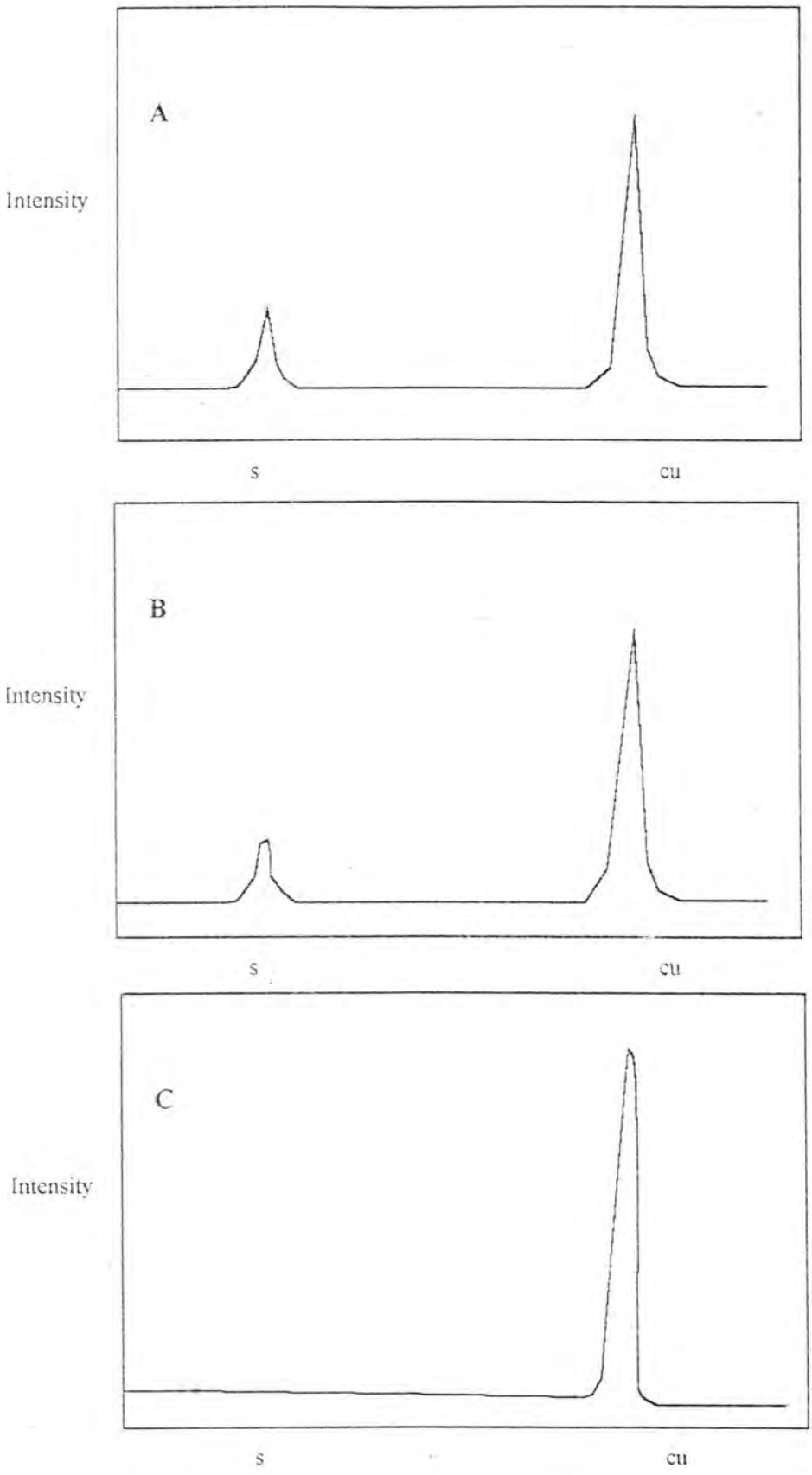

Figure 1: Analysis of Copper in sewage at 20^0C (A), 37^0C (B) and Control (C) after 6 weeks incubation

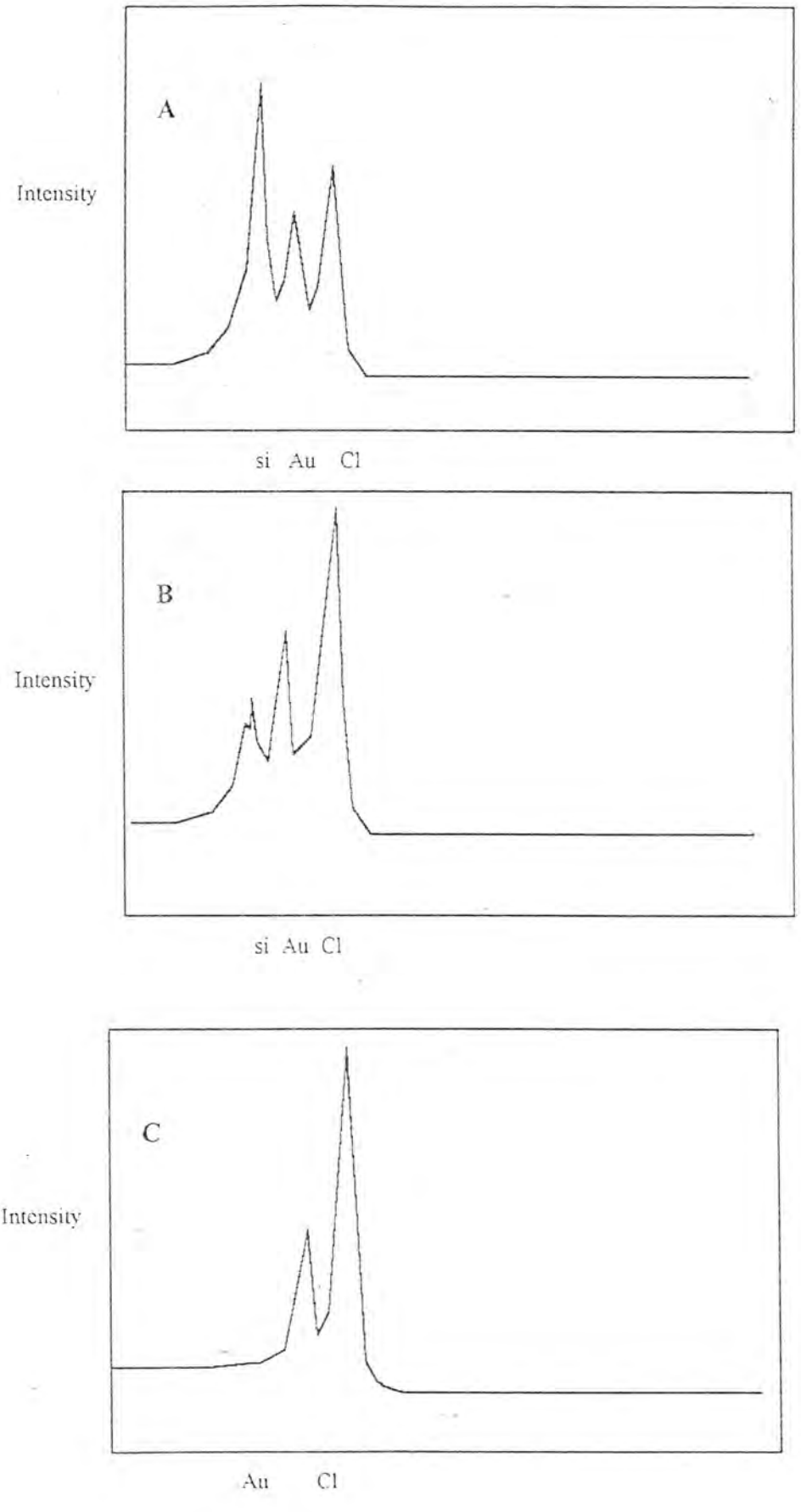

Figure 2: Analysis of plastic in sewage samples at 20^0C (A) 37^0C (B) and control (C) after 6 weeks incubation.

DISCUSSION

Microorganisms are responsible for the corrosion of some metals by producing acid(s) which may lead to corrosion by reducing the pH value of water (13).

The results described above indicated that the intial pH value of sewage sample was 8.92 then dropped considerably, during the first three weeks of storabe. This may be, probably, due to the formation of organic acid as a result of the metabolic activity of the organisms. Such metabolic activity increase is indicated by the increasing the total counts of organisms present in the sewage sample (Table 1). The acid produced may affect the solubility of the copper metal and hence increases the possibilities for corrosion. Shull (14) found a positive relationship between the decrease in the pH value and amount of dissolved copper in the water. He concluded that the carbon dioxide content of water, as indirectly measured by the pH, significantly affected the solubility of copper.

The turbidity values in copper and plastic at both 20°C and 37°C decreased, although the total counts of organisms increased. These results are in agreement with those of Goshko et al. ((7) who found a lack of correlation between standard plates count and the turbidity in some systems. LeChevallier (10) reported that the turbidity may vary in its nature and composition from region to region and its measurement will be influenced by particle size.

Data from Table 1 indicates that generally the greater counts of microorganisms were oobserved at 37°C than at 20°C in both copper and plastic pipes. This suggests that 37°C was closer to the optimum temperature than to 20°C, for growth of most those microorganisms present in the sewage.

The results also showed that differences in the distribution and proportion of the microofganisms colonizing the copper and plastic pipes may be apparent because of the sampling discrepancies. In copper sample, SEM observation indicated a number of cracks and pitting and a number of microorganisms were seen on the surface of the samples. This could be involved in the copper corrosion.

The EDAX analysis (Figure 1) indicated the massive build up of corrosion products to be predominately sulphur based. Although cracks can be seen in the copper samples, they are not seen in the plastic samples. In the plastic samples only pores can be discerned. The results of ESAX analysis of plastic (Figure 2) showed variation in the amount of chlorine and silicon between the samples incubated at 20°C and 37°Cm resoectively. These variations could be related to the differences in incubation temperature which allow some microorganisms to grow and metabolized faster than others.

The results reported above suggest that the Gram negative bacteria isolated from plastic and copper pipes in sewage gave effects in decreasing the pH value and hence increasing the possibilities for the deterioration of copper and plastic pipes.

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A Study on Bioaccumulation of Some Heavy Metals in Four Fresh Water Fish Species of Iraq.

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الخلاصة

تم تعيين تراكيز المعادن الثقيلة (الرصاص والمنغنيز والحديد والكوبلت والنيكل والنحاس والخارصين) في عضلات (لحم) وغلاصم اربعة انواع من اسماك المياه العذبة البني (Gunther) *Barbus Sharpeyi* والشبوط (Heckel) *Barbus grypus* والشلك *Aspius vorax* (Heckel) والقطان *Barbus xanthopterus* (Heckel) تم اصطيادها من نهري دجلة وديالى عند بغداد وقد بينت النتائج ان الانواع التي جمعت من نهر ديالى تقوم بتراكم اكثر كمية من المعادن الثقيلة مقارنة مع تلك التي تم جمعها من نهر دجلة وحتى ضمن البيئة الواحدة تظهر الانواع كميات متباينة من المعادن الثقيلة في انسجتها وبصورة عامة كانت تراكيز المعادن الثقيلة اكثر في الغلاصم مما هي عليه في اللحم وقد لوحظت اعلى قيم التراكم الحيوي في الشبوط واوطاها في الشلك .

ABSTRACT

The concentrations of the heavy metals (Pb, Mn, Fe, Co, Ni, Cu and Zn) in flesh and gills of four fresh water species of fish: *Barbus sharpeyi* (Gunther), *Barbus grypus* (Heckel), *Aspius vorx* (Heckel), and *Barbus xanthopterus* (Heckel) were investigated. The fish specimens were collacted from Tigris and Diyala rivers at Baghdad. The results indicated that the species collected from Diyala river accumulated more heavy metals than those of Tigris river, even within a single habitat the concentrations of heavy metals were different in the tissues of the above species. Generally, the concentrations of the geavy metals were more in gills than in flesh. The highest values of accumulation were observed in *Babus grypus* while the lowest were recorded in *Aspius vorax*.

INTRODUCTION

The human environment can only be viewed as a holistic unit whose components are closely knit and any change or perturbation to any part will influence the whole (1). In environmental pollution studies, there is often the need for routine monitoring of toxic constituents in aquatic substrates (2).

Fish form an important food item, they play a vital role not only in meeting our food demand but also in supplementing our protein requirement, they are also good indicators of water quality, and no perennial river can be considered in satisfactory condition unless a variety of fish can live and survive in it. Being an end product of aquatic food chain, fish reflect both satisfactory water

quality and a suitable habitat for food supply, shelter and breeding sites (3).

Several of the priority toxins accumulate in aquatic organisms and are magnified in the food chain, biological-accumulation of certain toxins including heavy metals, can result in acute effects in fish that are the ultimate consumers in the aquatic food chain. Hence, the hazard to human health results from consumption of the contaminated fish (3, 4, 5).

A variety of physicochemical and biological parameters interact to influence the availability of metals to aquatic life, poisonous effects on fish life also relate to the character of the watercourse, species of fish and season of the year (3, 6, 7). The relationship between acute toxicity and the concentration of metal in different tissues has an important value in diagnosing the cause of fish kills (8).

Latif et al. (9) suggested to do more research work on the heavy metals accumulation in different Iraqi fish species, in order to know the pattern of accumulation in more species and the outcome of this pollution on human life.

The present work was carried out to determine the concentrations of some heavy metals (Pb, Mn, Fe, Co, Ni, Cu and Zn) and their patterns of accumulation in four economically important species of fish in Iraq: *Barbus sharpey* (Gunther), *Barbus grypus* (Heckel), *Aspius vorax* (Heckel) and *Barbus xanthopterus* (Heckel) from two different water bodies within Baghdad district (Tigris river and Diyala river).

MATERIALS AND METHODS

Fish samples were collected from different sites of Tigris and Diyala rivers (Figure 1) during the period from January to May, 1990. A total of 58 specimens of the different species investigated in the present study were captured (Table 1). The dry method of digestion was used according to Latif (8) which is proved to be supperior to other methods by the author himself. Flame atomic atomic absorption measurements were made with a Pye Unicam SP9 atomic absorption spectrometer.

RESULTS

Data obtained from the present investigation are presented and illustrated in Table 2 and Figures 2,3. Each value represents the average of eight to eight to eleven replicate samples, the concentration of all heavy metals studied were expressed in µg/gm. dry weight.

Lead (Pb)

The concentration of Pb in gills was found to be higher than those of flesh in both *Barbus sharpeyi* (from Tigris river) and *Barbus xanthopterus* (from Diyala river). The total concentration (gill & flesh) in *Barbus xanthopterus* was higher than those of *Barbus sherpeyi* whereas the percent distribution in gill showed a contradictory pattern.

In case of *Aspius vorax* the concentration in gills was higher than those in flesh with respect to Tigris specimens, the opposite was observed in Diyala specimens and the total concentration was almost higher in Diyala specimens.

In *Barbus grypus* (from both habitats) the concentration in flesh was higher than those in gill, the total concentration of Tgris specimens was higher than Diyala specimens but the percent distribution in flesh showed the adverse pattern.

generally, the Pb concentration was highest in *B. xanthopterus*. In contrast, *B. sharpeyi* showed the lowest concentration among the species strudied, the percent distribution in gills and flesh showed the highest value in *B. sharpeyi* and *B. grypus* (from Diyala river) respectively.

Manganese (Mn)

Gills appears to accumulate higher concentration of Mn than flesh in all species except *B. grypus*. The percent distribution in

gills was found to be higher in *B. sharpeye* than those of *B. xanthopterus*.

Aspius vorax in both habitats showed similar distribution in gills while the total concentration was higher in Diyala specimens.

B. grypus showed the opposite pattern of accumulation, the total concentration and percent distribution in flesh were higher in Tigris specimens.

As awhole *B. grypus* (from Tigris river) showd the highest concentration whereas the lowest concentration was noticed in *Aspius vorax* (from Tigris). The highest ratio of accumulation in both gill and flesh was recorded in *B. sharpeyi* and *Barbus grypus* (from Tigris river) respectively.

Iron (Fe)

The content of iron in all species was high in comparison with other studied heavy metals exclude zinc. Generally the concentration in gills was higher than those in flesh, this is correct to all species but *B. grypus* which showed the opposite pattern.

The total concentration in *B. sharpeyi* was higher than those in *B. xanthopterus*, while in *Aspius vorax* (from Diyala) the concentration and percent distribution in gills were higher than the same species in Tigris river. As mentioned above, *Barbus grypus* accumulated more iron in flesh than in gill. The total concentration and percent distribution in flesh was higher in specimens captured from Tigris river.

In general, the highest amount of iron was observed in *B. grypus* (from Tigris), the opposite was noticed in *Aspius vorax* which was also collected from Tigris river. With respect to percent distribution in gills and flesh, *Aspius vorax* (from Diyala river) and *B. grypus* (from Tigris river) respectively showed the highest values.

Cobalt (Co).

The general pattern of Co accumulation was similar to that of Fe. No big variations were observed in Cobalt content between all species studied. The percent distribution in gills and flesh was as follows: *B. sharpeyi* and *Aspius vorax* (from both habitats) showed higher concentration in gills than in flesh, the opposite was correct for *B. xanthopterus* and *B. grypus* (from both habitats).

The relatively highest concentration and percent distribution in flesh was recorded in *B. grypus* (from Diyala river), whereas the highest percentage in gills was noticed is *Aspius vorax* from Diyala river also.

Nickel (Ni)

The concentration of Ni in *B. sharpeyi* and *B. xanthopterus* showed the same pattern which was noticed in Fe and Co, but the total concentration in the both species was similar.

Aspius vorax (from Tigris river) exhibited more concentration in flesh than in gill, the oppoite was observed in the same species which was captured from Diyala river and the total concentration in Tigris specimens was almost higher.

In case of *B. grypus* from both habitats flesh showed higher concentration of Ni than gill and the total concentration in Diyala specimens was more than double when compared with Tigris specimens.

With regard to the species altogether, it's clear from the data obtained that the highest concentration of Ni was observed in *B. grypus* (from Diyala river) and the opposite in *Aspius vorax* (from Diyala river), while the highest percent of distribution in both gills and flesh were found in *Aspius vorax* and *B. grypus* (both from Diyala river) respectively.

Copper (Cu)

Data obtaied from the present investigation show that all species accumulate a low concentrations of Cu in comparison with other studied metals.

In contrast to all studied metals, *Barbus sharpeyi* was accumulated more Cu in flesh than in gills and the opposite was found in *B. xanthopterus*.

Aspius vorax in both habitats showed the same pattern of accumulation as the concentration in gills was higher than in flesh and the total concentration in Diyala specimens was somewhat higher.

B. grypus showed the same pattern of accumulation that was noticed in *B. sharpeyi*, the total concentration and precent distribution in flesh was higher in Diyala specimens.

Generally, the highest concentration of Cu among all species studied was recorded in *B. gryous* (from Diyala river) the lowest concentration of Du was found in *Aspius vorax* (from Tigris river) but with the highest percent of distribution in gills.

Zinc (Zn)

On the contrary to Cu, Zinc showed the highest values in all species, its concentration exceeded that of Cu with 24-64 times.

The parent distribution showed two different patterns in both *B. sharpeyi* and *B. xanthopterus* as the concentration in gills was higher than in flesh with respect to *B. sharpeyi*, while *B. xanthopterus* showed the opposite pattern with higher total concentration.

Aspius vorax from both habitats showed the same pattern of distribution as the gills showed more concentration than flesh with similar percent of distribution while the total concentration in Diyala specimens was higher.

Zn concentration in *B. grypus* (from both habitats) showed the same pattern of distribution like other metals mentioned formerly.

With respect to the species studied altogether, it seems that the highest concentration of all heavy metals studied with highest percent of distributiono in flesh were found in *B. grypus* while the lowest concentration was recorded in *Aspius vorax* with highest percent of distribution in gills.

DISCUSSION

The accumulation of heavy metals and their distribution in different tissues of fish depend on many factors including environmental factors especially pH and hardness of the water (10), the concentration of the metal; exposure time; physiological condition of fish and also the fish species themselves.

The highest concetrations of (Zn, Cu, Ni, Co) and (Fe, Mn) were found in *B. grypus* maintained in Diyala river and Tigris river respectively. On the other hand the highest concentration of Pb was recorded in *B. xanthopterus*. Generally, Diyala specimens accumulated more heavy metals than those of Tigris, this was more clear in *Aspius vorax*. This results confirm by the foundation of AL-Mukhtar et al. (11) on the water quality of Diyala river.

The lowest value was found in *Aspius vorax* from Tigris river with respect to (Zn, Cu, Fe, Mn) whereas the lowest concentration of Pb was observed in *B. sharpeyi* from the same habitat, this may be due the variation in feeding habits and the occurrence within the water body.

Lead is perhaps the nost (ubiqutous) heavy metal pollutant in the environment, its concentration in aquatic organisms varied substantially within and between different sectors. Organisms that live and feed in the sediments have higher Pb concentrations than those feed in water, there was general correlations than those feed in water, there was general correlation between feeding habits and lead concentrations in the body tissues. Insectiveres had the highest concentrations of lead, herbivores had intermediate levels while carnivores had the lowest concentrations (1).

Al-Kugaisis (12) identified some insects in the gut content of *B. xanthopterus* while none of them was observed in *B. sharpeyi* in addition the algal species that he noticed in the gut content of *B. xanthopterus* indicate the occurrence of this species in

more polluted sectors in comparison with the other species. The results of the present study follow the aabove assertion and the high content of Pb in *B. xanthopterus* is probably due to its food habit, as the specimens of Al-Dubaisi study (12) were adopted for the determination of heavy metals in the present study.

There is no evidence that lead is a serious health problem in fish (acute lead poisoning is wxtremely rare) and there was no bilogical magnification of lead through aquatic trophic structure (1). In areas non contaminated by Pb, fish contain about 0.6ppm Pb as an average (13). The values recorded in the present investigation will accertain that the areas from where the samples were collected are somewhat contaminated with Pb.

Cross et al. (14) showed that Fe and Mn concentrations may be at equilibrium between the fish and their environment. In the present study it is found that Fe and Mn concentrations in *B. grypus* and *B. sharpeyi* which were captured from Tigris river were more than their concentrations in *B. grypus* and *B. xanthopterus* obtained from Diyala river. The opposite was observed with respect to *Aspius vorax* from both habitats.

Generally, manganese concentration in gills of all species was more than in flesh exclude *B. grypus*, this may indicate that Mn concentration in water was not high enough to cause adverse effect on Mn accumulation by gills (13).

Iron is concentrated to a considerable degree by fish, high levels are accumulated in gills (8), this was true in the studied species in general as Fe concentrations conctituted the second highest values after Xn.

Apart from Bazzaz (1) who showed that Cobalt was an effective inhibitor of electron transfer in mitochondris, there is no data available on the Cobalt content in fish to the best of our knowledge. In the present investigation no clear variations were observed among the species, almost all species showed similar concentrations of Co with the exception of *B. grypus* in Diyala river.

Nickel is not accumulated in significant amounts by aquatic animals and orally ingested Ni has a very low toxicity to man (15). Generally, all species studied but *Aspius vorax* maintained in Diyala river had more Ni in the flesh than in gill, this comes in accordance to the results obtained by (15), and the high content of Ni in the flesh of *B. grypus* from Diyala river may indicate that the sectors from where the specimens captured were more polluted.

The relatively high amoun of Copper in the flesh of *B. grypus* when compared with other species indicate that *B. grypus* is not capable of regulating Cu content and hence higher Cu concentration is found in this species (16). It is clear from the results that Cu concentration in all studied species was the lowest among other metals, this comes in accordance with previous studies (9, 13) the reason may be due to the fact that Cu is retained by liver but it is lost from the gills unless there is a continuous supply of Cu (15).

Zinc is an essential element and a common pollutant, it is readily accumulated by fresh water fishes from both food and water (17). Mount (18) found that fish accumulate Zn at a modest rate during chronic exposure. A part from *Aspius vorax* maintained in Tigris river all species showed highest concentrations of Zn in comparison with other studied metals.

The percentage distribution of the studied metals was generally more in gills than in flesh, this finding agrees with previous studies (8, 9, 13). In *B. grypus* the contradictory pattern of accumulation was observed where the percentage distribution was higher in flesh than in gills, this does not agree with previous studies on the same species (9, 13).

CONCLUSION

From the results of the present investigation we can conclude the following points:

Generally Diyala species accumulated more heavy metals than Tigris species, even within the same habitat the accumulation values vary from on species to another, generally, Tigris species can be ordered descendigly as follows accordin to their content or the metas altogether (*B. grypus*, *B. sharpeyi* and *Aspius vorax*) while Diyala species are ordered as (*B. grypus*, *B. xanthopterus* and *Aspius vorax*).

The percentage of accumulation was more in gills than in flesh with respect to *Aspius vorax* and *B. sharpeyi*, adverse pattern was observed in *B. grypus*, whereas in *B. xanthopterus* the concentrations of Pb, Mn and Cu were higher in gills than in flesh.

It is clear from the findings that considerable amount of heavy metals is bioaccumulated in four of the important fishes of Iraq. Although the concentrations did not reach the lethal levels but it may be a reason behind the abnormalities in both operculum and base of dorsal fin which are observed in some individuals (19).

The metals can be ordered as follows according to their degree of accumulation in the species altogerher (Zn>Fe Ni>Pb>Mn>Co>Cu).

As for the average wieghts of the samples under sstudy, *B. sharpeyi* was the heaviest followed by *B. grypus*, *B. xanthopterus* and *Aspius vorax*. The concentrations of the metals in the above species didn't follow the same sequence as above; since *B. grypus* showed the highest concentration followed by *B. xanthopterus*, *B. sharpeyi* and *Aspius vorax*.

It is evident from these findings that, there is no definite pattern of accumulation, it varies with variations in species studied on one hand and according to environmental conditions, habitats and physiological conditions of the fishes on the other hand and even within the single species the values differ from one tissue to another. Accordingly, there is a need for extensive futurestudies including the analysis of heavy metals in both water and sediment, some important chemical parameters, biological aspects and food habits these altogether beside the study of bioaccumulation of the important metals in different tissues of fishes in order to investigate the reasons of pollution depending on the above oriteris as a whole, this is in one word and in other word to prevent the contamination of rivers used as water sources ultimately increasing the fish productivity.

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Table 1: Numbers, Lengths and weights of fish species from the studied areas.

area	species	Number	Length (mm) (Range)	Length (mm) (average)	Weight (g) (average)
Tigris river	*Barbus sharpeyi*	10	308-320	317	337.2
	Barbus grypus	11	351-362	356	295.1
	Aspius vorax	9	239-251	248	78.6
Diyala River	Barbus xantho-pterus	11	248-260	257	173.2
	Barbus grypus	9	332-344	338	315.8
	Aspius vorax	8	217-234	229	77.9

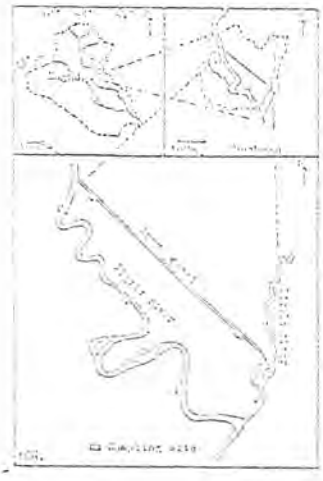

Figure 1: Map of Iraq showing the study area and sampling sites

Table 2: The average of heavy metals concentrations (µg/gm.) in the tissues of fish species from the studied areas.

Area	Metal / Tissue / Species	Pb		Mn		Fe		Co		Ni		Cu		Zn	
		flesh	Gill	flesh	Gill	flesh	Gill	flesh	Gill	flesh	Gill	flesh	Gill	flesh	Gill
Tigris river	*Barbus sharpeyi*	0.47	0.76	0.12	0.96	3.87	5.47	0.29	0.38	1.63	1.76	0.10	0.06	5.13	11.4
	Barbus grypus	2.16	2.04	4.66	0.17	23.16	1.01	0.56	0.15	2.49	1.62	0.26	0.03	21.07	5.61
	Aspius vorax	1.36	1.92	0.02	0.11	1.113	1.42	0.29	0.37	1.62	1.33	0.03	0.06	0.45	1.75
	Barbus xanthopterus	2.75	2.84	0.42	0.57	3.14	2.68	0.25	0.14	1.95	1.50	0.05	0.09	12.17	8.85
	Barbus grypus	2.35	0.41	0.75	0.31	10.9	2.03	2.15	0.13	9.45	0.86	0.60	0.05	34.55	7.08
	Aspius vorax	3.14	0.61	0.11	0.47	0.79	4.12	0.08	0.59	0.86	1.36	0.05	0.07	2.00	7.84

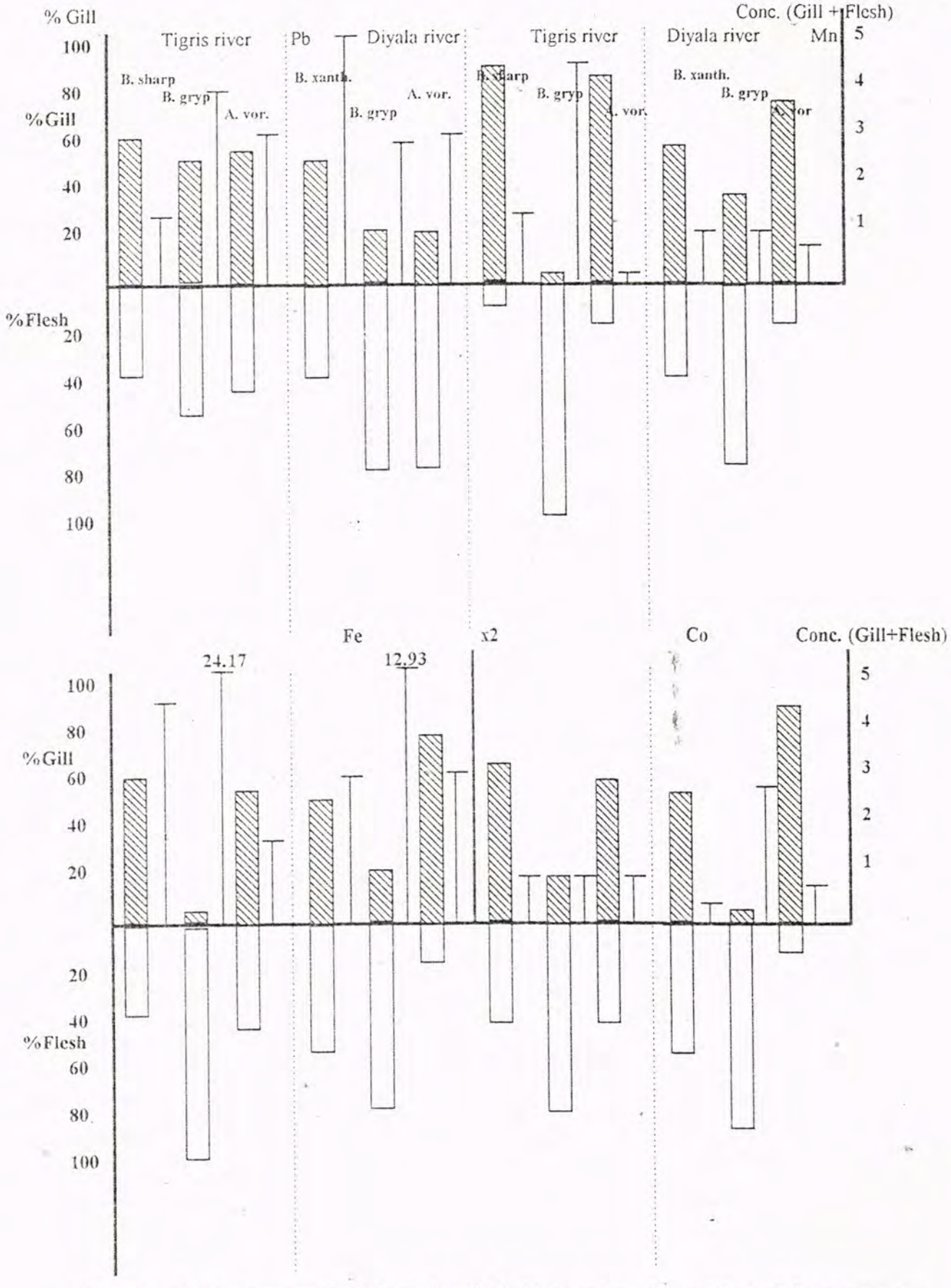

Figure 2: Total concentration (Conc. in gill+flesh) as µg/gm. dry weight and the percentage distribution of (Pb, Mn, Fe, Ce,) in gills and flesh of the studied species within Tigris and Diyala Rivers.

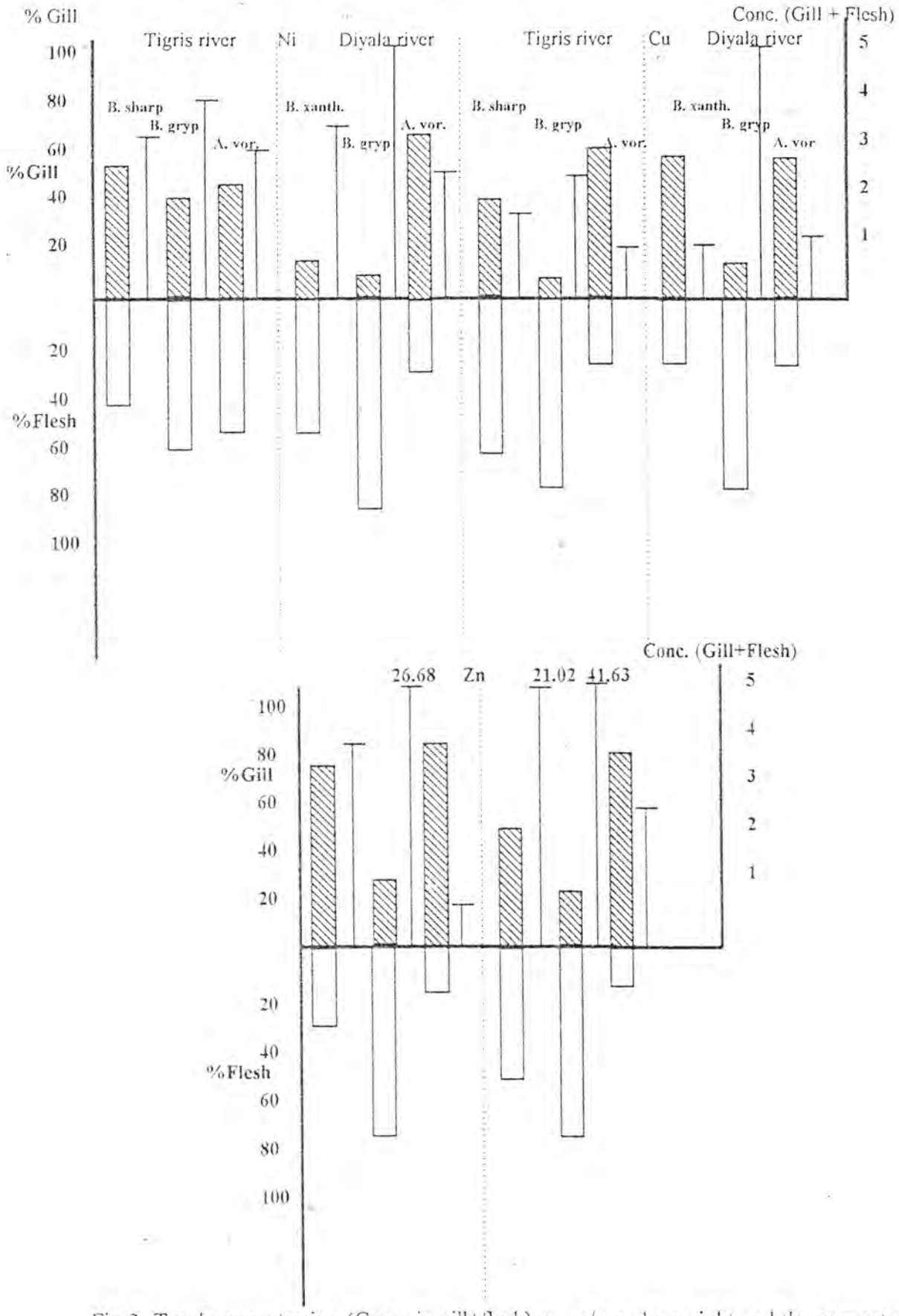

Fig 3: Total concentration (Conc. in gill+flesh) as µg/gm. dry weight and the percentage distribution of (Ni, Cu, Zn) in gills and flesh of the studied species within Tigris and Diyala rivers.

Recording of The Species of *Myxidium butschli*, 1882 (Sporozoa : Myxosporidia) for The First Time in Fishes of Iraq

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الخلاصة

تم تسجيل النوع *Myxidium pfeifferi* والنوع *Myxidium rhodei* Leger, 1905 من كيس الصفراء وكبد سمكة البني من بحيرة سد القادسية وسط العراق . يمثل ظهور هذين النوعين من الطفيليات اول تسجيل لهما في العراق وتسجيل خامس جنس من البوغيات المخاطية المسجلة من اسماك العراق . لم تظهر اصابة بهذا الجنس في ثمانية انواع اخرى من الاسماك من نفس المنطقة والمفحوصة خلال نفس الفترة (شباط – آب ١٩٩٣) . تم وضع مفتاح تشخيصي لتمييز الاجناس الخمسة من البوغيات المخاطية المسجلة لحد الآن في اسماك العراق .

ABSTRACT

Myxidium pfeifferi Auerbach, 1908 and *Myxidium rhodei* Leger, 1905 were recorded from the all bladder and liver, respectively, of the cyprinid fish *Barbus sharpeyi* from Al-Qaddisiya Dam Lake of mid Iraq. The occurrence of these parasites represents their first record in Iraq and the record of the fifth genus of myxosporidians to be reported from fishes of Iraq. Eight other fish species collected from the same site and examined during the same period (February to August 1993) showed no infection with any of *Myxidium* spp. An identification key was constructed to distinguish the five myxodporidian genera so far recorded in fishes of Iraq

INTRODUCTION

In order to improve the stocks of valuable commercial fisheries in their natural waters, a detailed knowledge of the parasites inhabiting such waters is required (1). To achieve such task, the Fish Research Centre of the I.A.E.C., since its establishment, surveyed different water bodies in Iraq for fish parasites. Among reults of such surveys are those of Al-Khateeb et al. (2) and Balasem et al. (3).

Severe epidemic diseases of fishes are frequently found to be due to myxosporidian infections (4-6). However, Rofers and Caines (7) stated that coelozoic myxosporidians generally do not produce significant pathological effects.

In iraq, four genera of myxosporidians were reported. *Myxobolus* and *Myxosoma* were first recorded by Herzog (8), *Unicauda* by Rahemo (9) and *Thelohanellus* by Abdûl-Ameer (10). The first two genera are so far represented in fishes of Iraq with nine and two species, respectively, while the last two genera are represented with one species each (11). The present paper is intended to report on the occurrence of two species of myxosporidian genus *Myxidium*. This record gives two

additional items to the parasitic fauna of fishes of Iraq.

MATERIALS AND METHODS

Fish samples were taken from Al-Qaddisiya Dam Lake on the Euphrates river at about 260 kms west of Baghdad during the period from February to August 1993. Description and map of this lake are given by Al-Alusi (12). Cill nets and beach seine nets (600-800 mm mesh size). Trawled with the aid of menchanical tractors, were used to catch the fishes.

After recording measueements, fishes were examined as soon as possible. Skin, gill and blood smears were prepared and examined under microscope. Smears were prepared from the internal organs of fishes, preserved in 5% formalin, examined and photographed under compound microscope type Olympus PM-10A. Drawings were done with the aid of a camera lucida. Shul'man's (13) account was used for the measurements (Fig. 1a) and description of *Myxidium* spp.

RESULTS AND DISCUSSION

A total of 82 specimens belonging to nine fish species were inspected for parasites. These included 4 *Alburnus caeruleus*, 2 *Aspius vorax*, 7 *Barbus esocinus*, 5 *B. Grypus* , 7 *B. Luteus*, 3 *B. Sharpeyi*, 26 *B. Xanthopterus*, 22 *Cyprinus carpio* and 6 *Liza abu*. Only two specimens of *B. Sharpeyi* were infected with *Myxidium*. The following is an account on the occurrence and description of *Myxidium* species. All measurements, unless otherwise indicated, are given in microns.

Myxidium pfeifferi Auerbach, 1908 (Fif. 1b)

Host : One male *B. Sharpeyi*, standard length 29.8 cm., total length 35.7 cm., total weight 450 gm.
Locality : Al-Qaddisiya Dam Lake.
Date of collection : 17 February 1993.
Habitat : Call bladder.

Spores fisiform with occasional central constriction ; poles of spore somewhat rounded. Surface of valves slightly striated longitudinally. Length of spore 12-15, width 5-5.5. Polar capsules narrow and pointed; length of polar capsules 4.5-5, width 3.5-4.5. Trophozoite)plasmodium) was not found.

Myxidium rhodei Leger, 1905 (Fig. 1c)

Host : One male B. Sharpeyi, standard length 29.0 cm., total length 34.5 cm., total weight 547 gm.
Locality : Al-Qaddisiya Dam Lake.
Date of collection : 12 August 1993.
Habitat : Liver.

Spora form and size quite similar to that of M. Pfeifferi. Spore narrower centrally; poles of spore pointed cuticle less striated in comparison with that of M. Pfiefferi. Length of spore 13-16, width 5.5-6. Polar capsules lie either along longitudinal axis of spore or slightly to on side; length of polar capsules 4-5.5, width 4-4.5. Trophozoite (vegetative stage as cyst) was not found.

The description and measurements of both *M. Pfiefferi* and *M. Rhodei* are so agreeable with those reported by both Shul'man (13) and Bykhovskaya-Pavlovskaya et al. (14). However, polymorphism and abnormality are well known in some species of *Myxidium* such as *M. Coryphaenoidium* as reported by Moser et al. (15).

As demonstrated by Shul'man (13), infection with *Myxidium* takes place through three wayes : a- ingestion of spores during fish feding on the mud, b- fish consumption of dead diseased fish, or c- predation of infected fish. The feeding habits of *B. Sharpeyi*, as demonstrated Al-Kanaani (16), explaines that only the first way of infection of *B. Sharpeyi* with *Myxidium* spp. Of the present investigation is possible. Hence, some

other freshwater fishes of Iraq are expected to gain such infection as they have fish species may result in detection of new hosts for *Myxidium* spp. In Iraq, *M. Pfeifferi* was reported from 12 fish species in the previous USSR, while *M. Rhodei* from 19 fish species (13).

Fif. (1) a- Measurement of *Myxidium*, A= length of spore, B= width of spore, C= length of polar capsule, D= width of polar capsule.
B- *Myxidium pfeifferi* (Bar = 5microns).
C- *Myxidium rhodei* (Bar = 5 microns).

In connection with the pathological effects of *Myxidium* spp. Ot the present study, no such effects were detected due to the light infection rate among examined fishes (two out of 82). However, *M. Oviforme* produce abscesses in the liver and was suspected of being a contributary factor in extensive salmon mortalities (17). This species is also reported to cause intensive inflammation in billiary ducts of the liver in severe infection (7).

Finally, in order to make use of the present paper, the following key, modified from Hoffman (18), is included to facilitate quick recognition of the five genera of the myxosporidians (inclusive of *Myxidium*) do far recorded in fishes of Iraq.

1- One polar capsule present.. Thelohanellus.
1- Two polar capsule present 2

2- One polar capsule present in each end od spore ..... Myxidium
2- Two polar capsules present in one end of spore 3

3-No : iodinophilous vacuole present ..... Myxosoum
3- Iodinophilous vacuole present 4
4- Spore ovale with single tail-like process (not extension of shell valves) present .....Unicauda
4- Spore oval, with no posterior processes .....Myxobolus

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Light and Electron Microscopic Srudies of The Juxtaglomerular Apparatus of The Camel (Camelus Dromedarius) Kidney.

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الخلاصة

تم دراسة المنطقة المجاورة للكبيبة . في كلى الجمل بواسطة المجهر الضوئي والالكتروني وتبين بان الخلايا النارزة للرنين لا تحتوي على حبيبات مما يدل على ان افراز الرنين في الجمال يتم بصورة غير حبيبيه.

ABSTRACT

The juxtaglomerular apparatus (JGA) of the camel *Camelus dromedarius* kindey was studied by light and electron microscopy. The camel's JGA was similar to other mammalian JGA in many respects, located in the glomerular hilum, consists of macula densa, vascular component and Goormaghtigh cell. The cells of the JGA lack the granules which suggest that renin storage in this species might be in a non-granular form.

INTRODUCTION

It has been well-established that most of the renin in the kidney is synthesized and stored in the myoepithelial cells of the afferent arteriole of the juxtaglomerular apparatus (JGA); ultrastructural and immunocytochemical findings have demonstrated the presence of renin in these cells (1-3). Renin is responsible for the production of angiotensin I from plsma angiotensinogen. The renin-angiotensin system is involved in a number of physiological activities including the maintenance of systemic arterial blood pressure, stimulation of aldosterone secretion from the zona glomerulasa of the adrenal cortex and the intra-renal control of glomerular filtration rate (4).

In mammals, JGA is composed of the afferent arteriole, the efferent arteriole, the macula densa (MD) of the distal tubule (DCT) in the triangular area delineated by these three structures, the polar Goormaghtigh (GO) cells (5). The JGA has been studied in human (3, 6) and different experimental animals including mouse (7, 8), rat (2), dog(9), sheep (10) gird (1, 12) and rabbit (13). The camel, *(Camelus dromedarius)* commonly known as the "shop of the desert" is a very important animal used for transport where no vehicle could be used on sand. This animal can withstand dehydration and live for 17 days on dry food in summer seson. The available information on the camel's kidney is mainly concerned with general morphology (14, 15). Recent electron microscopic study has described the ultrastructure of the camel's renal corpuscle (16). The present study concerns with the light and electron microsopy of the JGA of this creature.

MATERIALS AND METHODS

Five pairs of kidneys were collected from young-adultcamels of both sexes at Al-Najaf sloughter house. Small pieces of tissue were taken from different parts of the cortex. Tissues prepared for light microscopy were

fixed in variety of fixatives (10% formal saline, Zenker formalin and Helly's fluid), embeded in paraffin wax and sections cut at 5μm. Sections were stained specifically for juxaglomerular cells (GJCs) demonstration with alkaline crystal violet by the method of Harada (17), Endes's combined trichrome method (18) and Bowie's method (19).

Materials for electon microscopy were fixed in 4% glutaraldehyde in phosphate buffer, post fixed in 1% osmium tetraoxide and embeded in resin. Thick sections (1-2μm) were stained with methylen blue studied by light microscopy until the JGA were identified. Thin sections (400-600nm) were cut from selected areas, stained with lead citrate and uranyl acetate and viewed with Philips electron microscop;

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RESULTS

By light microscop; the JGA of the camel appeares to be composed of MD, the JGCs and the GO cells (Fig. 1). The MD cells appeared as tall cell in the DCT neighbouring to the GO cells (Fig. 1). The GO cells lie and filled the space between the two hilar arterioles, the MD cells of DCT and the glomerulus (Fig. 1). The JGCs of the AA lack the granules despite the use of several different specific stains and fixatives.

Ultrastructural examination revealed that the GO and JGCs were agranular (Fig. 2, 3) and the myoepithelial cells were seen to contain spherical mitochondria and a few cytoplasmic vacuoles.

DISCUSSION

The present study has shown that the JGA of the camel being similar in many respects to the JGA of other mammals (2, 3, 6-11). This species was found to possess all the cell types usually sen in the JGA, though the myoepitheloid cells appeaed devoid of secretory granules even under the electron microscope. Species differences in renin secretion are well known. The number of renin-positive cells may be estimated at light microscopic level using "Jg index" or by measuring the length of renin-positive part of the AA upstream from the glomerulus (20). Under normal conditions, myoepitheloid cell granules are very numerous in mouse and pig, intermediate in man and rat (7), but progressively decrease in rabbit, cat dog and monkey (21). They are lowest in guiñea pig, chines hamester (22), sheep (10) and Jird (12). In the guinea pig, there is only very sparse JG granulation which is often undetactable by light microscopy; granules appearing only when the JGC is stimulated by adrenalectomy (23). In the jird, Al-Ani (12) has observed only one JGC by electron microscopy which explain the unability to detect them by light microscopy (11, 12). However, the number of renin-positive cells vary with the functional state of renin-angiotensin system. Long-lasting stimulation of renin secretion "e. G. By Na-depletion (10), by experimental constriction of renal artery (24) or by furosimide treatment (8) "result in an increase, wheras inhibition" e. G. Na-loading (10, 25) or dehydration (25, 26) "results in a decrease of renin-positive cells.

Bucher et al (23) have suggested that there are two site of renin storage; granular and axtraglanular. It might be that in the camel, there is a high rate of renin turnover with little or no storage, as has been proposed for the kangaree desert rat (27), for although renal renin content has not been measured in this species, renin appears to have been found in all mammalian kidneys so far studied.

The present study has also shown GO cells lining between the two hilar arterioles, the MD of the distal tubule and the glomerulus, these cells are similar to other species's GO cells usually are non granular. However, Sottiurai and Malvin (9) have shown few granules in dogs fed for 21 days low Na-diet. GO cells are alaways in direct contact with all other component of the JGA and is believed by some workers to be

concerned with the control of renin secretion (5, 9). Further radioimmunoassay and immunohistochemical studies are needed to study the presence of renin in this species.

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Figure 1. Section through the juxtaglomerular apparatus of a camel. AA, affrent arteriole; EA, efferent arteriole; G, Glomerulus; GO, Goormahtigh cells; MD, macula densa, Endes stain, X. 400.

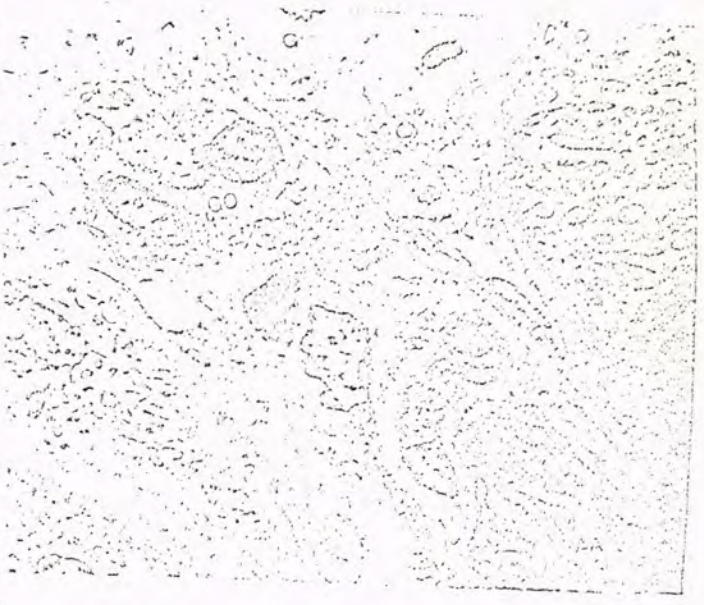

Figure 2. Section through affrent arteriole (AA) and Goormahtigh cells (GO) in the vicinity of glomerulus (G). X. 1450.

Figure 3. Section through afferent arteriole (AA) "enlargement to figure 2". Note the absence of granulation from the myoepitheloid cells (M). X. 3400

The Effect of Some Heavy Metals on The Fungus *Pythium plaroticum*

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الخلاصة

تم دراسة تاثير ثلاثة عناصر هي الرصاص ، الزنك والمنغنيز بالتراكيز (0.3, 0.25, 0.2, 0.15, 0.1, 0.05 جزء في المليون) على الفطر *Pythium pleroticum* . وبينت النتائج ان تركيز 0.15 جزء في المليون من الرصاص قد ادى بعد يومين من المعاملة الى خفض معنوي في تكوين ونضوج وعدد العلب البوغية الفارغة ، وكذلك ظهر انخفاض معنوي في نمو قطر مستعمرة الفطر في تركيز 0.05 جزء في المليون للرصاص والزنك ، بينما تطلب انخفاض عدد الحوافظ البوغية الناضجة والفارغة الى تركيز اعلى من الزنك . اما المنغنيز فلم يظهر أي تاثير معنوي على تكوين الحوافظ البوغية ، في حين حدث انخفاض معنوي في نمو قطر مستعمرة الفطر عند التركيز 0.1 جزء في المليون .

ABSTRACT

The effect of following concentrations of Lead, Zinc and Manganese (0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 ppm) on *Pythium pleroticum* was studied within 2 days of observation, 0.15 ppm of lead inginbited sporangial formation, maturation and reduced the number of empty sporangia. Colony diameter was significantly reduced by the pressence of 0.05 ppm of lead and Zinc, while higher concentration of Zinc was needed to reduce the number of mature and empyting sporangia. In case of Manganese there was no signigican effect on sporangia production but the diameter of colonies was significantly reduced at 0.1 ppm.

INTRODUCTION

The heavy metals among other chemical pollutant are introduced to the aquatic system from industrial discharges which present a threat to the aquatic organisms. A low concentration of heavy metals stimulate the growth but the higher concentrations inhibits the growth (1).

Zinc presumably has a particular affinity for the amindodependant-enzymes and inhibits it but not the others (2). In *Candida albicans* accumulation of significantly higher concentration of Zn^{2+} transport but may also reflect internal compartmentation (3). Zn^{2+} starved bacteria had slightly higher free lipid and phospholipid contents, protein and hexosse contents were lowered (4).

In *Saccharomyces cerevisae* Zn^{2+} is perferentially stored in the vacuole, the vacuolar membrane (tonoplast) possessing an ATP-dependent Zn^{2+} uptake system (5). Sarhan et al. 1989 reported that Cu, Zn inhibited spore germination and mycelial growth (6). It was found that many fungi require (0.1-0.003) ppm of Fe^{2+} for ordinary growth (7) on the other hand, there is very little informations about the effect of lead on fungi.

. The purpose of the present work is to examine the influence of different concntrations of Zinc, Manganese and Lead on the funus *Pytium pleroticom.*

MATERIALS AND METHODS

Standard solution prepared from lead as $Pb(NO_3)_2$, Zinc as $ZnCl_2$, Manganese as $MnCl_2$. All diluted with distilled water to give the following concentration 0, 0.25, 0.1, 0.15, 0.2, 0.25, 0.3 ppm then autoclaved at 121 °C for 30 min under 151 b.

Pythium pleroticm was isolated from wet soil of Al-Mustansiriyah University garden and it was described previously by Al-Rekabi et al (8). Corn meal Agar (CMA) incubated at 20±2 °C was used for fungal cultures in glass peridishe. One milliliters of chlorophenicol (250 mg/1L distilled water) was added to each petridishes to prevent bacterial growth, then CMA was prepared by replacing distilled water with one of the above prepared solutions.

After autoclaved 10 ml of media was poured into sterile glass petridishes 9 cm diameter.

In order to measure the colony diameter a disc of 5 mm diameter was removed from periphery of a culture growing on CMA. Each disc inverted and placed in the center of a petidish, inoculated plates were incubated at 20±2 °C. After five days the colony diameter was recorded using three replicates for each concetration.

The effect of heavy metals on sporulation was examined by transferring a disc (5 mm diameter) of media containing hyphal tips to petridishes containing 10 ml of each metal at different concetrations, three replicates used, one boiled *Pennisetum spicatum* seed was added. Distilled water was used in the control, as described previously (9). All petidishes kept at 20±2 °C and they were examined for the appearance of sporangia after one day, 2-days and 3-days.

Data Analysis

ANOVA and the F test of significance was conducted to compare means of different groups of manipulations. To define the position of difference levels between means of the groups the 95% confidence multiple comparison test was conducted.

RESULTS AND DISCUSSION

It is evident from Table (1) that all concentrations of $Pb(NO_3)_2$ used inhibited sporangia formation after 2 days. Concentrations of 0.15 ppm and higher concentration reduced sporangial formation byo approximately 50% compared with the control. Almost the same effect was noted after 3 days which means that time of exposure to Pb^{2+} has no effect on sporangia formation.

High concentration of $Pb(NO_3)_2$ also reduce maturation and the number of empty sporangia significantly, this result is in line with those obtained by Sadler and Trudinger (1).

The results in Table (2) show that Zn^{2+} at the high concentration also reduce the maturation and emptying of sporangia, these results are in agreement with Sarhan et al. (6). While the results in table (3) shows that the different levels of Mn^{2+} have no significant effects on the spore formation, maturation and emptying. This result is similar to that obtained by Parkin and Ross (10), who described high specificity of Manganese uptake in *Candida utilis*. Table (4) shows the effect of $Pb(NO_3)_2$ on the vegetative growth of *Pythium pleroticum*. It was evident that all concentrations used in this study significantly decreased thediameter of colonies. This might be due to its effect on the cell permeability (2). Although Zinc and Manganese are essential for the growth of fungi but in this work it was found that the presence of 0.05 ppm of Zn^{2+} and 0.1 ppm of Mn^{2+} was poisonus to *Pythium pleroticum*. These results are in line with (1, 4, 6).

Table 1: Effect of Lead on Sporangia production *Pythium pleroticus*

Conc ppm	Young Sporangia				Mature Sporangia				Empty Sporangia			
	2 days	± SE	3 days	± SE	2 days	± SE	3 days	± SE	2 days	± SE	3 days	± SE
0	14.67 a*	1.76	16.33 a	0.88	13.00 a	1.53	13.62 a	1.33	17.00 a	3.21	22.33 a	1.45
0.05	12.33 a	0.33	12.00 bc	0.57	9.67 a	4.67	10.62 ab	4.18	11.00 bc	1.73	14.33 b	2.33
0.1	8.67 bc	1.20	11.00 c	1.00	9.33 a	3.18	9.67 ab	2.33	7.67 cd	2.40	8.67 cd	1.86
0.15	6.00 cde	2.08	6.00 d	2.08	8.33 ab	4.37	6.67 bcd	2.33	5.00 efg	2.00	6.67 de	1.45
0.2	3.67 def	1.20	3.33 efg	1.20	3.33 bcd	0.88	3.33 cde	0.88	3.33 efg	1.33	3.67 ef	1.20
0.25	3.33 ef	1.45	3.33 efg	1.45	2.33 cd	0.88	2.33 de	1.33	2.33 fg	0.66	2.33 fg	0.66
0.3	2.66 f	0.88	2.66 g	0.88	2.00 d	0.57	2.00 e	0.57	2.00 g	1.00	2.00 g	0.57

* Numbers in the same column with the same letters are not significantly different at the (5%) level

Table 2: Effect of Zinc on Sporangia production of *Pythium pleroticus*

Conc ppm	Young Sporangia				Mature Sporangia				Empty Sporangia			
	2 days	± SE	3 days	± SE	2 days	± SE	3 days	± SE	2 days	± SE	3 days	± SE
0	12.33 a*	2.19	19.33 a	5.24	9.33 a	4.91	8.67 a	4.18	7.67 a	2.40	10.33 a	3.28
0.05	9.67 a	2.73	14.67 abc	1.76	10.33 a	3.28	9.33 a	3.18	5.33 abc	2.33	9.67 a	1.33
0.1	9.66 a	0.88	13.00 bcde	0.57	8.67 ab	4.18	5.00 ab	2.00	4.33 bcdef	1.33	3.00	0.00
0.15	5.67 cde	1.76	11.00 cde	1.53	9.66 a	0.88	3.33 bc	1.45	3.32 cdef	1.33	3.00 cd	0.57
0.2	3.67 cde	1.76	10.00 de	4.51	3.00 bcd	0.57	3.33 cde	0.88 def	2.32 de	0.66	3.33 ef	0.88
0.25	3.66 de	0.88	9.33 f	2.96	2.00 cd	0.57	2.33 de	0.66	2.30 ef	0.88	2.33 ef	0.88
0.3	2.00 e	0.57	2.00 f	0.57	1.66 d	0.66	0.66 e	0.33	2.30 f	0.88	1.00	0.00

* Numbers in the same column with the same letters are not significantly different at the (5%) level

Table 3: Effect of Manganese of Sporangia production of *Pythius pleroticus*

Conc ppm	Young Sporangia				Mature Sporangia				Empty Sporangia			
	2 days	± SE	3 days	± SE	2 days	± SE	3 days	± SE	2 days	± SE	3 days	± SE
0	6.00 a*	1.15	7.00 a	1.53	4.00 a	0.57	5.66 a	0.88	3.00 a	0.57	5.66 a	0.33
0.05	5.33 ab	1.76	5.00 ab	2.52	4.00 a	1.15	5.00 a	1.00	2.66 ab	0.88	3.66 bc	0.88
0.1	3.67 bc	1.20	4.76 abc	1.45	2.66 abc	0.88	5.00 ab	1.73	2.00 abc	0.57	3.00 cd	1.00
0.15	2.66 cde	0.66	3.00 bcde	0.57	3.33 a	0.33	3.33 bc	0.33	2.00 abc	0.57	2.33 de	0.66
0.2	1.33 def	0.33	2.00 cde	0.57	2.67 abc	1.20	1.66 bc	0.33	1.66 bc	0.33	1.00 ef	0.57
0.25	1.33 ef	0.33	1.66 de	0.33	1.33 bc	0.88	1.33 de	0.88	1.00 cd	0.57	0.33 fg	0.33
0.3	0.66 f	0.33	1.00 e	0.00	1.00 c	0.57	1.33 e	0.33	0.33 d	0.33	0.33 g	0.33

* Numbers in the same column with the same letters are not significantly different at the (5%) level

Table 4: Effect of Lead, Zinc and Manganese treatment on the colony diameter (mm) of *Pythium pleroticus*

Conc/ ppm	$Pb(NO_3)_2$	± SE	$ZnCl_2$	± SE	$MnCl_2$	± SE
0	8.43 a*	0.120	7.76 a	0.088	7.63 a	0.066
0.05	7.63 b	0.033	6.43 b	0.033	7.36 a	0.167
0.1	5.63 ce	0.318	6.26 c	0.066	7.06 bc	0.219
0.15	6.06 d	0.066	5.93 d	0.088	6.90 c	0.057
0.2	5.56 e	0.186	4.96 e	0.088	5.90 d	0.057
0.25	4.70 f	0.208	4.20 f	0.057	4.76 e	0.145
0.3	2.60 g	0.208	3.8 g	0.015	3.23 f	0.066

* Numbers in the same column with the same letters are not significantly different at the (5%) level

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Mutagenic Improvement of Citirc Acid Producing Ability of The Local Isolates of *Aspergillus niger*

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الخلاصة

تم الحصول على عزلات محلية للفطر *Aspergillus niger* من مصادر مختلفة هي التربة ، الفواكه والحمضيات . درست قدرة هذه العزلات على انتاج حامض الليمون بدرجات حامضية مختلفة ، وتم فحص تأثير العوامل المطفرة مثل النايتروسوكواندين على قدرة انتاج حامض الليمون وامكن الحصول على عزلات مطفرة ذات قيمة صناعية واعدة .

ABSTRACT

Local isolates of the fungus *Aspergillus niger* was obtained from different sources such as soil, vegetables and Citrus. Their ability to produce citric acid at different pH was studied, the effect of a mutagen nitrosoguanidine, on the citric acid producing ability was examined, and mutants of a promising industrial value were obtained.

INTRODUCTION

The use of *Aspergillus niger* for the production of citric acid has received enormous consideratin by scientists (1-6). Number of publication has increased particularly after the contribution of Currie (4). Many organisms can be used to produce citric acid, among them are, *A. Clavatus, Penicillium luteum, P. Citrinum, Paecilomyces divaricatum, Mucor piriformis, Ustuling Vulgaris,* however, only *A. Niger* is found to be of industrial importance. (7).

Citric acid can be produced in large scale by two major methods. The classical method is the surface culture in which large aluminum pans used as a containers for the medium solution to increase the efficiency of the aerobic fermentation (8). Among the factors affecting the products are the correct interaction of the various constituents of the medium, pH, and ratio of the surface area to volume of the fermented solution. The production of oxalic acid can be suppressed to a negligible or small amount depending on pH degree value. Doelger and Prescott (9) found that the most satisifactory pH range was 1.6-2.2. The best citric acid producing fungi possess the greatest tolerance to low pH value (4). The other important industrial method is the submerged fermentation, in which a well aerated and agitated medium solution was used. The method has offered a higher production but lower efficiency (10, 11) .

Many attempts were used to isolate an economical strain of the local *A. Niger* (12-14). Al-Hamando (13) reported that the yield of citric acid obtained by a local isolate of the fungi *A. Niger* was 20%. Other workers have used different sugar sources available such as, date syrup or cane molasses (12, 14), and showed that the y ield of citric acid was not satisfactory.

The aim of this work was to isolate high efficiency citric acid producing strains of

A. Niger using nitrosoguanidine as a mutagenic agent.

MATERIALS AND METHODS

***Isolate :**

A. Niger was isolated from soil, vegetables citrus and identified according to khan et al (15) and Raper et al (16).

***Spore suspension :**

Spore suspension medium was prepared according to Al-Obaidi method (12). The medium was distributed in agar slant and autoclaved at 120 C for 20 min. The slants were inoculated with the isolates and incubated at 30 C for 4-5 days.

***The Fermentation medium :**

The fermentation medium was prepared accoding to karow (10) using the following constituents in 1 l of the medium 150g sucrose, 0.5g NH_4NO_3, 0.5g Mg SO_4. $7H_2O$, 0.08g KH_2PO_4, 0.15 KCl, 0.01g $ZnSO_4$. $7H_2O$, 0.02g $MnSO_4$ $4H_2O$.

The medium was always adjusted to the required pH with 0.1 N HCl aliquate of the spore suspension 3ml was used for each 50 ml portion of the fermentation medium.

The fermentation was performed by three methods, The surface fermentation was done by using 250 ml conical flask. The second was the shaker-water bath method, in which a 250 ml flask was used and the incubation at 30 °C was carried out in a water bath-shaker at 120 rpm. The thrid method was the submerged culture in which an 81 batch fermentor was used with 400 cycle/min agitation speed and 5 3 l/min air flow rate. All types of the fermentation were carried out for 10 days and then the analysis of citric acid production was done.

*** Isolation of Mutants :**

Nitrosoguanidine (NTG) was used as a mutagen. Stationary cultures were treated with NTG (200 Mg/ml) in phosphate buffer (pH 6.5) at 28 °C for 25-30 min to induce mutants. Mutagenic treatment of fungal cells was carried out according to Savchenko and kaputtsevich (17).

***Biochemical Analysis :**

Citric acid was determined according to Stern (18), while suger was determined according to double et al (19).

RESULTS AND DISCUSSION

Seven isolates of the fungi *A. Niger* have ben obtained from local sources; two from soil, three from vegetables and two from citric. Their citric acid producing ability were examined and presented in table 91). The isolates S_5 and C_7 showed the highest citric acid producing efficiency, where their yield were 11.5 and 12.7% respectively. On the other hand, the isolate V4 obtained from vegetable, has shown the lowest citric acid producing efficiency, witth a value of 3.5%. These results were found to be similar to that obtained by sheikh et al (20). They reported a range of 2.66-10.95% of citric acid production efficiency for some isolates of *A. Niger*, although they found no distinct differences in their biochemical and morphological features.

The effect of pH on citric acid production of the isolates C_9 and S_5 was studied. The highest citric acid production efficiency was at pH range 1.5-2.5 for both isolates as shown in table (2).

At a pH above 3, the efficiency of citric acid production was decreased remarkably. These results were very close to that obtained by Doegler and Prescott (9), who reported a satisfactory pH range between 1.6-2.2. The highest efficiency was at pH 2, where a values of 33 and 37.5% were obtained for the isolate S_5 and C_9, respectively. This is very encouraging result because it is very close to the minimum requirement of an industrial strainsfor the production of citric acid. A value of not less than 35% is required for a commercial use (10).

The other part of this work was concerned with the subjection of two selected strain S_5 to a mutagen, nitrosoguanidine (NTG). An attempt to examine its mutagenic effect on *A. Niger* has been carried out. A concentration of 200 Mg/ml has shown a higher level of killing within two hours for the isolate S_5 and 6 hours for the isolate C_9. (Fig. 1).

The citric acid producing ability of the presumably mutant isolates were examined at pH 2 and the results were presented in table (3). The mutants S_{48}, S_{59} and S_{62} were derived from the isolate S_5 while the mutants S_{15}, C_{29} acid producing efficiency than the mother isolates (S_5 and C_9). The isolate C_{96} showed remarkable efficiency of producung citric acid (48%). The mutant isolates of the strain S_5 behaved in a simillar way, but S_{48} gave a lower yield than the mother strain, which gave 33% at pH 2.0. However, a value of 45% of the citric acid conversion was obtained for the mutant isolate S_{59}, while the mother strain C_9 gave 37.5%.

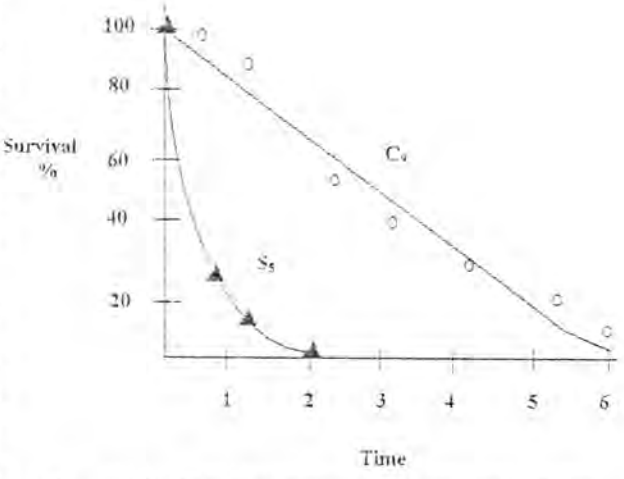
The fermentor size was scaled up to examine the efficiency of the two mutants S_{59} and C_{96}. Three methods were used for large scale production of citric acid as shown in table (4). The submerged fermentor gave the highest yield for both production were 49% and 55.5% respectively. It can be concluded that the local isolates of the fungus *A. Niger* canbe improved for commercial production of citric acid, Particularly, after inducing mutants with nitrosoguanidine.

Table (1). The percentage of citric acid production obtained from different local sources of *A. Niger*. The fermentation was performed by using sucrose medium at pH 3.5.

Isolates	Citric acid production &	Isolate source
S_8	4.6	Soil
S_5	11.5	Soil
V_4	3.5	Vegetable
V_{13}	7.8	Vegetable
V_{10}	10.2	Vegetable
C_9	12.7	Citrus
C_5	8.5	Citrus

Table (2). The effect different pH of the sucrose medium on the percentage conversion to citric acid of the isolates S_5, C_9

	pH	1.5	2.0	2.5	3.0	3.5	4.0
Isolate S_5	Citric acid product-ion %	22.5	33	27	20	10.5	5
	Unconv-erted suger %	19	17.0	16.6	18.7	18.0	21
Isolate C_9	Citric acid product-ion %	27	37.5	25	18.5	11.5	7
	Unconv-erted suger %	17	15.6	17.5	18	18.7	20.3



Figere (1) The Percentage of survival of the isolates S_5 and C_9 after treatment with nitrosoquanidine as a function of time.

Table (3). The percentage of citric acid production of the mutant isolates S_5 and C_9 after treatment with nitrosoguanidine (200 Mg/ml).

Isolates	Citric acid production %	Uncoverted sugar %
S_{18}	20	18.6
S_{59}	45	13.2
S_{62}	27	17.1
C_{15}	32	15.0
C_{25}	36	14.5
C_{36}	48	12.5

Table (4). The citric acid production of the mutant isolates S_{59} and C_{36} using scaled up methods.

		Surface fermentation	Shaker water bath	Submerged fermentation
S_{59}	Citric acid production %	37	44	49
	Unconverted sugar %	15.4	13.6	11.7
C_{36}	Citric acid production %	40	50	55.5
	Unconverted sugar %	12.5	12.1	10.4

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A Study on the Effect of Zn^{+2} , Pb^{+2} and Mn^{+2} on the Vegetative and Sporogenesis of *Achlya racemesa*

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الخلاصة

درس تأثير التراكيز التالية من أيونات الزنك والرصاص والمنغنيز على كل من النمو الخضري والتكاثر اللاجنسي للفطر *Achyla racemosa* ٠,٠٥ ، ٠,٠١ ، ٠,١٥ ، ٠,٢ ، ٠,٢٥ ، ٠,٣ ، جزء/ المليون لوحظ أن الرصاص حفز معنويا قطر المستعمرة بتركيز ٠,١٥ جزء / المليون أما التراكيز الأعلى من ذلك فقد ثبطت قطر المستعمرة . كان للزنك تأثيرا مثبطا عند نفس التراكيز أما المنغنيز فقد كان مثبطا عند تركيز ٠,٠٥ جزء / المليونتز . كان لكل من الزنك والرصاص والمنغنيز تأثيرا مثبطا لعملية انتاج الحوافظ السبورية وتكوين الحاجز وانطلاق السبورات السابحة وبتراكيز مختلفة . بصورة عامة كان النمو الخضري أكثر حساسية من التكاثر اللاجنسي .

ABSTRACT

Effect of 0.05 , 01 , 0.15 , 0.2 , 0.25 and 0.3 ppm of Zn^{-2} , Pb^{-2} and Mn^{-2} on the vegetative growth and asporogenesis of the fungus Achlya racemosa was studied . Pb^{-2} at 015 ppm significantly encourged the colony diameter , higher concentrations inhibited the growth , Zn^{-2} showed similar inhibitory effect , Mn^{-2} inhibited the growth significantly at 0.05 ppm . The three elements used showed significant supression to the production , septation of sporangia to zoospore liberation at different conc. In general the vegetative growth of Achlya racemosa was more sensitive to Zn^{-2} , Pb^{-2} and Mn^{+2} than sporogenesis .

INTRODUCTION

Heavy metalspollutants are known to inhibit a number of fungal activitie including respiration , mycelial growth spore production and germintation (1) , metal ions may become incorporated into aquatic system as algicides or accidentally discharge through neglegance ; however low concentration of heavy metals stimulates the growth .

Among heavy metals Zinc is known to be of many important biological functions , including stabilization of proteins and membranes , it is also a component of many metalloenzymes (2) , on the other hand Zn+2 was found to inhibit Ca+2 uptake in microsomal vesicle , it also inhibited Ca+2 atpase activity and the formation of phosphorylated intermediate of the enzymes (3).

Among heavy metals deficiency in molybdenum was found to depress the growth , dry weight and sporulation of *Aspergillus niger* (4) .

Sabi & Gadd 1990 (5) found that spore germintation is usually more readily poisend by metals than mycelial growth . Abnormal spore discharge was observed in *Achlya americana* subjected to different concentrations of Cadmium , Nickle and Copper (6) . The presence of Copper , Aluminium , Cadmium , Lead and Mercury

induced melanin pigmented chlyamydospore and hyphae (7).

There appears to be no detailed information available on the effect of manganese and lead on fungi but Ross & Perkins 1989 (8) pointed out that there is a highly specific uptake system for manganese in *Candida utils* .

The present study was designed to assess the effective concentration of Zinc , Lead and Manganese on the vegetative growth and sexual reproduction of *Achlya racemosa* .

MATERIALS AND METHODS

The fungus :

Achlya racemosa was isolated from Dijla river near AL-Rashdeyah , stock cultures were maintained after Dick 1975 (9).

Chemicals:

All chemicals used were analytical grade . The following concentrations of $Pb(NO_3)_2$, $ZnCl_2$ and $MnCl_2$ were prepared with Glass Distilled Water (GDW) : 0.5 , 0.1 , 0.15 , 0.2 , 0.25 , 0.3 ppm.

Vegetative Growth:

Colony diameter was used as a parmeter for vegetative growth cultures were refreshed by transferring a single hypha from the stock culture into the centre of a Raper ring inserted in Corn Meal Agar in a glass petridish incubated at 20 °C , when hyphal tips emerged from the Raper ring (after 2 days), a 5 mm disc of CMA containing hyphal tips was removed by sterile cork borer transferred into a glass petridish containing 9 mls of CMA. CMA was prepared by replacing the GDW with one of the solutions previously mentioned, in the control CMA was prepared by GDW, three replicates were prepared of each treatment, incubated at 20 °C , colonies diameters were recorded in the 3rd day of incubation.

A Aexual Reproduction:

A disc of CMA containing hyphal tips was transferred to glass petridesh containing one corn seed plus 10 mls of one of the conc. Of $Pb(NO_3)_2$ or $MnCl_2$. GDW was used as control, 3 replicattes of each treatment was prepared , cultures were incubated at 20°C examined after 24 , 48 and 72hours for sporangia formation by disecting microscope .

Data were analized using Analysis of Variance (ANOVA) and F test to compare means of different groups and manipulations : to define the position of differences between the means of the compared groups 95% confidence interval multiple comparision test was conducted .

RESULTS AND DISCUSSION

It is clear from Table (1) that Pb^{+2} at conc. Of 0.15 ppm significantly increased the vegetative growth of *Achlya racemosa* , higher conc. Were of depressing effect , 0.2 ppm of Zn^{+2} also depressed the diameter of colony significantly , however much lower conc. (0.5ppm) of Mn^{+2} showed the same effect (Table 2) . This sensitivity of *Achlya racemosa* towards Mn^{+2} may be due to the high specific manganese uptake system (8).

When sporulation is considered (Fig.1) , it is obvious that 0.1 ppm of Zn^{+2} inhibited the initiation of sporangia to 50% , on the other hand the deposition of septa at the base of sporangia was inhibited significantly at lower conc. (0.05ppm) this could be explained to the interference of Zn^{+2} with some synthetic enzymes necessary for septa formation .Liberation of zoospores and formation of sporeball tolerated higher conc.

Fig.2 showed that higher conc. Of Pb^{+2} (0..25ppm) significantly effected the sporangia initials while both of sporangia septation and zoospore liberation was reduced almost to half at 0.2 ppm , this inhibitory effect of Pb^{+2} may be attributed to poisonous interference of Pb^{+2} with some synthetic enzymes necessory for septation , or preventing the accumulation of wall vesicles in the septation area never the less its

effect on ATP necessory for this process may be considered .

from Fig. (3) it is clear that initiation of sporangia reduced to half at 0.2 ppm of Mn^{+2} , the sporeliberation was similary effected , but septation of sporangia occured at lower conc. (0.15ppm)

Not much difference was observed after 72 hr. Of inoculation as it is obvious from table 1 , 2 and 3 , this may reflect the negative effect of time of exposure to the heavy metals used in this study .

In contrast to AL-Rekabi et al (6) no abnormal spore discharge was observed in Achlya racemosa .

It would be of interest to examine the effect of Zn++ , Pb+2 and Mn+2 on germintation of spores which was found to be more sensitive than hyphal growth (5).

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Table (1) Effect of lead , Zinc & Manganese Cations on 3 days old colony diameter of Achlya racemosa

Conc. ppm	Diameter of Colony in mm.					
	Pb^{+2}		Zn^{+2}		Mn^{+2}	
0	32*.667 ± 2.33	a**	6.06 ± 0.66	a	6.63 ± 0.88	a
0.05	34.67 ± 203	a	5.93 ± 0.33	ab	6.30 ± 0.15	b
0.1	35.67 ± 3.84	ab	5.80 ± 0.20	ab	5.86 ± 0.03	c
0.15	37.67 ±1.45	b	5.80 ± 0.30	ab	5.90 ± 0.05	cd
0.2	22.67 ± 2.91	c	4.93 ± 0.14	b	5.03 ± 0.3	e
0.25	18.00 ± 1.73	d	4.16 ± 0.08	bc	4.80 ± 0.00	f
0.3	3.97 ± 1.07	e	3.33 ± 0.24	c	4.06 ± 0.06	g

* Each value is the mean of 3 replicates ± S.E.M.

** Any two mean followed by the same letter are not significantly different at 0.05 range test .

Table (2) . Effect of Zn+2 on sporangia initiation (I) Septation (S) and Zoopore Liberation (Z) of *Achlya racemosa* after 72 hours.

Zn^{+2} Conc. ppm	No. Of Sporangia after 72 Hours		
	I	S	Z
0	5.00 ± 1.00* a**	5.00 ± 1.00 a	10.00 ± 1.15 a
0.05	6.00 ± 5.7 a	6.00 ± 0.577 a	10.67 ± 3.28 a
0.1	6.00 ± 1.53 a	6.00 ± 1.53 a	7.33 ± 2.67 ab
0.15	3.00 ± 0.57 bcde	3.00 ± 0.57 ab	8.00 ± 1.15 ab
0.2	3.33 ± 0.88 cdg	3.33 ± 0.88 ab	3.67 ± 2.9 bcd
0.25	2.00 ± 0.57 de	2.00 ± 0.57 b	2.66 ± 0.33 cd
0.3	1.35 ± 0.33 e	6.50 ± 5.50 a	2.66 ± 0.88 d

* Each value represents the mean of three replicates ± standard error.

** Any two values followed by the same letter are not significantly different at 0.05 range test.

Table (3) . Effect of Pb+2 on sporangia initiation (I) Septation(S) and Zoopore Libration (Z) of Achlya racemosa after 72 hours .

Pb^{+2} Conc. ppm	No. Of Sporangia sfter 72 hours		
	I	S	Z
0	5.33* ± 0.88 a**	5.33 ± 0.882 a	19.33 ± 1.76 a
0.05	5.00 ± 0.57 a	5.00 ± 0.57 a	16.00 ± 2.31 a
0.1	3.00 ± 1.53 bcdef	3.00 ± 1.53 bcdef	14.67 ± 1.45 ab
0.15	2.67 ± 1.20 cdef	2.67 ± 1.20 cdef	11.00 ± 3.21 bc
0.2	2.00 ± 0.57 def	2.00 ± 0.57 def	9.00 ± 1.53 cd
0.25	2.66 ± 0.66 ef	2.66 ± 0.67 ef	5.00 ± 2.52 de
0.3	2.00 ± 0.57 f	2.00 ± 0.57 f	4.67 ± 2.03 e

* Each value represent the mean of 3 repliacates ± standard error.

** Any two values foilowed by the same letter are not significantly different at 0.05 range test

Table (4) . Effect of Mn+2 on sporangia initiation (1) Septation (S) and Zoopore Liberation (Z) of Achlya racemosa after 72 hours.

Mn^{+2} Conc. ppm	No. Of Sporangia after 72 hours		
	I	S	Z
0	12.33*±1.86 a**	12.67 ± 2.19 a	16.00 ± 1.15 a
0.05	9.00 ± 0.57 bcd	12.33 ± 1.86 a	12.33 ± 1.86 bcd
0.1	9.00 ± 0.57 cd	9.00 ± 0.57 bcdef	10.66 ± 0.88 cde
0.15	8.00 ± 0.57 def	9.00 ± 0.57 cdef	10.00 ± 1.15 de
0.2	5.67 ± 1.86 efg	8.00 ± 0.57 def	9.00 ± 2.08 ef
0.25	6.00 ± 2.31 fg	5.67 ± 1.86 ef	6.67 ± 1.76 f
0.3	3.66 ± 0.33 g	6.00 ± 2.31 f	3.33 ± 0.88 g

* Each value represent the mean of 3 repliacates ± standard error.

** Any two values followed by the same letter are not significantly different at 0.05 range test

Figure 1: Effect of Zinc on itiattion (i), septation (s) and zoospore Liberation (2) after 48 hours of Euro indicata standard error. Whan no bars are present the standard error was smaller than the syinton.

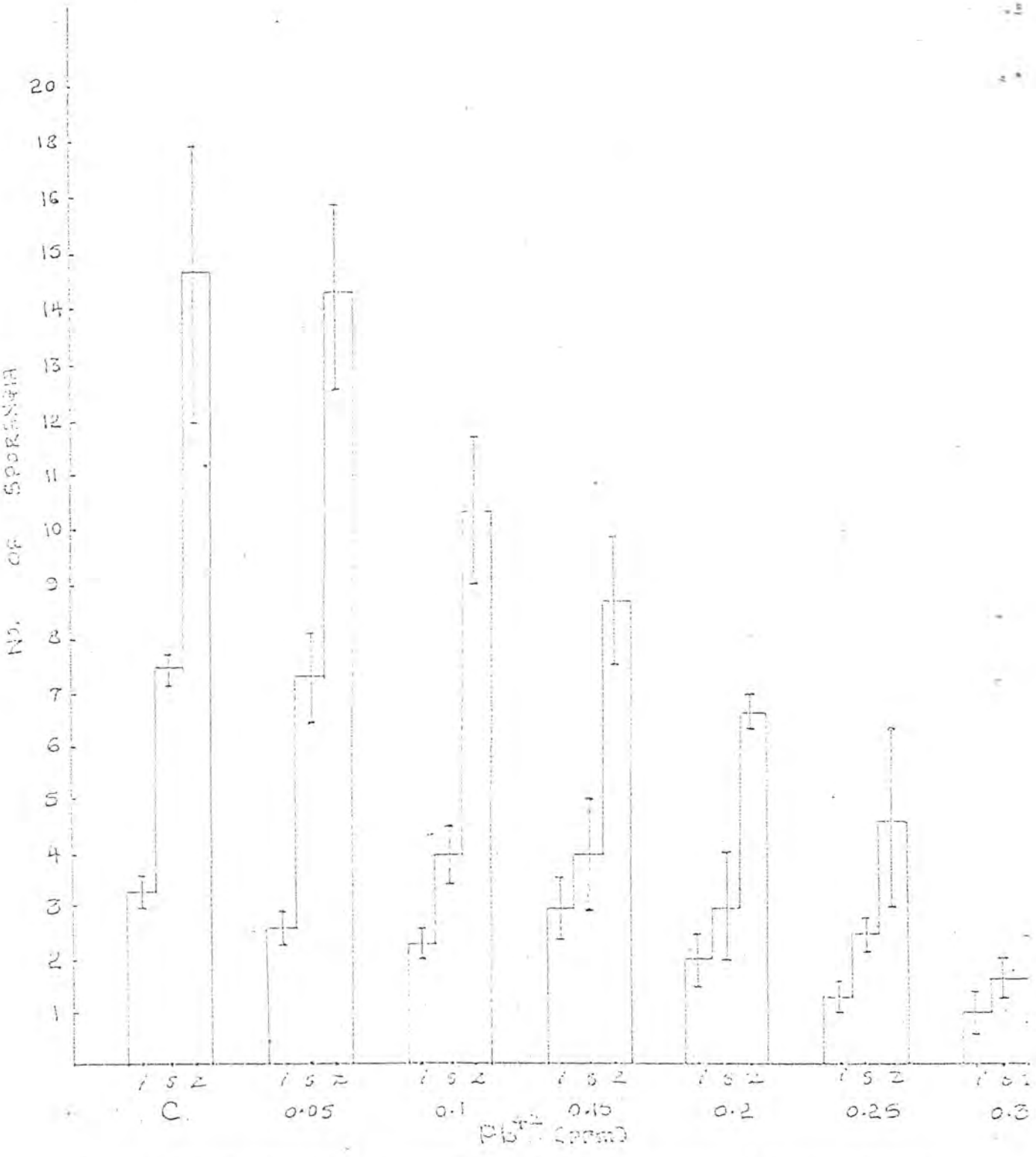

Figure 2: Effect of Lead on intiation(i), septation (s) and zoospore Liberation (z) after 48 hours. of Achlya Tuecmosa Bars indicate standard error.

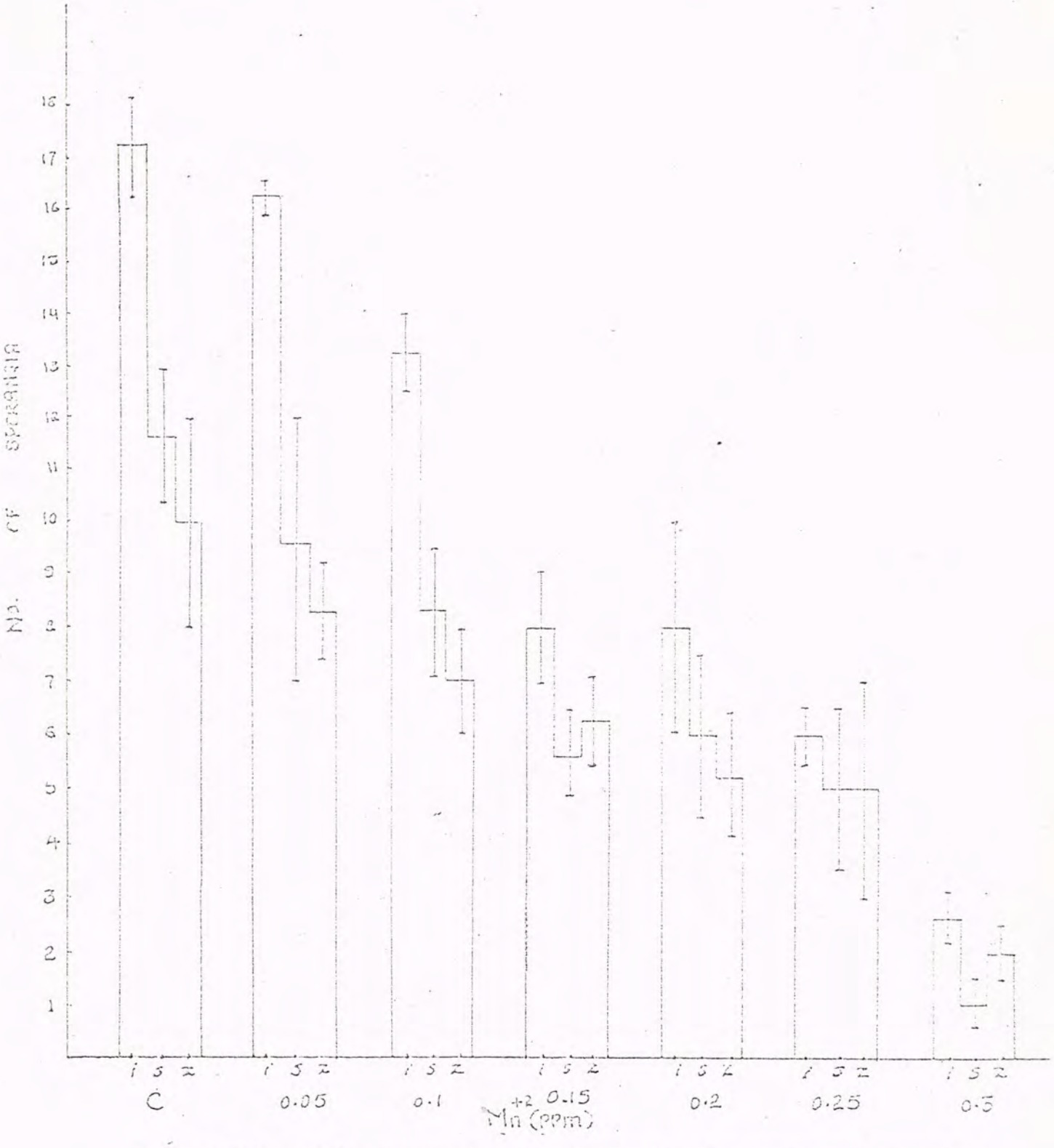

Figure 3: Effect of Manganese on initiation (i), septation of sporangia, and zoospore Liberation (z) after 48 hours. Bars indicate standard error.

Structural Studies of Mouse Seminal Vesicles in Intact Animals Following Estrogen Treatment

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الخلاصة

تمت دراسة الحويصلات المنوية في ذكور الفئران لسليمة (حيوانات السيطرة) والمحقونة بالمادة الزيتية المستعملة كمذيب للاستراديول 17B حيث تم حقن مجموعتين بـ 0.1, 5 مايكروغرام يوميا ولمدة ١٦ يوما (حيوانات التجربة). اظهرت دراسة هذه الحويصلات في حيوانات السيطرة تراكيب كيسية غير منتظمة الشكل ذات التواءات اولية وثانوية كثيرة التداخلات مع بعضها مكونة كثيرا من الفجوات والجريبات . ان النسيج الطلائي المبطن لهذه الاكياس هو افرازي عمودي او مكعبي كاذب غير مهدب ذات انوية بيضوية الشكل قاعدية الموقع. بعد مقارنة مقاطع حيوانات السيطرة مع مقاطع حيوانات التجربة في ذكور الفئران المحقونة بـ 5 مايكروغرام من الاستيراديول اوضحت تناقصها في عدد الالتواءات الطلائية وتوسعها محتوية على انوية دائرية الشكل كبيرة الحجم مركزية الموقع اضافة الى تأثير هذه المعاملة الى زيادة سمك الطبقة العضلية حسب جرعة الهرمون المحقونة. تبين من النتائج التي توصلت اليها بان الاستيراديول الذي يلعب دورا مهما في كبح افراز هرمون التستوستيرون يلعب دورا فعالا في نمو وافراز الحويصلات المنوية .

ABSTRACT

The effect of estradiol 17B upon structure of seminal vesicles in intact mice was studied using light microscopy (LM). Estradiol was injected daily for sixteen days. The normal mice seminal vesicle as revealed by LM was characterized by a large saccular lumen with highly folded walls, although the epithelial cells were pseudostratified columnar or cuboidal secretory not ciliated. Estradiol in low does did not appear to have an additive effect on epithelial structure. High does of estradiol treatment reduced the epitheial height, folds and increase both fibrous connective tissue and thickness of muscularis tunics. The present investigation demonstrates that estradiol which play an important role in suppression of testosterone releazing is very important in growth of seminal vesicles.

INTRODUCTION

The antiandrogenic effects of estrogen on male accessory sex organs have been demonstrated in a number of studies and remain of current interest because of frequent use of estrogen therapy with or without castration in the treatment of human prostatic adenocarcinoma [1]. This therapy is under close scruting because of undesirable side effects. There exists a wide range in the response of various accessory sex organs to the administration of both male and female steroid sex hormones [2] and the literatuure reports even further variations according to the dose, time course of treatment, castration or hypophysectomy and age of animal. Previous reports in theis area had focused upon the responses of the prostate gland neglecting other accessory structures such as the seminal vesicles.

The normal rat seminal vesicle has been described using light (L) and

The normal rat seminal vesicle has been described using light (L) and transmission electron microscopy (TEM) [3]. The sequential atrophic events following castration or treatment of intact animals with antiandrogen have been studied using LM and TEM [4]. Estrogen has been shown to minic these atrophic effects in the intact animal by decreasing the weight of the glands and epithelial height and by reducing secretion [5]. In castrated adrenalectomized and castrated non-adrenalectomized animals, certain doses of estrogen increased the weight was attributed to increased fibromuscular growth [6]. Also in the rat seminal vesicles, an additinve stimulation effect of cortison, megestrol or testosterone injected simultaneeously with estrogen has been reported.

Estrogen is clearly a potent inhibitor of gonadotropin release in the male rat and the regression of the accessory sex organs after estrogen treatment was related to supression of gonadotropin release [7]. Luteinizing hormone may be totally inhibited and follicle stimulation hormone was reduced in male rats after estrogen application [8].

Up to the auther's knowledge, there has been no published work on the morphology of mice seminal vesicles following estrogen treatment. The present study was undertaken to investigate more closely the morphological effects of high doses of estradiol 17B (E2) upon seminal vesicles of the intact mice.

MATERIALS AND METHODS

Thirty group-housed intact male albino mice were treated at 10 weeks of age with E2 at daily doses for 16 days. Group (N 10) received injections as oil, 0.1 µg and 5.0 µg, before tissue sampling. All preparations administered by injection were given as oily solution. The oily vehicle for injections was generally a mixture of one part of ethyl oleate to four parts of Archis oil. Estradiol 17B was prepared for injection by weighing the required amount of compound dissolving it in a small quantity of ethanol and making the resultant solution up to a volume of 100 mls with the oily vehicle in a clean volumetric flask. As 0.1 was generally injected intramusculary, calculation were based on the quantity of material in this volume. At the end of experiment the mice were killed by cervical disslocation and the organs were prepared for light microscopy and stained by Mallory's trichrome method.

RESULTS

Seminal vesicles of intact animals treated with oil revealed that the organ is elongated sac covered by a dense fibromuscular capsule with highly convoluted irregular lumen. Sections through the wall show complex primary folds and innumerable thinner secondary folds frequently joined by anastomoses forming many cypts and cavities.

The epithelium is usually a low pseudostratified with some basal and short columnar or cuboidal cells which are secretory but not ciliated. Cells have large oval nuclei oriented parallel to the long axis. The thin lamina propria is rich in elastic fibers which extend around the bases of the folds and sends projections into them. The muscularis is more or less in the form of an inner circular and an outer longitudinal layers. This is often difficult to discern in sections because of the convolutions of the vesicles (Figure 1).

Seminal vesicles from 0.1 µg E2 treated mice did not differ markedly in any respect from oil treated counterparts.

Histological examination of the seminal vesicle from animals treated with 5.0 µg E2 was markedly altered as shown in Figure (2). The number of epithelial folds was markedly reduced but were broader than those in controls. Connective tissue in the lamina propria was more abundent and the epithelial cells generally showed less cytoplasmic basophilia.

The nuclei of the epithelial cells were large, round centrally located rather than displaced towards the basal surface as in highly secretory state of control group. Muscular tunics was thicker relative to the overall cross-section and the adventitial layer was abundant.

DISCUSSION

Several mechanisms for the action of estrogen on male accessory sex organs have been suggested [9]. These include an indirect effect by the relative feedback on pituitary release of gonadotropins or by a direct effect on the prostate and seminal vesicles or supressing utilization of testosterone.

Direct effects of estrogen have been investigated *in vitro* which suggested that the mechanisms of action may involve competition for active centers on the androgen-metabolizing enzymes, depletion of cofactors during concurrent estrogen metabolism or product-inhibitions by estrogen [10]. Any of these mechanisms would effect a reduction in the size of the gland and secretory activity. The effect of estrogen on rodents has been known for several decades and was thought to be due to a reduction in the release of pituitory gonadotropins. Indeed both natural and synthetic estrogens can inhibit the release of LH and FSH in a variety of *in vivo* and *in vitro* systems [11].

In adult male mice both morphologica and fuctional integrity of the sex accessory tissues depend on the presence of adequate level of androgen at the target organ receptor site [12]. Thus the reduction of gonadotropin synthesis or release, the inhibition of testosterone biosynthesis or the inhibition of androgen at the target organ itself represents three potential mechanisms where by one may control androgen-sensitive tissue.

The present investigation demonstrated that the low does of E2 did not change the histological appearance of the seminal vesicle structure whereas the high dose had a quite definite suppressive effect on the antiandrogenic effects of cyproterone acetate reported in intact animals [13].

The results of this study were in agreement with previous studies [14, 15] concerning the concentration and length of treatment employed.

In contrast E2 prevented the circulating androgen from exerting its normal stimulative effects upon the seminal vesicle.

These findings were in agreement with the findings of other researchers that E2 antagonises the effect of circulating endogenous androgen [16,17,18].

Similar suppressive actions of E2 on other sex accessory glands have been reported in rat [11].

The fact that the seminal vesicles were histologically altered in large doses treatment is open to at least three interpretations:-

1. Estrogens in low doses may not interfere with the action of androgen and the nervous system.
2. The seminal vesicles may be more sensitive to androgen and large doses of E2 might have inhibited androgenic stimulation.
3. The vesicles may react more promptly to a decrease in androgenic activity and the adminstration of large doses of E2 might eventually have induced regressive changes.

Evaluation of these explanations needs further investigations.

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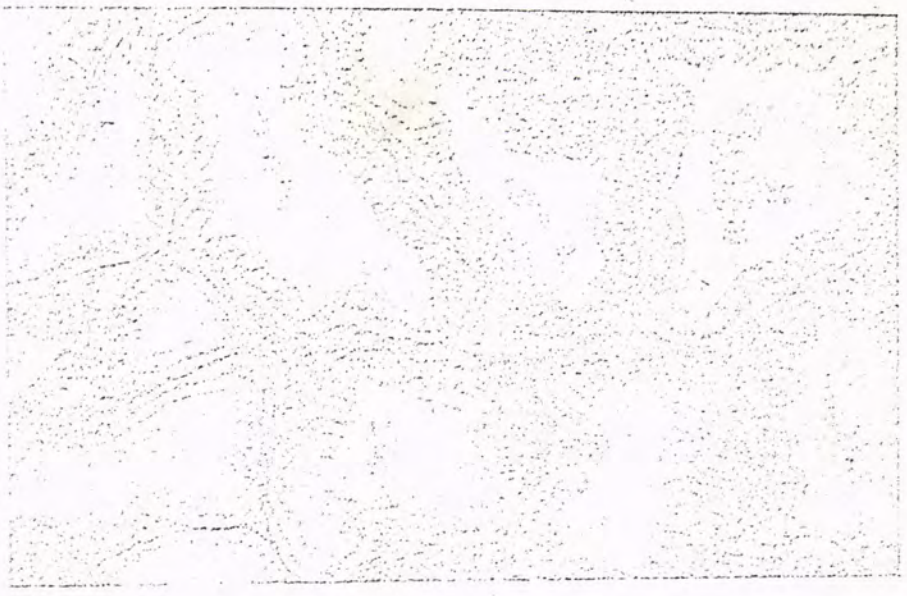


Figure 1: Histology of mouse seminal vesicles of untrerated control.

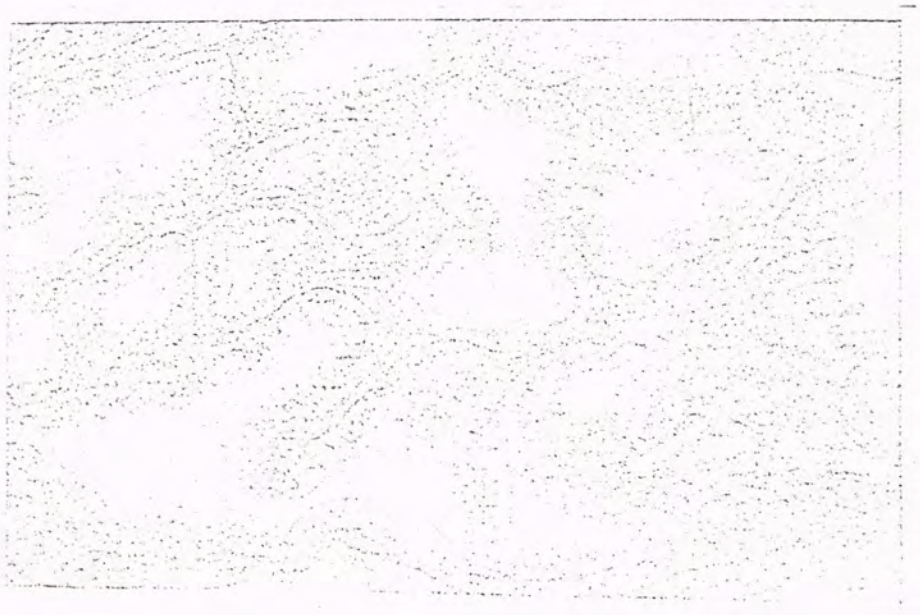
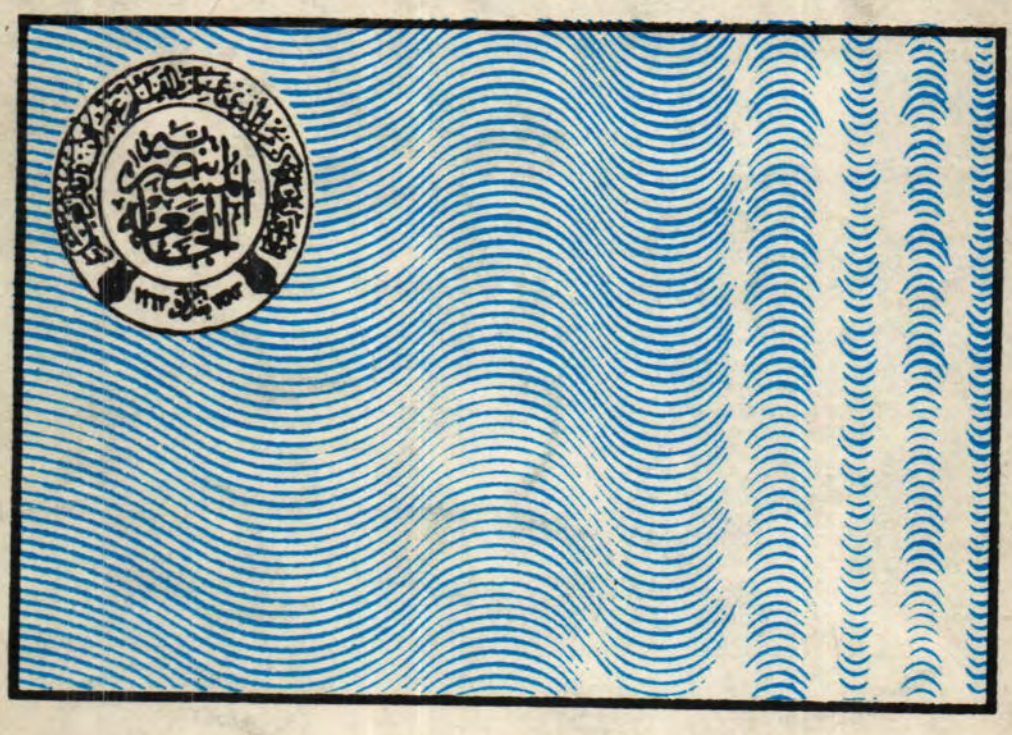


Figure 2: Seminal vesicle changes in the mouse after 16 days intramuscular administration of 5.0 µg E2/day.

الجامعة المستنصرية

كلية العلوم

مجلة
علوم المستنصرية



مجلد : (٨)

عدد (٣)

سنة : ١٩٩٧

مجلة علمية دورية تصدرها كلية العلوم في الجامعة المستنصرية

تعنون كافة المراسلات الى : سكرتير هيئة تحرير مجلة علوم المستنصرية

كلية العلوم - الجامعة المستنصرية

الوزيرية - بغداد - جمهورية العراق

تلكس : ٢٥٦٦ (مسباد - عراق)

هاتف : ٤١٦٨٤٩١ أو ١-٤١٦٨٥٠٠ (بداله) خط ٢٧٦

مجلة علوم المستنصرية

تعليمات النشر

١ـ تقوم المجلة بنشر البحوث الرصينة التي لم يسبق نشرها في مكان اخر بعد اخضاعها للتقويم العلمي من قبل مختصين وباي من اللغتين العربية او الانجليزية .

٢ـ يقدم الباحث او الباحثون طلبا تحريريا لنشر البحث في المجلة على ان يكون مرفقا بثلاث نسخ من البحث مطبوعة على الالة الكاتبة بترك فراغين (double space) بين سطر واخر على ورق ابيض قياس (A4) من النوع الجيد وتترك مسافة (٢٫٥) سم على جانبي كل صفحة .

٣ـ يطبع عنوان البحث واسماء الباحثين (كاملة) وعناوينهم باللغتين العربية والانجليزية على ورقة منفصلة شرط ان لاتكتب اسماء الباحثين وعناوينهم في اي مكان اخر من البحث وتعاد كتابة البحث فقط على الصفحة الاولى من البحث .

٤ـ تكتب اسماء الباحثين كاملة بحروف كبيرة (capital) في حالة استخدام اللغة الانجليزية وكذلك الحروف الاولى فقط من الكلمات (عدا حروف الجر والاضافة) المكونة لعنوان البحث وتكتب عناوين الباحثين بحروف اعتيادية صغيرة (small letters)

٥ـ تقدم خلاصتان وافيتان لكل بحث احداهما بالعربية والاخرى بالانجليزية وتطبع على ورقتين منفصلتين بما لا يزيد على (٢٥٠) كلمة لكل خلاصة .

٦ـ تقدم الرسوم التوضيحية منفصلة عن مسودة البحث وترسم على ورق شفاف (tracing paper) بالحبر الصيني الاسود وترفق ثلاث صور لكل رسم وتكتب المعلومات عنها على ورقة منفصلة ولا يجوز تكرار المعلومات ذاتها في الرسوم والجداول في وقت واحد الا اذا اقتضت ضرورة المناقشة ذلك .

٧ـ يشار الى المصدر برقم يوضع بين قوسين بمستوى السطر نفسه بعد الجملة مباشرة وتطبع المصادر على ورقة منفصلة ويستخدم الاسلوب الدولي المتعارف عليه عند ذكر مختصرات اسماء المجلات.

٨ـ يفضل قدر الامكان تسلسل البحث ليتضمن العناوين الرئيسة الاتية : المقدمة طرائق العمل النتائج والمناقشة الاستنتاجات . المصادر. وتوضع هذه العناوين دون ترقيم في وسط الصفحة ولا يوضع تحتها خط وتكتب بحروف كبيرة عندما تكون بالانجليزية .

٩ـ يتبع الاسلوب الاتي عند كتابة المصادر على الصفحة الخاصة بالمصادر ترقم المصادر حسب تسلسل ورودها في البحث يكتب الاسم الاخير (اللقب) للباحث او الباحثين ثم مختصر الاسمين الاولين فعنوان البحث مختصر اسم المجلة المجلد او الحجم العدد الصفحات (السنة) وفي حالة كون المصدر كتابا يكتب بعد اسم المؤلف والمؤلفين عنوان الكتاب الطبعة . الصفحات . (السنة) الشركة الناشرة . مكان الطبع .

مجلد : (٨) عدد (٣) سنة : ١٩٩٧

رقم الايداع في دار الكتب والوثائق ببغداد ٢٧٨ لسنة ١٩٩٠

البحوث العربية

رقم الصفحة

زيادة فعالية المبيدات الكيمياوية ضد الصرصر الالماني بأستخدام مستخلص فرمون التجمع

طارق محمد عبد
قسم علوم الحياة / كلية العلوم / جامعة الأنبار / الرمادي / العراق
(أستلم بتاريخ ١٩٩٤/١١/٢ ، قبل للنشر في ١٩٩٥/٤/٤)

الخلاصة

أجريت هذه الدراسة لقياس كفاءة فرمون التجمع في زيادة فعالية ثلاث مبيدات (بايرثرويدية , فسفورية , كارباماتية) لمكافحة الصرصر الالماني ولقد تبين من الدراستين المختبرية والحقلية اهمية استخدام الفرمون في زيادة فعالية المبيدات المختبرة قياساً بالمقارنة كما اشارت النتائج الى ضرورة زيادة تركيز الفرمون لزيادة الفعالية السمية للمبيد الكيمياوي كما اتضح من الدراستين تفوق المبيد البايرثرويدي بيرمثرين مع مستخلص الفرمون مقارنة بالمبيدين الكارباماتي بايكون و الفسفوري دايازينون كما اظهر المبيد بايكون مع مستخلص الفرمون سمية فورية في الدراسة الحقلية خلال الاسبوع الاول والثاني بعد المكافحة في حين اظهر المبيد بيرمثرين مع مستخلص الفرمون سمية متبقية عالية مقارنة بمبيد بايكون .

ABSTRACT

This research work was carried out to study the role of aggregation phenomone extract from german cocroach *Blatella germanica* in enhancement of three different incecticided against this medically important pest. Laboratory and field experiments showed clearly that phermonal extract increased the toxicity of permethrin, Baygon and Diazinone insecticides to german cocroach, when compared with the toxicity of these insecticides without the phermone extract. It was also found that Baygon with phermone extract gave high breakdown action to cockroach during the first and second weekafter the field conttrol, however, with permethrin the phermone extract has increased the residual activity of this insecticide to cockroach.

المقدمة

تعتبر ظاهرة التنفير في المبيدات الكيمياوية المستخدمة في مجال مكافحة الحشرات من العوامل المهمة في تحديد فعاليتها السمية (١و ٢و ٣) ونظراً لكون أغلب المبيدات الحشرية تمتاز بضغط بخاري عالي فأن أبخرة هذه المبيدات تؤدي الى نفور الحشرات المستلمة لها واختبائها او الهروب الى مناطق غير معاملة وبالتالي تقليل فعالية هذه المبيدات وعلى هذا الاساس فأن فعالية المبيدات تتناسب عكسياً مع القدرة على الطرد والتنفير وهذا ما أشارت اليه عدد من الدراسات حيث أوضحت زيادة فعالية القتل بالمبيد الفسفوري كلوربيرفوس مع مستخلص الفرمون بالمقارنة مع المبيد لوحده (٤) , وتفوق المبيدين كلوربيرفوس وبروبكسيور مع مستخلص الفرمون مقارنة بالمبيدين بدون فرمون (٥) , وأن استخدام فرمون التجمع مع المبيد الفسفوري العضوي سوماثيون أدى الى خفض التراكيز اللازمة لقتل الصرصر الالماني (٦) كما أن ذكور الصرصر الالماني تكتسب قدرة في تجنب المواد السامة وان هذه السلوكية تقلل من فعالية هذه المواد في القتل (٧) , اضافة الى ما تقدم فأن البيئة غير النظيفة وما يرافقها من النفايات والاوساخ تؤمن بيئة لاختباء هذه الحشرات تحول دون

نجاح عمليات المكافحة بالمبيدات الكيمياوية ان هذا يحتم استغلال بعض الصفات الحيوية والسلوكية والتي من بينها استخدام الفرمونات لزيادة الفعالية الابادية للمبيدات الكيمياوية وللحد من ظاهرة تجنب او النفور من المادة السامة وتحقيقاً لهذا الهدف اجريت هذه الدراسة لمعرفة اهمية استخدام الفرمون المستخلص من الحشرة مع المبيدات الكيمياوية المستخدمة في مجال الصحة العامة لمكافحة الصرصر الالماني .

المواد وطرق البحث

١- الدراسة المختبرية

تم تربية الصرصر الالماني في حاويات بلاستيكية سعة غالون واحد مزودة بغذاء صناعي جاف مكون من ٤٠٪ دقيق الذرة و ٤٠٪ مسحوق حليب نصف دسم و ٢٠٪ خميرة البيرة (٨) ومزودة بمنهل بلاستيكي ٢٠٠ مل لتأمين الماء وتمت التربية على درجة حرارة ٢٧ ± ١ م ورطوبة نسبية ٥٤ ± ٥ ٪ ولمنع هروب الصراصر من الحاويات استخدم الفازلين على ارتفاع ٢ سم من الحافة العلوية ونظراً لعدم معرفة التركيب الكيمياوي لفرمون التجمع (٥) فقد استخدم براز المستعمرة لاستخلاص فرمون التجمع (٩) حيث تم غربلة البراز عبر منخل رقم ٢٠ (٠،٨ ملم) وغسلت المادة التي حصل عليها بعد الغربلة بالكحول المثيلي لمدة ٢٤ ساعة واستخدام ورق الترشيح Wh.No1 للحصول على الراشح ذو اللون الاصفر , تم امرار الراشح على كبريتات الصوديوم اللاماتية لسحب الماء ثم حفظ الراشح على درجة الصفر المئوي بعدها تم تعيئة شرائح من ورق الترشيح وبمساحة ٢١ سم (٧ × ٣) تم معاملتها بـ (٠،١) مل من المحلول السام وبتركيز (٠،٨٤ ملغ مادة فعالة لكل سم) لكل من المبيد البايرثرويدي بيرمثرين ٢٥٪ مسحوق قابل للبلل والمبيد الفسفوري العضوي دايازينون ٥٠٪ سائل مركزقابل للاستحلاب والمبيد الكارباماتي بايكون ٢٠٪ سائل مركز قابل للاستحلاب واضيف مستخلص الفرمون بواقع ثلاث مستويات صفر , ٢،٥ , ٢٥ ملغ لكل شريحة خلطاً مع محلول المبيد وتركت المعاملات ثلاث ساعات لكي تجف واستخدمت ثلاث مكررات لكل مبيد ولكل مستوى من مستخلص الفرمون اضافة الى مكررات المقارنة والتي استخدم فيها الماء المقطر والكحول المثيلي و وضعت هذه الشرائح في علب بلاستيكية ٩×٩×٦،٨ سم وتركت شريحتين غير معاملة على جانبي الشرائح المعاملة ولكل علبة (٤) ولمنع هروب الصراصر تم معاملة السطوح الداخلية لجدران العلب والى ارتفاع ٢ سم من الحافة العليا من مادة الفازلين ونقلت عشر حوريات بالعمر لكل مكرر بعد شل حركتها وذلك بتعرضها على درجة حرارة ٦م لمدة ١٠-١٥ دقيقة (١٠) ثم نقلت هذه العلب الى حاضنة مثبتة علىدرجة حرارة ٢٧±١م ورطوبة نسبية ٤٥±٥٪ وتم حساب النسبة المئوية للقتل بعد ١٢ , ٢٤ ساعة من الترعيض وتم تحليل النتائج احصائياً باستخدام اقل فرق معنوي LSD في تشخيص الفروق الاحصائية بين المعاملات (١٩) .

٢- الدراسة الحقلية

أختبرت فعالية المبيدات المشار اليها في الدراسة المختبرية مع مستخلص فرمون التجمع حقلياً حيث انتخبت بناية مستشفى صدام العام بمدينة الرمادي كموقع لتنفيذ هذه التجربة حيث تتفاوت شدة الاصابة بين المتوسطة في بعض مرافقها كردهات المرضى وبين الشديدة في المطبخ المركزي , الكافتريا , المطابخ الثانوية اضافة الى غرف العمال و دورات المياه , استخدمت مصائد كارتونية بقياسات ١٢٣×١٢٨×١٨٧ ملم مزودة بفتحتين على بعد ٥٠ ملم من كل جانب وتم وضع شريط من مادة لاصقة (ارترارات) على قاعدة المصيدة (لالتصاق الصراصر المصطادة بها ومن خلال ذلك حساب الكثافة العددية للصراصير) ويتوسطها عشر غرامات من الخبز الابيض كطعم غذائي للصرصر الالماني (١١) وتم تهيئة محاليل سامة بتراكيز ١ , ٠،٥٪ من كل من المبيدات بيرمثرين

فعالية محللة للدم عند نموها على اوساط اطار الدم الحاوية على المضاد الحياتي الريفامبسين جدول رقم (٧) مما يدل على ان صفة انتاج الهيمولايسين في هذه العزلات لاتشفرها بلازميدات غير مقترنة وانما تسيطر عليها جينات كروموسومية وهذه النتيجة جاءت مطابقة مع نتائج منشورة (١٠ ، ٣١) .

تؤكد هذه الدراسة وجود اكثر من عامل ضراوة واحد حيث اظهرت غالبية العزلات البكتيرية مقاومة مفردة او متعددة للمضادات الحياتية المستخدمة في حين كان عدد قليل من هذه العزلات منتجاً للهيمولايسين . كما نستنتج بأن الجينات المشفرة لمقاومة المضادات الحياتية محمولة على بلازميدات قابلة للانتقال بالاقتران في جميع العزلات المقاومة المنتجة بينما كانت الجينات المشفرة لانتاج الهيمولايسين محمولة على الكروموسوم في غالبية العزلات المدروسة باستثناء عزلة واحدة وجد انها تحتوي على البلازميد المشفر لانتاج الهيمولايسين والقابل للانتقال بالاقتران . وبالنسبة لنتائج التحليل الوراثي لبعض عوامل الضراوة الاخرى التي اشير اليها في هذه الدراسة فهي قيد الاعداد والطبع في بحث آخر (لنفس المؤلف) .

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جدول ١ : العزلات البكتيرية التي استخدمت في هذه الدراسة .

نوع العزلة	عدد العزلات	النموذج المعزولة منه	مصدر العزلات
Escherichia coli	28	خروج مرضى الاسهال	مستشفى اليرموك التعليمي في بغداد
Escherichia coli	27	خروج اطفال مصابين بالاسهال	مستشفى صدام للأطفال في بغداد
Shigella dysenteriae	3	خروج مرضى الاسهال	مختبر الصحة المركزي في بغداد
Shigella sonnei	3	خروج مرضى الاسهال	مختبر الصحة المركزي في بغداد
Shigella flexeneriae	2	خروج مرضى الاسهال	مختبر الصحة المركزي في بغداد
Shigella boydii	1	خروج مرضى الاسهال	مختبر الصحة المركزي في بغداد
Salwonella typhi	4	خروج مرضى الاسهال	مختبر الصحة المركزي في بغداد
Salmonella typhiaurium	1	خروج مرضى الاسهال	مختبر الصحة المركزي في بغداد
Klebsiella pneumoniae	3	خروج مرضى الاسهال	مستشفى اليرموك التعليمي في بغداد
Vibrio cholerae inaba	1	خروج مرضى الاسهال	مختبر الصحة المركزي في بغداد
Vibrio cholerae ogava	1	خروج مرضى الاسهال	مختبر الصحة المركزي في بغداد
Vibrio cholerae (NAC)	1	خروج مرضى الاسهال	
مجموعة من سلالات عالمية من البكتريا المعوية وهي :			
Kscherichia coli 10536	1	خروج مرضى الاسهال	كلية الطب البيطري / جامعة بغداد
Klebsiella pneumoniae 10031	1	خروج مرضى الاسهال	كلية الطب البيطري / جامعة بغداد
Salmonella typhi 13314	1	خروج مرضى الاسهال	كلية الطب البيطري / جامعة بغداد
Shigella sonnei 11060	1	خروج مرضى الاسهال	كلية الطب البيطري / جامعة بغداد
Enterobscter cloacae 15337	1	خروج مرضى الاسهال	كلية الطب البيطري / جامعة بغداد
Salmonella spp.	13	خروج الطيور المصابة بالاسهال	قسم التقنية الحياتية للدراسات العليا / جامعة بغداد
Salmonella spp.	17	خروج الدواجن المصابة بالاسهال	قسم التقنية الحياتية للدراسات العليا / جامعة بغداد
Escherichia coli	3	خروج الابقار المصابة بالاسهال	كلية الطب البيطري / جامعة بغداد

جدول ٢ عدد العزلات البشرية والبيطرية المقاومة لكل مضاد من المضادات الحياتية المستخدمة .

العزلة	مصدرها	عدد العزلات المفحوصة	عدد العزلات المقاومة للمضادات الحياتية					
			امبيسلين	تتراسايكلين	ستربومايسين	كنامايسين	كلورامفينيكول	ريفامبيسين
اشرشيا القولون	انسان	55	48	36	15	25	12	2
اشرشيا القولون	ابقار	3	3	2	1	1	0	0
سالمونيلا	انسان	6	3	3	2	0	2	0
سالمونيلا	دواجن	17	0	3	0	1	0	0
سالمونيلا	طيور	13	3	2	0	0	0	0
شكيلا	انسان	10	3	4	5	0	1	0
كليسيلا	انسان	4	3	0	0	0	1	0
انتيروباكتر	انسان	1	1	0	0	0	0	0
ضمات الكوليرا	انسان	3	0	0	0	0	0	0

جدول ٣ توزيع وانتشار الصفات المساهمة في الامراضية بين عزلات البكتريا المعوية تحت الدراسة

العزلة	مصدرها	عدد العزلات المفحوصة	انتاج الهيمولايسين	الفعالية المحللة لليوريا *	انتاج كبريتيد الهيدروجين *	القابلية على استهلاك *	
						السترات	اللاكتوز
اشرشيا	انسان	55	5	0	0	0	55
القولون	ابقار	3	0	0	0	0	3
سالمونيلا	انسان	6	0	0	6	0	0
سالمونيلا	دواجن	17	0	0	17	0	0
سالمونيلا	طيور	13	0	0	13	0	0
شكيلا	انسان	10	0	0	0	0	0
كلبسيلا	انسان	4	0	4	0	4	4
انتيروباكتر	انسان	1	0	0	0	1	1
ضمات الكوليرا	انسان	3	2	0	0	3	0

* ان التحليل الوراثي لهذه الفعاليات يضمه بحث اخر لنفس المؤلفين تحت الاعداد والطبع .

جدول ٤ : العزلات التي تشترك في صفة انتاج الذيفانات المعوية غير المستقرة حرارياً (LT) ، انتاج الهيمولايسين والمقاومة للمضادات الحياتية .

العزلة البكتيرية	انتاج الذيفان المعوي LT*	انتاج الهيمولايسين HLY	مقاومة المضادات الحياتية					
			امبيسلين AP	تتراسايكلين Tc	ستربتومايسين Sm	كناميسين Kn	كلورمفينيكول Cm	ريفاميسين Rif
Escherichia coli GMB	-	+	-	-	-	-	-	-
Escherichia coli GM9	-	+	+	-	-	-	-	-
Escherichia coli GM10	-	+	+	-	-	-	-	-
Escherichia coli GM14	-	+	+	-	-	-	+	-
Escherichia coli GM16	-	-	+	-	-	-	-	-
Escherichia coli GM17	-		-	-	-	-	-	-
Escherichia coli GM19	-	-	+	-	-	-	-	-
Escherichia coli GM32	-	-	+	-	-	-	-	-
Vibrio cholerae inaba	-	-	-	-	-	-	-	-
Vibrio cholerae ogava	-	-	-	-	-	-	-	-
Klebsiella pneumoniae Gm39	-	-	+	-	-	-	-	-
Shigella dysenteriae GA6	-	-	+	-	-	-	-	-

* نتائج هذا التحليل من بحث اخر تحت الاعداد والطبع .

جدول ٥ : وتائر تردد انتقال بلازميدات المقاومة الناتجة من اقتران العزلات الواهية *E. Coli GM19, E. Coli CM10, E. Coli GM97 E. Coli GM8* مع السلالة المستلمة *E. Coli MM294* على التوالي .

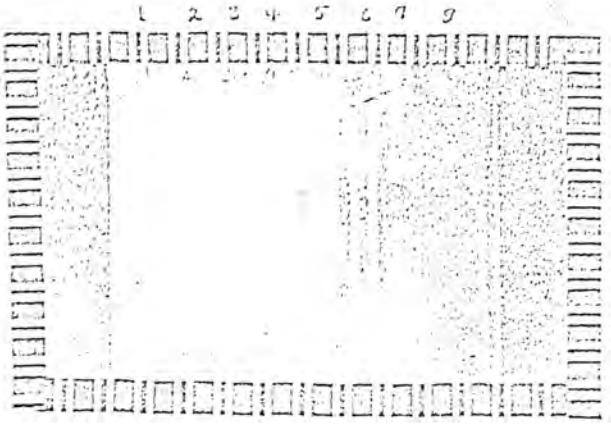
السلالات المقترنة	العدد الكلي للسلالة المستلمة / ملليتر = 1.2×10^{8}			عدد مستعمرات الخلايا الطافرة تلقائياً	
	الاوساط الانتقالية	عدد المستعمرات النامية الموجبة في تنفيذ 10^{-3}	تردد الانتقال	الواهبة	المستلمة
GM8 ×MM294	Tc + Rif	6	$10^{-4}\times5$	0	0
	blood + Rif	0	0	0	0
GM9 × MM294	Ap + Rif	12	$10^{-4}\times1$	0	1
	Tc + Rif	9	$10^{-4}\times7.5$	1	0
	Cn + Rif	5	$10^{-4}\times4.1$	0	0
	blood + Rif	0	0	0	0
GM10 × MM294	Ap + Rif	15	$10^{-3}\times1.25$	0	0
	Tc + Rif	12	$10^{-3}\times1$	0	1
	blood + Rif	0	0	0	0
GM19 × MM294	Ap + Rif	12	$10^{-3}\times1$	0	2
	Tc + Rif	7	$10^{-3}\times5.8$	0	1
	Cn + Rif	0	0	0	0
	blood + Rif	0	0	0	0

جدول ٦ : وتائر تردد انتقال بلازميدات الهيمولايسين الناتجة من اقتران العزلات الواهبة *E. Coli GM17, V. cholerage ogawa, V. cholerage inaba* مع السلالة المستلمة *E. Coli* HB101 على التوالي .

السلالات المقترنة	العدد الكلي للسلالة المستلمة / ملليتر = 1.2×10^{8}			عدد مستعمرات الخلايا الطافرة تلقائياً	
	الاوساط الانتقالية	عدد المستعمرات الموجبة النامية في تنفيذ 10^{-3}	تردد الانتقال	الواهبة	المستلمة
GM17×HB101	blood + Sm	15	$10^{-4}\times6$	0	0
inaba×HB101	blood + Sm	0	0	0	0
owada×HB101	blood + Sm	0	0	0	0

جدول ٧ : وتائر تردد انتقال البلازميدات العائدة الى العزلات E. Coli GM10 E. Coli GM9, E. Coli GM8 E. Coli GM19 الى السلالة E. Coli HB101 على التوالي عن طريق التحويل الوراثي .

السلالات المتحولة	العدد الكلي للسلالة المستلمة / ملليتر = 1.0×10^{8} تركيز الدنا البلازميدي 0.05 مايكروغرام			عدد مستعمرات الخلايا الطافرة تلقائياً	
	الاوساط الانتقالية	عدد المستعمرات الموجبة النامية في تنفيذ 10^{-2}	تردد الانتقال	الواهبة	المستلمة
HB101×GM8	blood + Rif	0	0	0	0
HB101×GM9	blood + Rif	0	0	0	0
HB101×GM19	blood + Rif	0	0	0	0
HB101×GM19	Ap + Rif	5	$10^{-3}\times1$	0	1
	Tc + Rif	2	$10^{-3}\times4$	0	0
	Cn + Rif	0	0	0	0
	blood + Rif	0	0	0	0



صورة ١: توضح البلازميدات المعزولة من البكتريا التي تشترك في صفة المقاومة للمضادات الحياتية وانتاج الهيمولايسين والعزلات المنتجة للهيمولايسين غير المقاومة للمضادات الحياتية

العمود الاول : يمثل البلازميد المعزول من العزلة *E. coli* GM8

العمود الثاني : يمثل البلازميد المعزول من العزلة *E. coli* GM9

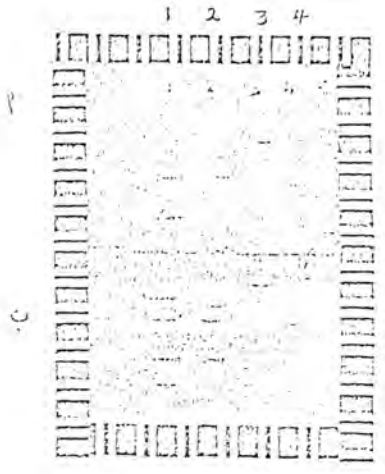
العمود الثالث : يمثل البلازميد المعزول من العزلة *E. coli* GM10

العمود الرابع : يمثل البلازميد المعزول من العزلة *E. coli* GM17

العمود الخامس : يمثل البلازميد المعزول من العزلة *E. coli* GM19

العمود السادس : يمثل البلازميد المعزول من العزلة *V. cholerae ogawn*

العمود السابع : يمثل البلازميد المعزول من العزلة *V. cholerae inaba*



صورة ٢: توضح انتقال البلازميدات المشفرة لمقاومة المضادات الحياتية عن طريق الاقتران

أ- بلازميدات المقاومة المعزولة من مستعمرات الخلايا المقترنة :

العمود الاول : يمثل البلازميد المعزول من مستعمرات الخلايا المقترنة الناتجة من تزاوج العزلتين *E. coli GM6* و *E.coli MM294* .

العمود الثاني : يمثل البلازميد المعزول من مستعمرات الخلايا المقترنة الناتجة من تزاوج العزلتين *E. coliGM9* و *E. coli* MM294

العمود الثالث : يمثل البلازميد المعزول من مستعمرات الخلايا المقترنة الناتجة من تزاوج العزلتين *E. coliGM10* و *E. coli* MM294

العمود الرابع : يمثل البلازميد المعزول من مستعمرات الخلايا المقترنة الناتجة من تزاوج العزلتين *E. coliGM19* و *E. coli* MM294

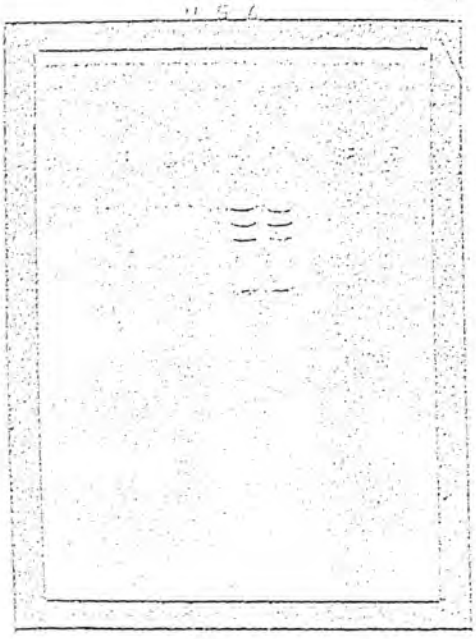
ب- بلازميدات المقاومة المعزولة من العزلات الاصلية الواهبة :

العمود الاول : البلازميد المعزول من العزلة الواهبة *E. coli GM6*

العمود الثاني : البلازميد المعزول من العزلة الواهبة *E. coli GM*

العمود الثالث : البلازميد المعزول من العزلة الواهبة *E. coli GM10*

العمود الرابع : البلازميد المعزول من العزلة الواهبة *E. coli GM19*

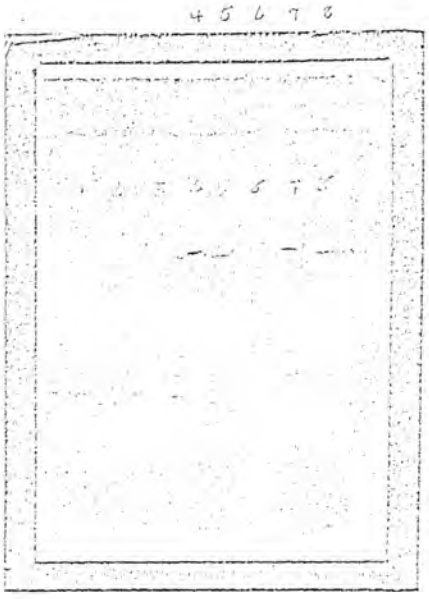


صورة ٣: توضح انتقال بلازميد الهيمولايسين عن طريق الاقتران من العزلة *E. coli* GM17 الى السلالة المستلمة *E. coli* HB101

العمود الرابع :يمثل بلازميد الهيمولايسين المعزول من مستعمرات الخلايا المقترنة الناتجة من تزاوج العزلتين اعلاه .

العمود الخامس : بلازميد الهيمولايسين المعزول من العزلة الاصلية الواهبة *E. coli* GM17

العمود السادس : عدم وجود أي بلازميد في السلالة الاصلية المستلمة *E. coli* HB101



صورة ٤: توضح وجود البلازميدات في الخلايا المقترنة الناتجة من تزاوج العزلات الواهبة *ogawa* و *inaba* مع سلالات *E. coli* HB101 المستلمة

العمود الرابع : يمثل البلازميد المعزول من مستعمرات الخلايا المقترنة الناتجة من تزاوج العزلة *ognawa* مع *E. coli* HB101

العمود الخامس : يمثل البلازميد المعزول من مستعمرات الخلايا المقترنة الناتجة من تزاوج العزلة *inaba* مع *E. coli* HB101

العمود السادس : عدم وجود أي بلازميد في السلالة المستلمة *E. coli* HB101

العمود السابع : يمثل البلازميد المعزول من العزلة الاصلية الواهبة *ognawa*

العمود السادس : يمثل البلازميد المعزول من العزلة الاصلية الواهبة *inaba*

دراسة مسببات الاسهال والعوامل المؤثرة عليه لدى الاطفال دون سن الخامسة من العمر لبعض مناطق محافظة ديالى / العراق .

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(أستلم بتاريخ ١٩٩٤/١/٢ ، قبل للنشر في ١٩٩٤/٥/٣١)

ABSTRACT

This research includs a study of diarrhoeal problem and identification of the factors affecting the incidence . A total of (200) mothers having children less than (5) years of age suffering from diarrhoea through visting many of health centers in Diyala province summer / 1993 . Five species of intestinal parasite were diagnosed , *Giardia lamblia* was the common parasite and three species of intestinal bacteria were identified , the *Escherichia coli* found to be the common bacteria . The percentage of infection among the children less than one year age higher than other ages . Mixed feeding children were highly infected especialy borne to young and illiterate mothers , and among children living within large families.

الخلاصة

يتضمن هذا البحث دراسة مشكلة الإسهال والعوامل المؤثرة على المصاب حيث شملت مقابلة (٢٠٠) أم من اللواتي لديهن أطفال دون سن الخامسة من العمر واللواتي يقمن بمراجعة عدد من المراكز الصحية في محافظة ديالى بسبب إصابة أطفالهن بمرض الإسهال خلال صيف / ١٩٩٣ .

دلت نتائج هذه الدراسة على تشخيص خمسة أنواع من الطفيليات المعوية المرافقة للإسهال . حيث كانت *Giardia lamblia* أكثر الأنواع إنتشارا ، وثلاثة أنواع من الجراثيم المعوية وكان *Escherichia coli* أكثر الأنواع شيوعا . سجلت نسبة اصابة الأطفال ذو عمر سنة واحدة أكثر من غيرهم من الفئات العمرية الأخرى . وظهرت أعلى نسبة للإصابة بين الأطفال الذين كانت أمهاتهم من غير المتعلمات والصغيرات السن والذين يتغذون على الحليب الطبيعي والإصطناعي معا والذين يعيشون ضمن أسر كبيرة العدد .

المقدمة

يعتبر الإسهال من الأعراض المرضية الخطرة تسببه عدة أنواع من الطفيليات والجراثيم المعوية والرواشح ، ويعتبر أحد الأسباب الرئيسة للوفيات بين الأطفال دون سن الخامسة من العمر ، حيث يقدر عدد الذين يتعرضون للإسهال في دول العالم الثالث بحوالي (٥٠٠) مليون طفل ، يموت منهم (٥-١٨) مليون طفل سنويا (1) . قام (2) بدراسة مسببات الإسهال لـ(٣٠٠) طفل في بغداد ووجد أن ٧٧٪ من الإصابات بين أطفال

السنة الأولى من العمر وسجل الجراثيم المعوية الآتية ونسبها Escherichia coli ١٤٪ ، Salmonella ٨٪ ، Shegilla ٣,٥ ٪ ، والأولة المعوية Giardia lamblia ١١٪ ، Entamoeba histolytica ٤٪ . ودرس (3) مسببات الأسهال بين أطفال أربيل وسجل نسبة إصابة بـ Salmonella ٦,٧٪ و Esherichia coli ١٤٪ و Shigella ٠,٦٪ . ووجد (4) أن Rotavirus المسؤولة عن (٣٠-٤٠٪) من إصابات الإسهال بين أطفال بغداد .

كما وجد (5) أن ١٨٪ من الأطفال المراجعين لمستشفى الطفل العربي في بغداد مصابين بالإسهال .

فقد درست (6) لـ(٢٠٠) حالة إسهال في بغداد ووجد بأن معدل الإصابة بالطفيليات المعوية هي ٦٣,٥٪ ، كانت أعلى نسبة للإصابة بالطفيل Blastocystes hominis ٢٩٪ ، وأقل نسبة للإصابة بالطفيل Ancylostoma doudenale ٠,٥٪ .

ولإفتقار هذه المحافظة لمثل هذه الدراسة فقد أجري هذا البحث وذلك :-

١. تشخيص أنواع الطفيليات والجراثيم المعوية المسببة للإسهال وتحديد نسب الإصابة بها .

٢. دراسة العوامل المساعدة لانتشارها .

المواد وطرق البحث

تم اختيار مستشفى جلولاء والمركز الصحي في السعدية/ ديالى لمقابلة (٢٠٠) أم من اللتي لديهن أطفال دون سن الخامسة من العمر واللواتي راجعن هذه المواقع بسبب إصابة أطفالهن بالإسهال للفترة من تموز ولغاية أيلول /١٩٩٣ . حيث تم فحص خروج هؤلاء الأطفال للتحري عن الطفيليات المعوية بطريقة مباشرة باستعمال المحلول الملحي Normal Saline Soultion والأيودين Iodin Soultion . كما تم عزل الجراثيم المعوية واتبعت طريقة الزرع بالتخطيط في أطباق تحوي على أكار الماكونتي Macconkey agar و Salmonella shigella agar وذلك بنقل ملئ الناقل من عينات البراز المحضرة سابقاً ، حضنت هذه الأطباق بدرجة حرارة (٣٧)م ولمدة (٤٨) ساعة .

وتم تشخيص هذه الجراثيم بالإختبارات الكيميائية الحياتية باستعمال IMVIC test ، تم تدوين المعلومات الخاصة بـ(عمر الطفل ، جنسه ، سكنه ، عمر الأم ، تعلمها ، مهنتها ، عدد أفراد الأسرة ، عدد الغرف في الدار ، نوع الرضاعة ، استعمال الأم لمحلول الإرواء الفموي) ، ودونت النتائج في جدول تبين العلاقة بين هذه الإصابات ، وتم تحليل النتائج احصائيا .

النتائج والمناقشة

يبين الجدول رقم (١) تشخيص خمسة أنواع من الطفيليات المعوية والتي قد تسبب الإسهال ـ أربعة منها عائدة للأولى وهي :-

Trichomonus hominis , Entamoeba histolytica , Giardia lamblia , Entamoeba coli .

علما أن الطفيليين الآخرين غير مرضيين .

وواحد عائدة للديدان وهي Hymenolepis nana ، وكان معدل الإصابة بهذه الطفيليات ٤١,٥٪ وهذا يتفق مع دراسة (7) .

جدول رقم (١) يبين النسب المئوية للإصابة بالطفيليات المعوية .

العدد الكلي للمفحوصين = (٢٠٠) طفل ، عدد الذكور = (٩٥) طفل ، عدد الإناث = (١٠٥) طفلة .

الطفيل	العمر	١-١٢ شهر		١٣-٢٤ شهر		٢٥-٣٦ شهر		٣٧-٤٨ شهر		٤٩-٦٠ شهر		عدد المصابين		عدد المصابين الكلي	٪ الاصابة
	الجنس	ذ	أ	ذ	أ	ذ	أ	ذ	أ	ذ	أ	ذ	أ		
G. lamblia		٥	٩	٤	١١	٥	–	٤	٥	٢	١	٢٠	٢٦	٤٦	٢٣
E. histolytica		٢	٦	٣	١	١	–	٣	٥	٢	١	١١	١٣	٢٤	١٢
T. hominis		–	–	١	١	١	–	٣	١	١	٢	٦	٤	١٠	٥
E. coli		–	–	–	–	١	١	–	–	–	–	١	١	٢	١
H. nana		–	–	–	–	–	١	–	–	–	–	–	١	١	٠,٥
المجموع		٧	١٥	٨	١٣	٨	٢	١٠	١١	٥	٤	٣٨	٤٥	٨٣	
المجموع		٢٢		٢١		١٠		٢١		٩				٨٣	
٪ الإصابة		١١		١٠,٥		٥		١٠,٥		٤,٥		١٩	٢٢,٥		٤١,٥

ذكور : قيمة F المحسوبة عند مستوى احتمال ٥٪ = ٨,٥*

اناث : قيمة F المحسوبة عند مستوى احتمال ٥٪ = ٨,٩*

لوجود ملوثات عديدة لمياه الشرب نتيجة تسرب مياه المجاري إلى نهر ديالى وتواجد أعداد كثيرة من الجواميس بالقرب من محطة تصفية المياه ، كما وجد أن ٩٠٪ من عوائل الأطفال المصابين لا يعقمون الفواكه والخضر قبل تناولها بل يقتصرون على غسلها بالماء فقط ، إضافة إلى أن ٢٩٪ من المصابين من القرى والتي لا يتوفر لهم قدرا كافيا من البيئة الصحية لحماية أطفالهم من مختلف أنواع التلوث ، كإلقاء المخلفات القذرة إلى الجداول ألتي قد تستخدم كمصادر لمياه الشرب . وسجل أعلى نسبة للإصابة بالطفيل Giardia lamblia ٢٣٪ وهذا يتفق مع ما جاء به (8) .

ولنفس الأسباب المذكورة وإضافة إلى مقاومة الطور المتكيس لهذا الطفيل نوعا ما لفعل الأدوية المضادة وخاصة عند عدم التقييد بجرعات ومدة استعمال العلاج . وكانت الإصابة بالطفيل Entamoeba histolytica عالية نوعا ما ١٢,٥٪ وهذا يتفق مع دراسة (9) وذلك لعدم إهتمام الأطفال بنظافة أيديهم وخاصة بعد التغوط .

كما سجلت الإصابة بالطفيليات T. hominis ٥٪ و Ent. coli ١٪ . وسجل أقل نسبة للإصابة بالطفيل H. Nana ٠,٥٪ . وظهرت نسبة إصابة بين الإناث أعلى من الذكور ٢٢,٥٪ ، ١٩٪ على التوالي (6) . كما سجلت أعلى نسبة للإصابة بين الأطفال السنة الأولى من العمر ١١٪ وهنا يتفق مع دراسة (10) ، وأقل نسبة للإصابة بين الفئات العمرية (٤٩-٦٠ شهر) وكانت ٤,٥٪ . وأكدت نتائج التحليل الإحصائي على وجود فروقات معنوية بين الفئات العمرية المختلفة لكلا الجنسين والمصابة بهذه الطفيليات .

يبين الجدول رقم (٢) أن معدل الإصابة بالجراثيم المعوية عالية ٣٤٪ وهذا يتفق مع ما جاء به (2) . وذلك لتلوث ثدي الأم عند الرضاعة المتكررة دون تنظيفه ، إضافة الى تلوث الملهاة المتكررة استعمالها دون تعقيم بين ٦٠٪ من الأطفال وكذلك تلوث مياه الشرب في بعض الاحيان ، لاعتماد ١٢٪ من المصابين على مياه الأنهار كمصدر للشرب ، وسجل أعلى نسبة للإصابة بالجرثومة Escherichia coli ١٨٪ وهذا يتفق مع ما سجله (11) . كما كانت نسبة الإصابة بـ Salmonella و Shigella ٩٪ و ٧٪ على التوالي ، وأكدت نتائج التحليل الإحصائي على وجود فروقات معنوية بين الفئات العمرية المختلفة لكلا الجنسين والمصابة بهذه الجراثيم ، ولم يتم تشخيص المسببات المرضية لـ ٣٠,٥٪ من الأطفال المصابين بالإسهال في هذا البحث .

جدول رقم (٢) يبين النسبة المئوية للإصابة بالجراثيم المعوية .

العدد الكلي للمفحوصين = (٢٠٠) طفل ، عدد الذكور = (٩٥) طفل ، عدد الإناث = (١٠٥) طفلة .

العمر / الطفيل / الجنس	١-١٢ شهر		١٣-٢٤ شهر		٢٥-٣٦ شهر		٣٧-٤٨ شهر		٤٩-٦٠ شهر		عدد المصابين		عدد المصابين الكلية	٪ الإصابة
	ذ	أ	ذ	أ	ذ	أ	ذ	أ	ذ	أ	ذ	أ		
E. coli	٣	٦	٥	٣	٤	٤	٢	٣	٢	٣	١٦	٢٠	٣٦	١٨
Salmonella	٣	٢	١	٣	٢	٢	٢	١	–	٢	٨	١٠	١٨	٩
Shegella	٢	٢	١	٢	١	٣	٢	–	–	١	٦	٨	١٤	٧
المجمـــوع	٨	١٠	٧	٨	٧	٩	٦	٥	٢	٦	٣٠	٣٨	٦٨	
المجمـــوع	١٨		١٥		١٦		١١		٨		٥			
٪ الإصـــابة	٩		٧,٥		٨		٥,٥		٤		١٥	١٩		٣٤

ذكور : قيمة F المحسوبة عند مستوى احتمال ٥٪ = ١٤,١٩*

اناث : قيمة F المحسوبة عند مستوى احتمال ٥٪ = ٣,٥*

جدول رقم (٣) تأثير التحصيل الدراسي للأم على نسبة الإصابة بين الأطفال .

العدد الكلي للمفحوصين = (٢٠٠) طفل .

التحصيل الدراسي للأم	عدد الاطفال المصابين	٪ الإصابة
غير متعلمة	١٠٨	٥٤
متعلمة	٩٢	٤٦
خريجة الدراسة الابتدائية	٥٢	٢٦
خريجة الدراسة المتوسطة	٢٧	١٣,٥
خريجة الدراسة الاعدادية	٨	٤
خريجة معهد أو كلية	٥	٢,٥

يبين الجدول رقم (٣) أن نسبة الإصابة بين الأطفال لأمهات (أميات) ٥٤٪ ، وبين الأطفال لأمهات (متعلمات) ٤٦٪ ووجد أنه كلما زاد تعلم الأم ووعيها في الأمور الصحية كانت الإصابة بين أطفالها أقل ما يمكن وذلك لإدراكها بمخاطر الطفيليات والجراثيم المعوية المسببة للإسهال وكيفية الوقاية منها قدر الإمكان (12) . حيث سجلت أعلى نسبة للإصابة بين الأطفال لأمهات (خريجات الدراسة الإبتدائية ٢٦٪ ، وأقل نسبة للإصابة بين خريجات المعاهد والكليات ٢,٥٪).

يبين الجدول رقم (٤) أن نسبة الإصابة بين الأطفال الذين يرضعون طبيعيا منخفضة ٢٢,٥٪ وذلك لأحتواء حليب الأم على الأجسام المضادة المقاومة للأمراض ، كما يحتوي على قيمة غذائية جيدة يتوفر فيه أنواع الفيتامينات والحوامض الأمينية الأساسيةلتغذية الطفل ونموه إضافة إلى كون حليب الأم معقما وسهل الهضم وغير ملوث ولا يربك معدة الطفل ، بينما كانت الإصابة بين الأطفال الذين يتغذون على الحليب المختلط عالية ٤٤,٣٪ وهذا يتفق مع دراسة (13) ، نتيجة تلوث هذا الحليب عند الإستعمال وفقدانه للأجسام المضادة المهمة .

يبين الجدول رقم (٥) أن أعلى نسبة لأنتشار الإسهال كان بين الأطفال الذين يعيشون ضمن أسر كثيرة العدد في أفرادها (سبعة أو أكثر) في الدار الواحدة (12) ، ٣٥,٥٪ ، نتيجة التزاحم وسهولة انتقال المسببات المرضية عن طريق التلوث بالتلامس ، كما سجلت أقل نسبة للإصابة بين الأطفال الذين يعيشون ضمن أسر قليلة العدد (٣-٤) ، ٣١,٥٪.

يبين الجدول رقم (٦) أن أعلى نسبة لأنتشار الاسهال كان بين الأطفال الذين يعيشون في الدور التي تحتوي على غرفتين أو أقل ٤٢,٥٪ ، وأقل نسبة للإصابة بين الأطفال الذين يعيشون في الدور التي تحتوي على خمسة غرف أو أكثر ١٧٪ ، وهذا يتفق مع دراسة (13) . وبينت الدراسة أيضا أن ٢٥٪ من الأطفال المصابين بالإسهال غير مسجلين في مراكز رعاية الأمومة والطفولة .

إن نتائج هذه الدراسة ذات فائدة كبيرة وذلك لأجل متابعة وتقويم الرعاية الصحية والتي تهدف إلى السيطرة على مسببات الإسهال أو تقليلها في العراق قدر الإمكان ، ولإفتقار هذه المحافظة لمثل هذه الدراسة فقد أجري هذا البحث للكشف عن أنواع الطفيليات والجراثيم المعوية المسببة للإسهال و العوامل المؤثرة على إنتشاره بين الأطفال ولتكون عونا لدراسات مستقبلية أخرى .

جدول رقم (٤) تأثير نوع الرضاعة على نسبة الإصابة بين الأطفال .

العدد الكلي للمفحوصين = (٢٠٠) طفل

نوع الرضاعة	عدد الأطفال المصابين	٪ الإصابة
طبيعي	٥٠	٢٢,٥
طبيعي / إصطناعي	٨٠	٤٢,٥
إصطناعي	٧٠	٤١,٥

جدول رقم (٥) تأثير عدد الأفراد في الدار على نسبة الإصابة بين الأطفال

العدد الكلي للمفحوصين = (٢٠٠) طفل .

عدد الأفراد في الدار	عدد الأطفال المصابين	٪ الإصابة
٤-٣	٦٣	٣١,٥
٦-٥	٦٦	٣٣
أكثر من (٧)	٧١	٣٥,٥

جدول رقم (٦) تأثير عدد الغرف في الدار على نسبة الإصابة بين الأطفال .

العدد الكلي للمفحوصين = (٢٠٠) طفل .

عدد الغرف في الدار	عدد الأطفال المصابين	٪ الإصابة
أقل من (٢)	٨٥	٤٢,٥
٤-٣	٨١	٤٠,٥
أكثر من (٥)	٣٤	١٧

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الدور المحتمل لبعض الحشرات في نقل وانتشار مرض خياس طلع النخل والفطريات المصاحبة له في جنوب العراق

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(استلم بتاريخ ١٩٩٣/٩/١٥ ، قبل للنشر في ١٩٩٤/٥/٢١)

ABSTRACT

Three species of insects were found associated with inflorescence rot disease of date palm caused by *Mauginiella scaettae* Cav. : *Carpophilus hemipterus* L. Nititulidae : Coleoptera , *Arenipses sabella* Hamps. Pyralidae : Lepidoptera , *Physiphora demendata* Ulidiidae : Diptera. All of them were isolated from infected spaths. *Manuginiella scaettae* was found in all samples in higher frequency Compared with other associated fungi isolated from spaths and the external surfaces of larvae and adults of the three insects species infecting spaths. *Mauginiella scaettae* and the other associated fungi were found in the digestive canal of larvae *Arenipses sabella* Hamps. But not in Carpophilus hemipterus and *Physiphora demendata.*

الخلاصة

وجدت ثلاثة انواع من الحشرات مصاحبة لمرض خياس طلع النخيل والذي يسببه الفطر *Mauginiella scaettae* Cav. وهي خنفساء الجافة ذات البقعتين *Carpophilus hemipterus* L. Nitidulidae : Coleoptera وعثة التمر الكبيرة *Arenipses sabella* Hamps. Pyralidae: Lepidoptera والذباب الملون الاجنحة Physiphora demendata Ulidiidae Diptera: ، عزلت جميعها عن الطلع المصاب . الفطر Mauginiella scaettae Cav وجد متكرراً بين انواع الفطريات الاخرى المعزولة عن الطلع المصاب وكذلك المعزولة عن السطوح الخارجية لأجسام كاملات ويرقات الانواع الثلاثة من الحشرات التي جمعت من عينات الطلع المصاب . كما تم عزل الفطر *Mauginiella scaettae* والفطريات المصاحبة له عن القناة الهضمية ليرقات الحشرة *Arenipses sabella* ، في حين لم يظهر ضمن الفطريات المعزولة عن القنوات الهضمية ليرقات الحشرات *Carpophilus hemipterus*, *Physiphora demendara* .

المقدمة

يعتبر النخيل من الاشجار الاقتصادية المهمة في العراق من خلال ما تنتجه من التمور وما لهذه المادة من فوائد غذائية هامة ، اذ تحتوي على عناصر ذات قيمة غذائية عالية كالمواد السكرية والاملاح المعدنية وبعض الفيتامينات اضافة الى نسبة من البروتينات والدهون ، (١) ، (٢) .

أن التمور تشكل مادة اولية في صناعة الخل والدبس والكحول والسكر اضافة الى ان الياف وسعف النخيل هي مادة اولية لصناعات اخرى (٣) . الا ان هذه الاشجار عرضة للاصابة بامراض وآفات عديدة من اهمها مرض خياس النخيل المتسبب عن الفطر .

Mauginiella scaettae Cav. والذي ينتشر في العراق مابين منطقة ديالى شمال شرق العراق وشط العرب جنوب العراق (١) .

أن العديد من انواع الحشرات تنجذب الى الطلع المصاب بتأثير رائحته تنجذب الى الطلع المصاب بتاثير رائحته الخاصة كما ان بعضا منها يكون غذائها الاساس طلع النخيل ، ومن هذه الحشرات عثة التمر الكبيرة *Arenipses sabella Hamps* التي تنتشر في وسط وجنوب العراق وتتغذى يرقاتها خلال شهر اذار مارس واوائل شهر نيشسان (ابريل) على الجزء العلوي من الطلعة ، اذ تحفر يرقاتها اخاديد متعددة في راس غلاف الطلعة ، وبعد انفتاح الغلاف وظهور العناقيد الزهرية تقوم اليرقات بالتغذي على الازهار (٤ ، ٥) .

ونظرا لقلة الدراسات على دور الحشرات في نقل وانتشار مرض خياس طلع النخيل والفطريات المصاحبة له ضمن الطلعة الواحدة وضمن طلع النخلة الواحدة ومن نخلة الى نخلة اخرى في بساتين النخيل جاءت فكرة اجراء هذه الدراسة .

المواد والطرق المستخدمة

تم جمع عينات عن طلع مصاب بمرض خياس طلع النخيل واخرى سليمة من المرض للاصناف (فحول فكري وغنامي ، واناث خضراوي وبريم) ، عن مناطق كتيبان ، شط العرب وابي الخصيب في محافظة البصرة ، وعينات عن منطقة الشطرة في محافظة ذي قار جنوب العراق ، خلال شهري آذار (مارس) ونيسان (ابريل) . وضعت العينات في اقفاص معدنية بقياس ٤,٥ * ٦,٥ سم مغطاة بقماش من الشيفون الناعم ، تركت الاقفاص في المختبر بدرجة حرارة (٢٠-٢٦) م لحين ظهور الحشرات الكاملة وتم دراسة ما يلي :

١- الفطريات والحشرات المصاحبة لمرض خياس طلع النخيل : اخذت كميات قليلة من الطلع المصاب الموجود في كل قفص وزرعت على انفراد في اطباق بتري ذات ١٤,٥ سم تحتوي على الوسط الغذائي PDA ، وبعد مرور ٧ ايام من الحصى في حاضنة درجة حرارتها (٢٣ ± ١)° م فحص الاطباق وتم تشخيص الفطريات بواسطة المجهر الضوئي الاعتيادي . في حين ارسلت كاملات الحشرات التي ظهرت في الاقفاص الى متحف التاريخ الطبيعي في بغداد لغرض التشخيص .

٢- دور الحشرات في نقل مرض طلع النخيل والفطريات المصاحبة له :

أ- اخذت ١٥ يرقة من كل نوع من انواع الحشرات التي ظهرت في الاقفاص باستعمال ملقط معقم ووضعت منفردة في اطباق بتري قطرها ١٤,٥ سم حاوية على وسط غذائي PDA ثم حضنت في درجة حرارة (٢٣ ± ١)° م ولمدة ٧ ايام ، ثم ازيلت اليرقات منها . لغرض تحديد الفطريات المتواجدة على السطح الخارجي ليرقات الحشرات من الفطريات النامية على سطح الوسط الغذائي .

ب- اخذ عدد من كاملات الحشرات بواسطة الملقط المعقم ووضعت بشكل انفرادي في اقفاص معقمة حاوية على طلع غير مصاب وتركت في حاضنة بدرجة حرارة (٢٣ ± ١)° م ورطوبة نسبية (٥٥-٦٠)٪ واضاءة ١٢ ساعة يعقبها ١٢ ساعة ظلام . ثم عزلت الفطريات النامية على الطلع وشخصت ح . تم تشريح عدد من اليرقات لانواع الحشرات المصاحبة لمرض خياس طلع النخيل بعد تعقيمها بغمرها في محلول هايبوكلورات الصوديوم ١٠٪ ولمدة ثلاث دقائق لقتل الفطريات النامية على السطوح الخارجية لليرقات . ثم زرعت قنواتها الهضمية على وسط غذائي PDA وحضنت بدرجة حرارة (٢٣ ± ١)° م ولمدة ٧ ايام وشخصت الفطريات النامية على الوسط الغذائي .

النتائج والمناقشة

وجدت ثلاثة انواع من الحشرات مصاحبة لمرض خياس طلع النخيل والذي يسببه الفطر . *Mauginiella scaettae* Cav. وهي خنفساء الثمار الجافة ذات البقعتين *Carpophiuls hemipterus* L

Nitidulidae Coleoptera التمر الكبيرة Arenipses sabella Hamps. Pyralidae: Lepidoptera ، والذباب الملون الاجنحة *Physiphora demendata* Ulidiidae: Diptera (جدول ١) . جميع هذه الانواع جمعت من الطلع المصاب ، ومن المحتمل ان يكون لهذه الحشرات دور مهم في نقل وانتشار مرض خياس طلع النخيل وذلك من خلال التصاق حبيبات الطلع الحاملة للفطر المسبب للمرض على اجسام تلك الحشرات او من خلال تغذي تلك الحشرات على حبيبات الطلع المصابة بالمرض .

الفطر . *Mauginiella scaettae* وجد متكرراً بين انواع الفطريات الاخرى المعزولة عن الطلع المصاب وكذلك المعزولة عن السطوح الخارجية لاجسام كاملات ويرقات الانواع الثلاثة عن الحشرات التي جمعت عن عينات الطلع المصاب (جدول ٢ ، ٣) .

لقد اشار العديد عن الباحثين الى ان الحشرات تلعب دوراً مباشراً او غير مباشر في نقل بعض الامراض الفطرية والفايروسية ، فقد عزل سلمان وجماعته (٦) الفطر *Fusarium oxysporium* عن جسم الحلم *Eriophyes manigiferae* كما وجودا ان الحفرة Chrysomya albiceps تحمل الحلم *E. Manigiferae* على شعيرات جسمها وارجلها مما يؤكد ان هذه الحشرات تلعب دور غير مباشر في نشر الفطر .

ووجد شاهين (٧) ان المن *Myzus persica* عن العوامل الحشرية الرئيسية في نقل عرض التفاف اوراق البطاطا الفايروسي في اليمن .

واظهرت النتائج التي توصل اليها حجاب وجماعته (٨) ان الذبابة البيضاء *Bemisia tabaci* Genn مسؤولة عن نشر بعض الفايروسات المرضية المصاحبة لامراض تبرقش واصفرار الطماطة Tomato yello trop ، تبرقش واصفرار البطاطا والتفاف حواف اوراق البطاطا عن النباتات المصابة الى النباتات السليمة في مصر .

كما توصل حجاب (٩ ، ١٠) من خلال النتائج التي حصل عليها ان الحشرة *Aphrophora solicina* G. والحشرة *Balclutha hortensis Lindb* . عاملان جديدان مهمان في نشر الـ (Mycoplasma) المسبب لمرض Rubus stunt disease لنباتات Raspberry في هنكاريا ونشر (M. L. B.) الاجسام المشابهة للمايكوبلازما مصاحبة لمرض الاخضرار التويجي لنباتات Strawberry من النباتات المصابة الى النباتات السليمة في مصر وعلى التوالي .

ان خنفساء الثمار الجافة ذات البقعتين لها القابلية على الطيران لمسافة كيلو متر في اليوم الواحد بارتفاع درجات الحرارة اثناء النهار مما يساعد الحشرة الكاملة على الوصول الى راس النخلة واصابة الطلع المتفتح (٤) . وتشير نتائج الجدول ٤ الى ان الفطر *Mauginiella scaettae* Cav والفطريات المصاحبة له تم عزلها عن القناة الهضمية ليرقات الحشرة *Arenipses sabella* Hamp. ولم يظهر وجوده ما بين الفطريات المعزولة عن القنوات الهضمية ليرقات الحشرات *Carpophilus hemipterus* و *Physiphora demendata* .

ان بعض الفطريات المتواجدة على الاعشاب والتي تؤكل من قبل الحيوانات لوحظ انها تقاوم العصارات الهضمية وتنبت على روث تلك الحيوانات مثل الفطر *Fusarium* والفطر *Cladosporium* . ووجد من خلال التشريح الداخلي للضفادع وجود بعض الخنافس داخل قناتها الهضمية وعندما تم تشريح تلك الخنافس وجدت سبورات الفطر *Basidiobolus ranarum* داخل قناتها الهضمية (١) .

ومن خلال النتائج التي تم التوصل اليها في هذه الدراسة يتبين الدور الذي تقوم به هذه الانواع من الحشرات في وبائية مرض خياس طلع النخيل في البساتين المصابة والتي تؤدي الى خسائر كبيرة في انتاجية محصول التمر ، لذلك اصبح من الضروري مكافحة هذه الانواع من الحشرات باستخدام المبيدات الحشرية الفعالة وذلك قبل بزوغ الطلع وفي فترة بزوغه . كما يتوجب اقتلاع الطلع المصاب بمرض خياس طلع النخيل من النخلة المصابة لكون رائحة خياس الطلع تجذب بعض الانواع من الحشرات ومن ثم تصبح تلك الطلعة المصابة

مصدر لعدوى الطلع السليم . حيث تكون تلك الحشرات واسطة لنقل العزل الفطري والسبورات لفطر خياس طلع النخيل والفطريات المصاحبة له ضمن طلع النخلة الواحدة او الى طلع النخيل السليم المجاور للنخلة المصابة .

جدول ١ : الطريات والحشرات المصاحبة لمرض خياس طلع النخيل

المصدر	مكان وتاريخ الجمع	الحشرات المصاحبة		الفطريات المعزولة
		الطور	النوع	
فحل فكري	ابو الخصيب اذار (مارس) ١٩٨٩	Adult Larvae	*Carpophilus hemipterus**	*Mauginiella scaettae* *Rhizopus sp.* *Fusarium rosium*
انثى خضراوي	شطرة نيسان (ابريل) ١٩٨٩	Adult Larvae Pupa	*Arenipses sabella***	*Mauginiella scaettae* *Penicillium sp.* *Rhizopus sp.* *Tricothecium roseum*
انثى ابزيم		Adult Larvae	*Physiphora demeddata*	*Mauginiella scaettae* *Fusarium roseum* *Trichothecium reseum*
فحل عنامي	شط العرب نيسان (ابريل) ١٩٨٩	Adult Larvae Pupa Adult Larvae Pupa	*Arenipses sablla* *Physiphore demeddata*	*Mauginiella scaettae* *Rhizopus sp.* *Aspergillus sp.* *Fusarium roseum*
فحل فكري	شطرة اذار (مارس) ١٩٨٩	Adult Larvae Pupa Adult Larvae Pupa	*Arenipses sabella* *Carpophilus hemipterus*	*Mauginiella scaette* *Fusarium roseum* *Trichothecium roseum*
انثى خضراوي	كتيبان اذار (مارس) ١٩٨٩	Adult Larvae Pupa	*Carpophilus hemipterus.*	*Mauginiella scaettae* *Penicillium sp*

* الرتبة Coleoptera ، الفصيلة Nitidulidae

** الرتبة Lepidoptera ، الفصيلة Pyralidae

*** الرتبة Diptera ، Ulidiidae

جدول ٢ : الفطريات المعزولة عن السطح الخارجي لاجسام يرقات الحشرات المصاحبة لمرض خياس طلع النخيل

يرقة الحشرة	الفطريات المعزولة
Arenipses sabils	*Mauginiella scaettae* *Trichothecium roseum* *Fusarium roseum*
Carpophillus hemipterus	*Fusarium roseum* *Mauginiella scaettae* *Rhizopus sp.*
Physiphora demendata	*Mauginiella scaettae* *Trichothecium roseum* *Aspergills sp.* *Fusarium roseum*

جدول ٣ : الفطريات المعزولة عن السطح الخارجي لاجسام كاملات الحشرات المصاحبة لمرض خياس طلع النخيل

انواع الحشرات	
Carpophilus hemipterus	*Fusarium sp.* *Mauginiella scaettae* *Aspergillus sp.*
Physiphora demendata	*Mauginiella scaettae* *Fusarium sp.* *Aspergillus sp.* *Trichothecium roseum*
Arenipses sabella	*Mauginiella scaettae* *Aspergillue sp.* *Trichothecium roseum* *Fusarium sp.* *Penicillum sp.*

جدول ٤ : الفطريات المعزولة عن القناة الهضمية ليرقات الحشرات المصاحبة لمرض خياس النخيل

انواع الحشرات	انواع الفطريات المعزولة
Arenipses sabella	*Aspergillus niger* *Hauginiella scaettae* *Fusarium sp.*
Carpophilus hemipterus	*Rhizopus sp.* *Fusarium sp.*
Physiphora demendata	*Rhizopus sp.* *Alternaria sp.*

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