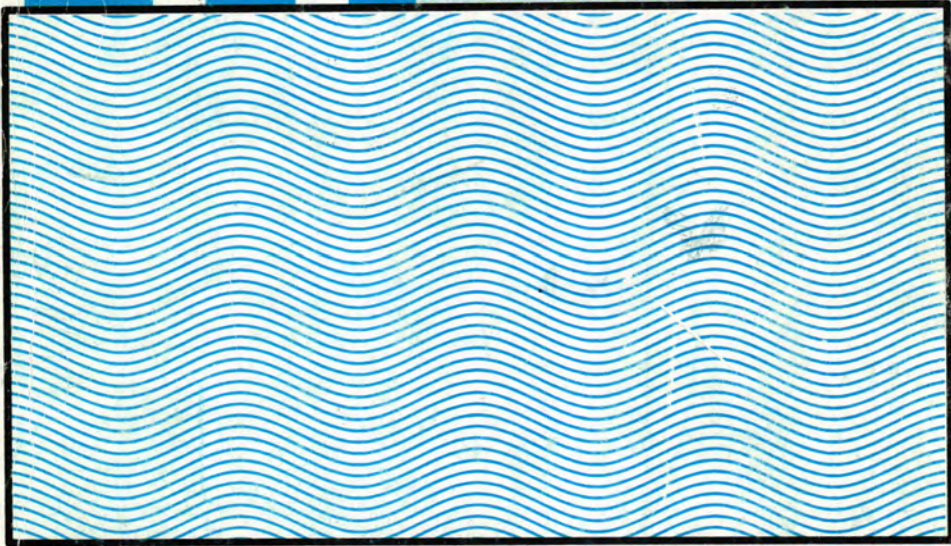


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Effect of The Major Chemical Constituents of Plant Extracts on Phytopathogenic Fungal Species

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الخلاصة

تم اختبار فاعلية المستخلص الكحولي الخام لستة أنواع من النباتات الطبيعية التي تشمل نباتات فرشة البطل، الحلفاء، الجهنمية، ورد نيسان، جرجسونيا وعين السبزون والمكونات الكيمائية لهذه المستخلصات ضد الفطريات التي تصيب النباتات وهي فطريات الترنازيا الترنازا، اسيرجلس فلافس و فيوزاريوم اوكسيبورم تم استخلاص المستخلص الخام بطريقة الاستخلاص المستمر باستخدام الميثانول الحار في المرحلة الاولى ثم محلول الميثانول في الماء (٨٠ / ٢٠ حجم / حجم). اما المكونات الاساسية المعزولة من المستخلصات الخام هي الفلافونويدات، الالكولويدات، الصابونيات، والترينيات. واختبرت فاعلية كل من هذه المكونات ضد الفطريات المذكورة أعلاه. اظهرت النتائج ان جزء الصابونيات لنبات عين البزون قد وجد اكثر فاعلية من باقي المكونات ضد الفطريات قيد الدراسة.

ABSTRACT

Crude extracts of six plant species including *Callistemon*, *Lanceolatus*, *Impirata cylindrica*, *Bougainvilla glabra*, *Girgensohnia oppositiflora*, *Glausium corniculatum*, and *Vinca rosea*, and their major constituents were tested for their antifungal activity against phytopathogenic fungi. The fungal species were *Alternaria alternata*, *Aspergillus flavis*. And *Fusarium oxysporum*. The crude extracts were obtained by continuous extraction method using hot methanol in the first step, followed by hot aqueous methanol (80 / 20 V/V). The separated fractions isolated from the crude extracts were Flavonoides, Alkaloids. Saponines, and Terpenes; each of them were tested against the above mentioned fungal species. The Saponin fraction of the plant *Vinca rosea* was found to be the most active constituent agaonst the tested fungi.

INTRODUCTION

In Iraq a variety of plants are widely used in folk medicine (1&2). Some of these plants have been systematically studied for their chemical and biological properties (3-5).

Many reports published in this respect indicate that these plants possess antimicrobial (6&7) algicidal and molluscicidal, and antihelmentic activity (8).

The antifungal activity of some plants and their major chemical constituents have been reported against pathogenic, phytopathogenic, phytotoxic, and toxin producing fungal species in different countries (9).

However little attention has been given to this matter in Iraq inspite of the wide distribution of such species as *Alternaria alternata*, *Aspergillus flavus*, and *Fusarium oxysporum*, and the considerable economic losses in agricultural crops caused by them (10-12), in addition to the health hazards caused by their toxic secondary metabolites (13).

Therefore the antifungal activity of six plant species and their major constituents was assayed against these fungi as an attempt to use plant extracts or their main components as natural fungicides, considering the abundancy of these plants in Iraq.

MATERIAL & METHODS

Plant Material

The following plant species *Callistemon lanceolatus* (Sm) Dc (Myrtaceae) : *Imperata cylindrica* (L) P. Beauv. (Gramineae) : *Bougainvillea glabra* Choisy. (Nyctaginaceae) : *Girgensohnia oppositiflora* (pall) Fenzl. (chenopodiaceae) : *Glaucium corniculatum* (L) Rud. (Papaveraceae) : *Vinca rosea* L. (apocyanaceae). Were collected from different parts of Iraq.

They were identified and authenticated by the Iraqi National Herbarium in Baghdad. The aerial parts of these plants were air dried at room temperature and ground to powder from *Extraction*.

The powdered plant material of each plant (40g) was extracted with hot methanol, then hot aqueous 80% methanol; the two extracts were combined and concentrated in *vaccuo*.

The detection of major constituents was proceeded by the Froth test for saponins. MgHCl test for flavonoids 40% H₂SO₄ for terpenes, and Dragendorff for alkaloids.

Isolation of Major Constituents

Each concentrated plant extract was divided into four parts for isolation of the major plant constituents.

- Part 1 : (Terpenes)

It was partitioned with CH₂CL₂ (4 x 100 ml. Portions) and concentrated in *Vaccuo*. TERPENES were separated on silica gel TLC plates developed in (CH₂CL₂ : MeOH) (25:1).

Further purification was made using another solvent system, (EtOAC : Aceton) (9:1) (9)

- **Part 2 : (Alkaloids)**

This part was combined with 100 ml of 5% HCl. And the acidic solution was washed with EtOAC (3 x 100 ml portions).

Ammonia solution was added to the aqueous acidic layer to get PH 9, and the preprecipitate was extracted with CH₂CL₂ (3 x 100 ml portions). This was evaporated to dryness to give total ALKALOIDS (9).

- **Part 3 : (Saponin)**

This part was dissolved in distilled water and partitioned with Butand (3 x 100 ml portions) and the combined butanolic extracts were evaporated to dryness. Minimum amount of methanol was added to the residue and the SAPONIN was precipitated by ether and purified on silica gel TLC plates developed in (CH₂CL₂ : Methanol : water) (6.5 : 3.5 : 0.5), Location of the spot was determined by spraying the plates with chlorosulphonic acid spray reagent (5).

- **Part 4 : (Flavonoids)**

The fourth part was dissolved in distilled water and separated on silica gel TLC plates developed in (Butanol : Acetic acid : water) (40 : 10 : 2.5). Location of the spot was determined by spraying the plates with methanolic LOH (9).

Analytical TLC was carried out using TLC sheets of silica gel 0.25mm (Kluka). Preparative TLC was made using glass plates (20 x 20 cm) coated with silica gel 0.5mm (Fluka).

Fungal species

Alternaria alternata (Fr.) keissl and *Fusarium oxysporum* Schlecht was isolated from tomatoes in Baghdad region, while *Aspergillus flavus* link ex Fr. Was isolated from corn at the southern part of Iraq. They were identified according to Ellis, 1971 (14), Booth, 1971 (15) and Raper & Fennel, 1965 (16) respectively.

Antifungal assay

The agar diffusion method was carried out using petri dishes containing PDA (Oxoid) with one hole in the center (10 mm in diameter). Aliquouts of 0.2ml of each extract or chemical constituents were added into each hole and left at room temperature for 24th, to permit the extract diffusion in the medium. Methanol was used as experimental control.

Each fungal species was inoculated in three spots around the hole at equal distances. After 4 days of incubation at 25 c the diameter of the colonies and other morphological aspects were observed macroscopically and microscopically.

RESULTS & DISCUSSION

The major chemical constituents of the plants under study was saponin and the highest amount of saponin compounds were obtained from *Vinca rosae* and *Girgensohnia oppositiflora* (4.5% and 3% respectively) as shown in Table -1. This will lead us to the conclusion that the antifungal activity of these plant extracts is mainly due to the saponin fraction.

There was variable inhibitory effects against the same fungal species (Table -2) which indicate different chemical grouping of these saponins (16 & 18). The saponin fraction of the plant extracts has shown a selective antifungal activity against the fungal species tested, with the exception of the saponin fraction obtained from *Vinca rosae* which showed complete inhibition activity against all fungal species under study.

The mehanolic extract of *Glaucim corniculatum* contained in addition to the saponin, alkaloids of isoquinolin type (18) at 1% concentration which showed a good slective inhibitory action against the fungs *F. oxysporum*.

The effects of the plant extracts and their major constituents on the morphological aspects of the tested fungi were noted on the abundancy of mycelial growth, pigmentation, sporulation in addition to the diameter to the colonies.

CONCLISIONS

The results of this work showed the following :

- 1- The antifungal activity of these plant extracts is mainly caused by the saponin fraction.
- 2- The variable inhibitory effects of saponin fractions against the same fungal species indicate different chemical grouping of these saponins (17 & 18).
- 3- The saponin fraction from the methonlic extract of *Vinca rosae* was the most effective agent against all the tested fungi.

Table -1 : The major constituents of plant species under study

Plant species	Plant family	Constituents	% Yield
Callistemon lanceolatus	Myrtaceae	Flavonoids	0.0
		Saponins	0.1
		Alkaloids	0.0
		Terpenes	0.0
Impirata cylindrica	Gramineae	Flavonoids	0.0
		Saponins	0.7
		Alkaloids	0.0
		Terpenes	0.0
Bougainvillea glabra	Nyctaginaceae	Flavonoids	0.0
		Saponins	0.3
		Alkaloids	0.0
		Terpenes	0.0
Girgensohnia oppositiflora	Chenopodiaceae	Flavonoids	0.0
		Saponins	3.0
		Alkaloids	0.0
		Terpenes	0.2
Glaucium corniculatum	Papaveraceae	Flavonoids	0.0
		Saponins	1.3
		Alkaloids	1.0
		Terpenes	0.0
Vinca rosea	Apocynaceae	Flavonoids	0.0
		Saponins	4.5
		Alkaloids	***
		Terpenes	0.0

*** = Present but not weighed.

Table 2 : Inhibitory action of different plant constituents on fungal species

Plant species	Plant Constituents	Inhibition zone on Fungal species		
		(1)	(2)	(3)
Callistemon lanceolatus	Flavonoids	-	-	-
	Saponins	+	+	+
	Alkaloids	-	-	-
	Terpenes	-	-	-
Lmpirata cylindrica	Flavonoids	-	-	-
	Saponins	+++	+	+
	Alkaloids	-	-	-
	Terpenes	-	-	-
Bougainvillea glabra	Flavonoids	-	-	-
	Saponins	++	+	+
	Alkaloids	-	-	-
	Terpenes	-	-	-
Girgensohnia oppositiflora	Flavonoids	-	-	-
	Saponins	++++	+++	++
	Alkaloids	-	-	-
	Terpenes	-	-	-
Glaucium corniculatum	Flavonoids	-	-	-
	Saponins	++++	++	++++
	Alkaloids	+++	++	++
	Terpenes	-	-	-
Vinca rosea	Flavonoids	-	-	-
	Saponins	+++++	+++++	+++
	Alkaloids	++	++	++
	Terpenes	-	-	-

(1) *Alternaria alternata*

(2) *Aspergillus flavus*

(3) *Fusarium oxysporum*

- = No inhibitory action.

+ = Inhibition zone diameter between 12-16 mm.

++ = Inhibition zone diameter between 17-22 mm

+++ = Inhibition zone diameter between 23-30 mm.

++++ = Inhibition zone diameter between 31-35 mm.

+++++ = No growth.

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Comparison Between Chemical, Physical and Laser Methods on Destruction of Local Isolates of Some Pathogenic Bacteria

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الخلاصة

عزلت اربعة انواع من البكتريا المرضية، اثنان منها سالبة لغرام (*Pseudomonas aeruginosa*, *Escherichia coli*) واثنان موجبة لغرام (*Staphylococcus aureus*, *Bacillus subtilis*) من مرضى يعانون من التهابات الجروح، الحروق، الجهاز التنفسي اضافة الى اسهال الاطفال. شخّصت البكتريا اعتماداً على الفحوصات الزرعية والشكلية والكيميائية. عرضت العزلات اعلاه (على افراد) الى المعاملة الكيميائية بمركب الـ NTG والفيزياوية بالاشعة فوق البنفسجية والى نوعين من الليزر هما ليزر النايتروجين (337 نانوميتر)، وبكثافات طاقة (42.4, 84.9, 1273) جول/سم² وليزر ثاني اوكسيد الكربون (10.6 مايكروليتر) بكثافات طاقة (188.6, 377.3, 754.5) جول /سم². أظهرت الخطوط البيانية لبقاء عزلات البكتريا حية ان الاشعة فوق البنفسجية كانت الافضل قسي قتل البكتريا يليها ليزر ثاني اوكسيد الكربون ثم ليزر النيتروجين واخيراً المعاملة الكيميائية.

ABSTRACT

Four pathogenic bacteria : (*Pseudomonas aeruginosa*, *Escherichia coli*) as gram negative, (*Staphylococcus aureus*, and *Bacillus subtilis*) as gram positive were isolated from patients complaining of wound-burn infections, infants diarrhea, lower respiratory tract infections. The bacteria were identified according to the cultural, morphological and biochemical examinations. The four isolated bacteria have been, separately, exposed to nitrosoguanidine (as a chemical method), UV light (as a physical method) and two types of laser light. The lasers used were : nitrogen laser (337nm) with fluences of (42,4, 84,9, 1273) J/cm² and CO₂ laser (10.6 μm) with fluences of (188.6, 377.3, 754.5) J/cm². The survival curves of the test bacterial isolates showed that the best killing method was the UV light followed by CO₂ laser, nitrogen laser and chemical method.

INTRODUCTION

Nitrosoguanidine (MMNG) which is an alkylating chemical agent, is used for killing the bacteria as well it is commonly used in the laboratory for creating mutations. It is the most potent chemical mutagen, which induces clusters of

closely linked mutations in the segment of the replicating chromosome. MNNG is fairly selective, it results with some bases and produces as specific kind of DNA damage^(1,2). While the physical method based on the use of UV light (260nm) to irradiate the test bacteria, UV light kills all kinds of microorganisms due to its short wavelength (10-400 nm) and its high energy. Certain substances such as purines and pyrimidines which then enter more reactive or excited state, are readily absorbed by UV light. DNA is most effected by UV light especially at a wavelength of (260)nm. But the maximum absorption of DNA by the UV light is in the wavelength (254 nm), which gives also maximum mutagenicity. Pyrimidines are absorbed strongly at (254nm) and become very reactive, pyrimidine hydrates and pyrimidine dimers are the two major products of UV absorption by pyrimidines^(2,3).

Recently, the laser is used as a promising tool for bacterial treatment because of its coherence, monochromaticity, collimation, directionality and its photon energy⁽⁴⁾. Mainly the wavelengths of the known lasers lied in three regions, the ultraviolet region which have photochemical effects, the visible region with either photochemical or photodestructive effect, and in the infrared region which have photothermal effect^(5,6). This study aimed to compare some of the conventional methods (physical and chemical) with two laser methods (CO₂ and N₂) for destruction of some common pathogenic bacteria (*P. aeruginosa*, *E.coli*, *S. aureus*, and *B. subtilis*).

MATERIALS AND METHODS

Bacterial Specimen

The following four pathogenic (test) bacteria (two G + and two G-) were used:

<u>Bacteria</u>	<u>Source of isolaes</u>
Bacillus subtilis	Contaminated wound and burn
Staphyococcus aureus	Lower respiratory tract (trabstracheal aspiration of sputum)
Escherichia coli	Infants diarrhia
Pseudomonas aeruginosa	Wound and burn infections

These bacteria were identified according to cultural characteristics, microscopic examinations and biochemical tests^(7,8,9).

The culture of each isolate was transferred from nutrient agar slant to brain-heart infusion broth, incubated at 37°C for 24 hours. Each culture broth was centrifuged by 6000 xg for 10min at 4°C. Cell pellets were washed twice with phosphate buffer (pH. 7), then mixed by a vortex and suspended in the normal saline solution.

Preparation of Isolates for Treatment

For chemical treatment, MNNG in the concentration of $100\mu\text{g} / 100 \text{ m}\ell$ was used, $10\text{m}\ell$ of phosphate buffer (pH=7) was used to suspend the washed bacterial cells in flask of $100 \text{ m}\ell$, $0.1 \text{ m}\ell$ of MNNG was added after mixing, placed in a shaking incubator at 37°C , (30 rpm) for (10, 30, 60, 120)min⁽¹⁾.

For the physical treatment; bacterial cells were washed with normal saline solution for 3 times, the suspensions then were distributed in sterilized petridish ($5 \text{ m}\ell$ each). The cells were exposed to the UV light for different periods of times (1,2,3,4 5 min) with contin imovement to open petridish at a distance of 10 cm from the UV lamp.

Two lasers (CO_2 and N_2) were used to study the laser effect. Nitrogen laser with the following parameters, (337 nm) wavelength, 10ns 1.5 mJ pulse energy and 30Hz repetition rate.

The exposed area to the laser beam was 0.63cm^2 . Bacterial cells were suspended in normal saline solution containing $1 \text{ m}\ell$ of each kind of bacteria, then transferred to sterile eppendrof tubes and treated by nitrogen and carbon dioxide lasers. The exposure times for the prepared bacteria for the nitrogen laser were (10, 20, 30) min. According to these parameters, the nitrogen laser fluences were (42.4, 84.9, 127.3)J/cm².

Carbon dioxide pulsed laser of the following parameters (10.6 μm wavelength, 100ns pulse duration, 0.8J pulse energy and 1Hz repetition rate) were used. The exposure times were (2.5, 5, 10) min, then the fluences were (188.6, 377.3, 754.5)J/cm²⁽¹⁰⁾.

A (genetic ED-500) detector was used to measure the pulse energy. The measurements were recorded before and after each exposing time.

The laser beam was screened by a mask of a circular aperture (0.9 cm in diameter) which represented the required exposing diameter.

The eppendrof tube was located on a fixed base. A mounted mirror was used to adjust the reflected laser beam on the required area of the eppendrof which were suspended in a normal saline solution containing $1\text{m}\ell$ of each test bacteria, then they were transferred to sterile eppendrof tubes.

RESULTS AND DISCUSSION

Figure (1,2,3,4) show the survival curves of *P.aeruginosa*, *E.coli*, *S.aureus* and *B.subtilis*, respectively, after subjection to the chemical, physical and the two lasers means. The slope value for each curve was determined, the slope value is equal to $\Delta y/\Delta x^{(11)}$. (Δy represents the number of dead colonies, while Δx represents the exposure time in min).

So the

$$\text{Slope value} = \frac{\text{Number of dead colonies}}{\text{Exposure time (min)}}$$

The increase in the slope value is due to the increment in Δy . So from the slope value results the effect of each chemical, physical, and laser methods on test bacteria (illustrated in table 1) could be better discussed as follows :-

Ultraviolet light was found to have the highest effect in destruction of the bacteria cells, as shown in above mentioned figures, The slope values of UV light for *P.aeruginosa*, *E.coli*, *S.aureus*, and *B.subtilis* were (6.9×10^9 , 4.9×10^9 , 1.9×10^9 , and 3.9×10^9) bacteria cell per exposure time, respectively, Such results were obtained due to the mode of action of UV radiation. Its effect is concentrated on damaging bacterial DNA, indicating that such a physical method has the most efficient killing ability (on the bacteria cells) among other methods used. Efficiency to destruct pathogenic bacteria depends on the intensity of the UV light, exposure time, and the mechanism of UV action on the bacterial cell (1,2,3).

Effect of CO₂ laser on (*P.aeruginosa*, *E. coli*, *S. aureus*, *B.subtilis*) comes next to that of UV light (table 1). CO₂ slope values were (1.3×10^8 , 9.7×10^7 , 1.6×10^8 , 4.7×10^8) bacterial cells per exposure time, respectively. CO₂ laser is easily absorbed by the water content of the bacterial cell due to its wavelength which falls in the middle of infrared region. This range is heavily absorbed by water. Bacteria protoplasm (which is encased by the cell membrane) is dense, gelatinous and prominent site for many of the cell's biochemical and synthetic activities. Major component of the protoplasm is water which exceeds all other component in quantity (70-80%). It serves as a solvent for the cell pool. Most of the incident fluences of CO₂ laser have a very short penetration depth. When the cellular water is heated to its boiling point (100°C), the heat causes complete destruction of the all cellular protein (by inactivation (coagulation) of protein and DNA) as well as the cell itself. The increase in the intracellular temperature and pressure cause explosion of the cell, throwing off steam and cellular debris. The steam of the debris rises from the site of impact and is carbonized in the laser beam. So, it is obvious that the CO₂ effect was photothermal^(4,5). For this reason the slope values were high when it is compared with other treatment.

Nitrogen laser had the third destructing effect on the bacteria. Its slope values were (3×10^7 , 2.8×10^7 , 1.9×10^7 , 2.5×10^7) bacterial cells per exposure time to the four tested bacteria respectively. Due to its wavelength (337nm), the nitrogen laser has an emission in the ultraviolet region, which can breakdown (if absorbed) the molecular bounds directly, leading to produce small molecules. The nitrogen laser has an action of photochemical effect due to its wavelength. This is happened when a change can occur as a result of direct excitation of electronic bounds. The cellular component, protein and lipids, absorb photons and become electronically excited. This photoexcitation leads to rupture of molecular bounds

and formation of molecular fragments⁽¹²⁾. The depletion of such excited states may be the cause of diminished yield of biomolecular pyrimidine photoproducts. Near UV irradiation of DNA (typically at 313 or 334 nm) a large number of pyrimidine dimers is induced⁽⁶⁾. Such compounds are known to have lethal effect through mutation^(2,3).

So, the difference between CO₂ and nitrogen laser was in their wavelengths, this will result in the different mechanisms of action and effect on the treated bacterial cells. From the above results, the CO₂ laser effect needs shorter exposure time to cause obvious effect during treating the bacterial cells, (photochemical effects needs more exposure time to form the pyrimidine dimers).

Previous figures show that the chemical treatment through exposing (*P. aeruginosa*, *E.coli*, *S.aureus*, and *B.subtilis*) to nitrosoguanidine has the lowest effect on their colonies when the calculated slope values were only (1.7×10^6 , $1 \times 10^+$, 1.18×10^7 , and 1.7×10^7) per exposure time, respectively (table 1). The effect of MNNG on the bacterial growth is due to the hydrolysis of MNNG to diazomethane, and when MNNG adds methyl groups to the guanine, mispair with the thymine will be created. Due to this action, nitrosoguanidine may cause specific mispairing. When MNNG changes a base structure, its basepairing characteristics will be altered, leading to produce a specific kind of DNA damage^(1,2,3).

Table (1) Slope values for the numbers of colonies as a function of the exposure time for the test bacteria after treatment with chemical, physical and laser methods

Bacteria	Treatment method	Slope value [bacterial cell/ exposure time(min)]
<i>Pseudomans aeruginosa</i>	UV light	6.9×10^9
	CO ₂ laser	1.31×10^8
	N ₂ laser	3×10^7
	NTG	1.7×10^6
<i>Escherichia coli</i>	UV light	4.9×10^9
	CO ₂ laser	9.71×10^7
	N ₂ laser	2.8×10^7
	NTG	1×10^6
<i>Staphylococcus aureus</i>	UV light	1.9×10^9
	CO ₂ laser	1.6×10^8
	N ₂ laser	1.9×10^7
	NTG	1.18×10^7
<i>Bacillus subtilis</i>	UV light	3.9×10^9
	CO ₂ laser	4.7×10^7
	N ₂ laser	2.5×10^7
	NTG	1.6×10^7

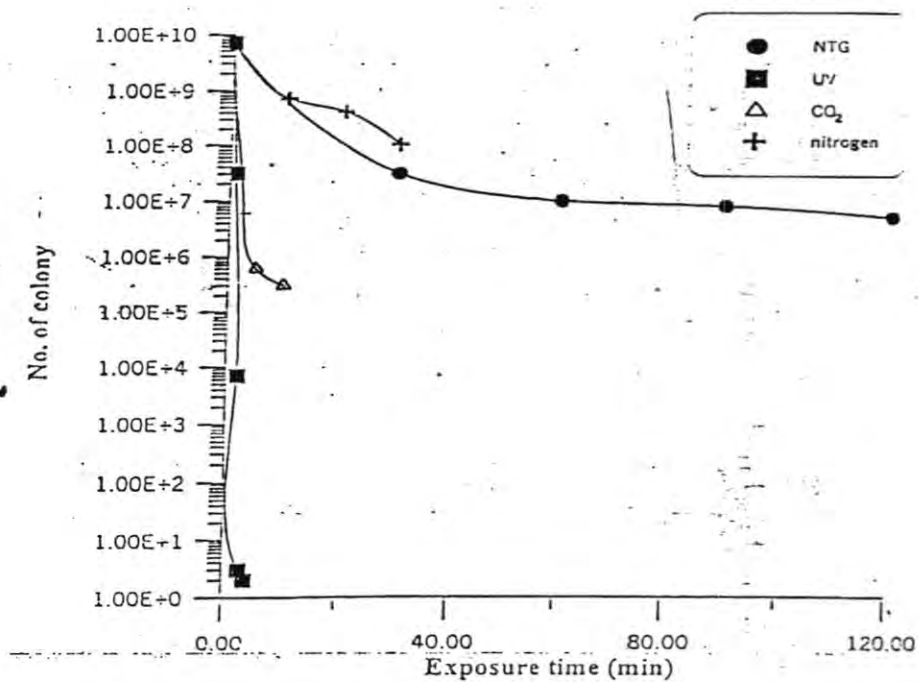


Figure (1) : Survival curve of *Pseudomans* after treatment with chemical, physical, and two types of laser methods

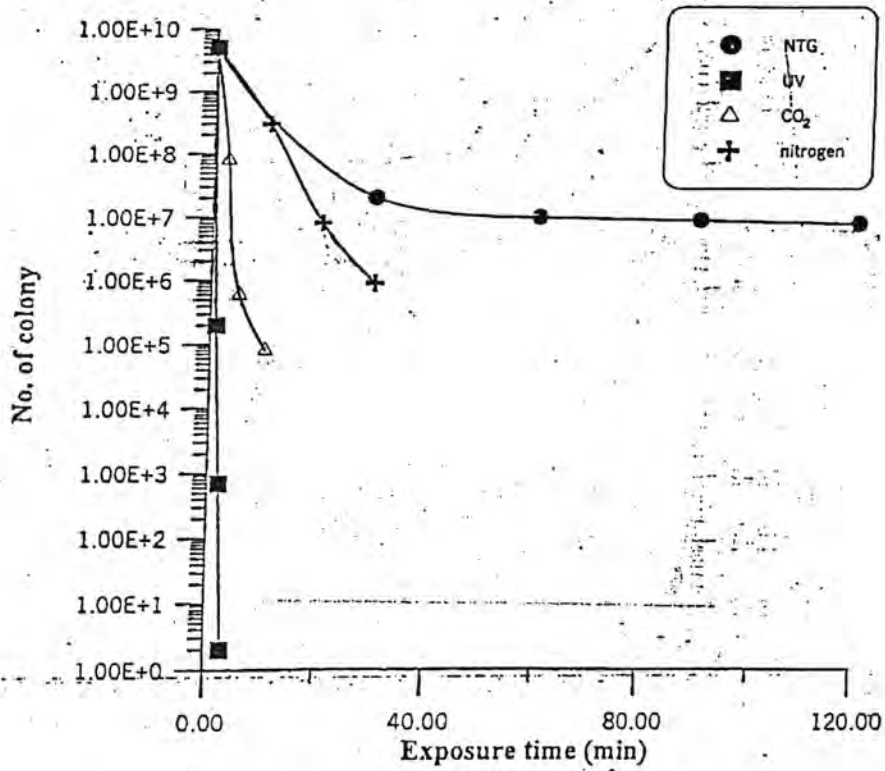


Figure (2) : Survival curve of *Escherichia coli* after treatment with chemical, physical, and two types of laser methods

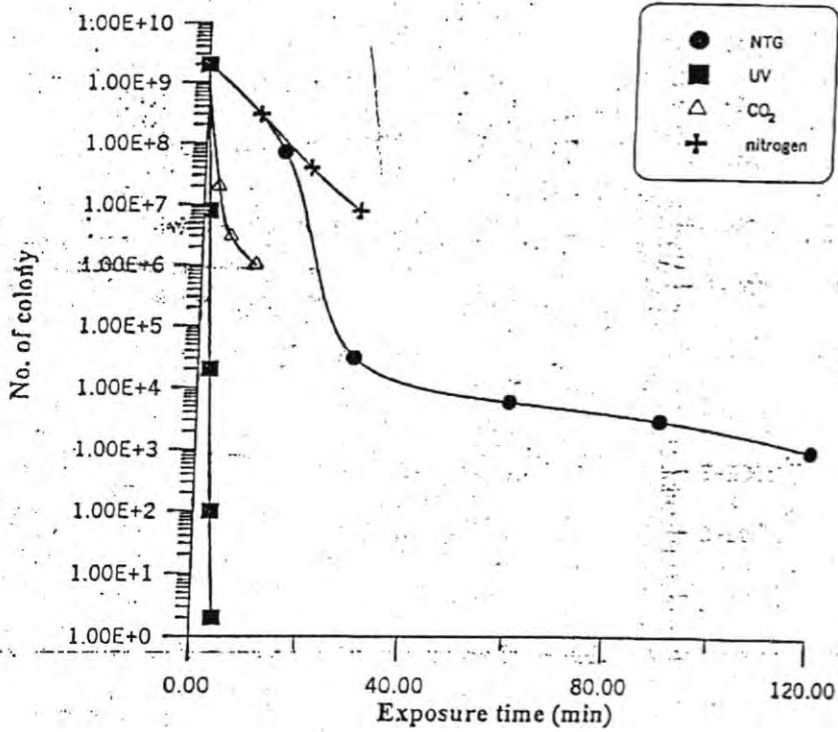


Figure (3) : Survival curve of *Staphylococcus aureus* after treatment with chemical, Physical, and two types of laser methods

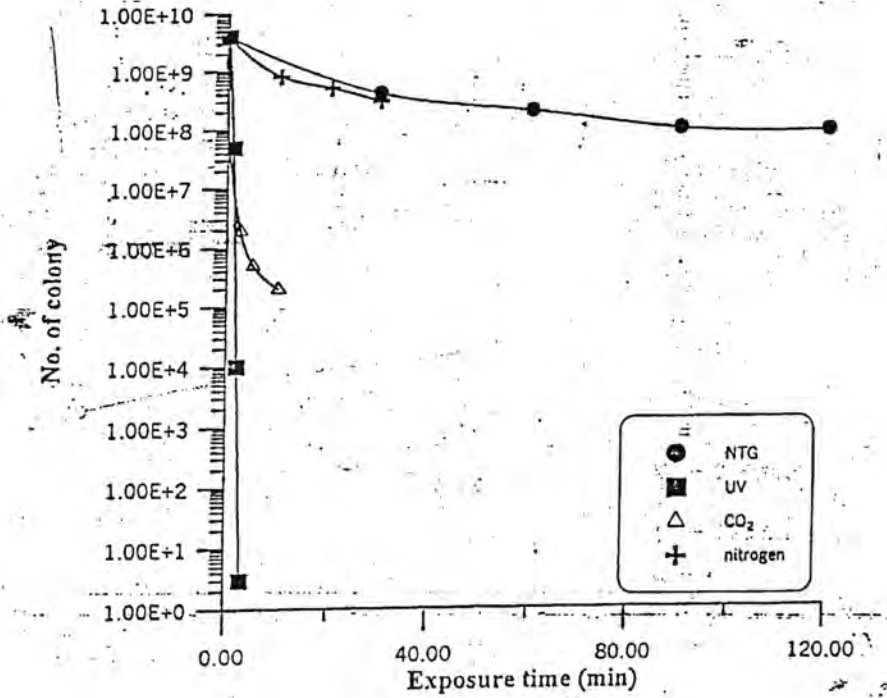


Figure (4): Survival curve of *Bacillus subtilis* after treatment with chemical, physical, and two types of laser methods

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The Influence of Heterophile Antibodies & ABH – Like Substances Stimulation on The Regulation of Antibody Response

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الخلاصة

يعرف ان لعاب الانسان يثبط تلازن الاضداد البشرية، بينت النتائج ان بعض اشكال الفعالية الذاتية قد تؤثر او تعمل عند المستوى الحيوي وكذلك فان مجاميع ABH الذاتية والمفرزة لها قابلية تكوين اجسام ضدية ضعيفة تجاه مجموعة AB شبيهه RBC في الاقسام بالمقارنة مع تلك غير المفرزة، تم دراسة ومناقشة انعكاس هذه الميكانيكية بين الاقوام البشرية المختلفة في العراق.

ABSTRACT

It is establish that human saliva can inhibit the agglutination of human antibodies. Results showed that some from of autologus activity may also operate at biological level and that the secretors of antologous ABH were found to make weaker antibodies to human group AB & sheep group AB – like RBC than non secretors. The implication of these finding in relation to different races of Iraq is discussed.

INTRODUCTION

The Antigens A, B and H of the Blood group system are not confide to the red cells, but are found in a soluble form in the body fluide. Not all individual, however secrete their coresponding ABH substances ; a proportion are not secretor, that is, their fluid are free from or contain only trace amounts of these substances⁽¹⁾.

It is considered that a secretion of the ABH group – specific substances is controlled by a pair of allelo – morphic genes Se and se.

The production of anti-A and anti-B antibodies by exogenous stimulation implies that such antibodies are hetrophile in origen, i.e., it can react with antibodies determinants on a variety of microbial cell membrane to which the host never been immunized⁽¹⁾. Furthermore, studies have been indicated that sheep red blood cells possess a significant amount of blood group A & B may found in areas unrelated to humans^(2,3).

The present study were undertaken to establish and evaluate the significance of ABH-like substances with respect to cross-reacting anti-A and anti-B antibodies in both human serum and saliva.

MATERIALS AND METHODS

Blood and saliva were collected from healthy blood donors.

An agglutination test were performed to demonstrate the antibody titer using two fold dilution following the procedure of Vos et.al.⁽⁴⁾. The reactions of human sera to micro-organism were evaluated following the procedure of Springer et.al.⁽⁵⁾ using gram negative and gram positive bacteria such as *E.coli* and *Streptococcus pyogens*. Bacteria were grown for 48 hours at 37°C and seperated from the culture by centrirugation after which the cell were suspended in distilled water and heated in boiling water for one hour.

The presence of ABH-like factor on these cells was assayed by the standard haemagglutination inhibition test following the procedure of Al-Agidi & Shukri,⁽⁶⁾. The Autologous ABH-like activity was measured by the ability to inhibit the reaction of human for ABH-like positive sheep RBC by saliva.

The non-autologous ABH-like substance was measured by the conventional inhibition test using human antibodies reactive for human group A, B & O – RBC. Serum and saliva IgA were assayed following Al-Agidi and Roberts⁽⁷⁾.

RESULTS

The dsitribution pattern of autologous ABH-like activity and non-autolgous ABH is shown in Table 1. The results revealed that autologus ABH-like substances is related to the presence of non-autologous ABH-like inhibitory activity occur oftenly in the saliva subjects who secrete A or B substances than in those who only secrete H substances.

Table 1: Incidence of autologous cross reaction ABH-like structure in the saliva of healthy donors

Donors	No. Tested	Cross Reacting ABH	
		Positive%	Negative%
A secretors	53	67.9	32.1
A&B secretors	24	79.1	20.9
B secretors	36	58.3	47.3
H secretors	90	25.5	74.5
Total	203	M = 57.4	M = 43.6
ABH secretors	52	1.9	98.1
Grand Incidince	255	39.2	60.8

A strong correlation exist between the development of low antibody values in the serum and the presence of autologous ABH-like substances in the saliva

(Table 2). The finding of total absence of cross reacting A & B antibodies in salive of individuals who have autologous ABH-like substances in their secretion suggest a direct link between the presence of ABH-like structure in saliva and the development of cross-reacting antibodies for A and B.

Table 2 : The effect of autologus saliva ABH substances on the development of Antibodies reactive for human A, B cell & ABHS Susbstances positive sheep RBC

Group	Secretary Status	No. Tested	Serum Antibody Titer			
			Human A,B cell		Sheep ABH	
			Total	M±SD	Total	M±SD
Saliva With Autologous ABH-	1.A-	36	484	18±5.5	255	11.2±2.6
	2.AB-	19	12	6±2.1	73	8.1±2.6
	3.B-	21	228	15±3.6	122	10.2±3.1
	4.H-	210	210	18±8.0	144	12.2±4.2
	5.ABH-	99	934	15±3.6	552	14.2±2.3
Sliva Only	1.A-	17	388	29±6.1	285	28.1±7.6
	2.AB-	5	43	9.2±2.3	61	10.1±4.3
	3.B-	15	444	41.6±5.6	369	30.2±6.9
	4.H-	67	1278	40.8±8.1	1255	42.3±7.2
	5. ABH-	155	3489	38.1±7.0	3214	35.7±9.3

Saliva Antibody Titer			
Human A,B cell		Sheep ABH	
Total	M±SD	Total	M±SD
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
41	4.1±0.9	3	3.2±0.9
10	1.8±0.02	7	9±0.001
48	4.6±9	44	4.7±0.9
281	8.9±2.1	258	7.9±1.8
667	7.0±1.6	573	6.2±1.9

The biological importance of ABH-like system is their association with the synthesis regulation of immunoglobulin (Table 3). This finding indicates that total serum and saliva levels of IgA may be lower in secretors of ABH than in non-secretors.

Table 3: IgA levels in serum and saliva of secretors & non secretors of ABH – like substances

Sample	No. Tested	IgA / Secretory Activity / M±S.D	
		Positive	Negative
Serum	25	322±55.1	255±71.3
Saliva	25	0.82±0.03	0.82±0.03

The 4 list the observed percentage of strong and weak reacting ABH-like antibodies in group of Arab and Kurds residing in the same geographical area, and showed a significant relationship between strong and weak reacting anti-ABH between these two population groups.

Table 4: Pattern of Distribution of Cross – Reacting Antibodies to ABH-like Substances on Sheep RBC in various race groups

Group	No. Tested	Secretary Activity		P value
		Strong %*	Weak %**	
Arabs	112	58.8	41.2	X ² =102.5
- Kurds	84	79.7	20.2	

* Strong: Titer of 1:20 or more.

** Weak : Titer of 1:10 or more

DISCUSSION

The expression of ABH blood groups in man is controlled by the action of genes which is independent of that regulate their expression in serum⁽⁸⁾. The distribution of ABH were documented in Iraqi population⁽⁶⁾. In the present study emphases was directed toward establishment whether the recognition of these autologous inhibitory factors in saliva could be assessed by deferent forms of cross-reacting ABH-like antigen-antibody system.

The demonstration of autologous activity with non-autologous suggest that there may be deference in sugar content of some glycoprotein^(9,10). Although the chemical nature of the ABH-has not been yet assassed. There is, of course, the possibility that “non-self” structure were produced under the influence of acquired viral or bacterial genetic material incorporated in the glycoprotein producing system.

It is important to establish the influence of autologous ABH on the development of cross reacting anti-A and anti-B in serum and saliva . Results showed a strong association between the development of low antibody value in serum and the presence of autologous ABH in saliva, while the finding of total absence of cross reacting A & B antibody in saliva of subjects who have

autologous ABH in their secretion, may indicate a direct link between the presence of autologous ABH in saliva and the development of cross-reacting A&B antibodies.

The relationship between strong and weak reacting anti-ABH was found to be significantly deferent between Arabs and Kurds which indicate an indirect or direct exposure to ABH-positive agent like microbial factor which may vary between race group or that some form of selective advantage enable kurds to be better responders than Arabs, i.e., Arab are not as often exposed to ABH positive agent and, therefore, show a reduced levels of antibody response, while genetic variations must play a role as been suggested^(6,7).

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Natural Promastigotes Infections of Sandflies (*Phlebotomus Paptasi Scolpi*)

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الخلاصة

لقد تم اجراء دراسة ميدانية خلال عامي ١٩٩٨ و ١٩٩٩ لتحديد نوع الحشرة الناقلة لطفيليات اللشمانيا في بورتين مرضيتين في المنطقة الوسطى من العراق. وقد تم تشريح (٤٩٤٧) حشرة انثى من ذباب الارمل (الحرمس)، وقد وجد ان (٥١) واحدة منها كانت مصابة بالطفيليات. وقد شخّصت هذه الطفيليات على انها طفيليات اللشمانيا. والعمل جاري الان على ادامة عزلات من الحشرات المصابة مباشرة تمكنا من الوصول الى النوع في دراسة لاحقة قريبا.

ABSTRACT

A field study in central region of Iraq performed during 1998 and 1999 to determine the possible vectors of *Leishmania sp.* In two traditional foci of Leishmaniasis in central Iraq. About 4974 females of sandflies dissected, only 51 ones were infected. The parasites identified as *Leishmania sp.* We are trying to isolate the parasite as soon as possible.

INTRODUCTION

Many researchers interested during last century on the studies of Iraqi sand flies and their possible relations with both cutaneous and visceral Leishmaniasis in Iraq^(1,2,3,4,5,6,7,17). Baghdad considered as endemic area with both cutaneous and visceral leishmaniasis^(1,7,12,13,14,15,18,19).

The disease is probably transmitted among people and animals by anthropophilic species of the genus of *Phlebotomus* and *Sergentomyia*, *Phlebotaminae*, *Psychodidae*, *Diptera*.

The suspicion is directed towards the anthropophilic species of *Phlebotomus papatasi*, *P. alexandri*, *P. sergenti*, *p. clydi*, *Sergentomyia baghdadis*, *S. sintoni*, *S. squamipleuris* and *S. palestinensis*. In the central region

of Iraq the *P. papatasi* consisted about 95% of the total number of sand flies species^(1,2,3,6,14,15,18,20,21).

Visceral leishmaniasis is common in rural area around Baghdad and about 92% of the patients are in the first three years of age^(12,13,14,19,22,23) the traditional foci of this pest disease are Diala bridge, Medain, Sowira, Aziziya, Kan Beni Saad, Taji, Falowja, Abougrab, Mahmodiya, Latifiya, Nommaniya, Missan, Thiqrar etc^(1,2,5,6,12,13,14,15,16,20,23).

The vectors of visceral Leishmaniasis in Iraq are deferent from these of the Mediterranean area^(6,7,8,9,10,11). This is due probably to the wide variation in Iraqi fauna caused by the geographical situation (Mecan 1965 in 8).

The central region of Iraq is a very wide alluvial plain; its elevation is from 36m. to 300m.a.s.l. The climate is of a desert and semi-desert. Last few years the dusty days increased due to dry season. The region is covered by the tress of *dactyli fera*, *citrus siensis*, *morus aibus*, *pyrus cpmminus*, etc. The main occupationso fvvillagers are agriculture, poultry farms, and cattles.

MATERIALS AND METHODS

Two stations selected in this endemic area according to the following:

1. The high number of reported cases of Visceral Leishmaniasis on the few three last years.
2. The high density of sand flies
3. The possible canine Leishmaniasis, and high number of deferent species of rodents.
4. To use the data of previous studies which had been conducted in the area as a base line data.

First station is Abougrab, about 20km west of Baghdad. Second one is Mahmodiya about 50km south of Baghdad.

Field studies started from the end of April till the beginning of November 1998 and 1999.

Sticky papers with castor oil and CDC light traps of malaria used to collect sand flies biweekly. The flies collected from different places, like human dwellings, cattle shelters, near rodent's barrows, Dog pits, vegetables farms, and open-land. These techniques offered very important information about the density, behavior, distribution, biting time, and resting-places of the sand flies.

The identification of males was based on the genitalia. The females identification by the spermatheca, cibirum and pharynx^(1,6,7,9). The collected SF brought alive to laboratory and dissected next days. The gut was exposed to search of flagellate. When parasite presented inoculation take place take place to

NNN-media and bulb c. The females kept alive with 0.5ml gentamicin 500 μ /ml, and 0.6% NaCl. Smears of midgut of feeding sand flies were done on sterile slides washing in 95% alcohol, then fixed with 95% alcohol before staining with Giemza, this simple technique offered the isolation of *Leishmania sp.* From the infected females, but its unfortunately unsatisfactory to determine the species of parasite.

RESULTS AND DISCUSSION

About 4947 females of feeding sand flies were dissected during 1998 and 1999. Only 51 ones were infected with parasites. The dominated species was *Phlebotomus papatasi* Scolpi which formed about 965 in total number^(1,7,14,20). The species *P. Alexendir*, *Sergentomiya baghdadis*, *S. palesteinsis*, *S. sequamipluris* and *S. sentoni* presented in very small number^{(2,3)*}.

Parasites found in 51 females of *P. papatasi*. Unfortunately the culture was contaminated and the bulb-c were dead. Parasite was identified as *Leishmania sp.* With five slides of blood meal (mid gut) of the infected females'. Its impossible to announce that these parasites are *L. donovani* or other species of *Leishmania sp.* Before improved that by isoenzyme electrophoresis. In wide term, the isolation of parasites from the vector in large numbers, and in traditional focus of visceral leishmaniasis (about 25 cases since 1967) in central Iraq is important step in this way. Also we have reported two new cases of visceral Leishmaniasis in Feb. and March 2000 in two neighbors dwellings in the endemic focus. This is so important and encouraged singe that we are near the finding of the vector of visceral leishmaniasis in central Iraq. Sukker^(18,20) isolated *Leishmania sp.* From 4 infected *P. papatasi* in Al-Nommaniya district Wasset province, in addition of Mohsen (in 22), he also found one infected females of *P. papatasi*.

Killic-kendrlie (in 20) thought that *P. papatasi* might be the vector of visceral and cutaneous leishmaniasis in Iraq.

The habits of feeding (anthropophilic species) and wide distribution of *P. papatasi* in compression with the other species of sand flies in Iraq causing this species as a suspected vector of infantile leishmaniasis. Many other investigators^(1,6,7,13,15,17,19,22) think that *Sergentomiya baghdadis* is also suspected as a possible vector of both visceral and cutaneous Leishmaniasis in Iraq. We dissected 42 females of this last species with out any kind of flagellates, few others ones to five different species were negative also.

Thus it seem that this study is important in the way, and may be issued to find the vector of visceral leishmaniasis in the central region of Iraq.

*The entomological investigation presented in separated papaer

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Depressed Phagocytic Capacity of The Neutrophil Leucocytes In Patients With Atopic Dermatitis in Iraq

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الخلاصة

تم اجراء دراسة مقارنة للقدرة الالتهامية للخلايا البيضاء في مرضى الالتهاب الجلدي المتماحل الشديد والبسيط Sever and mild atopic dermatitis وقد تم استخدام طريقة خلايا الخميرة في هذه الدراسة. لوحظ ان الخلايا البيضاء العذلة في مرضى الالتهاب الجلدي المتماحل الشديد أظهرت نقص في وظيفتها البلعمية، بينما كانت عملية البلعمة في مرضى الالتهاب الجلدي المتماحل البسيط طبيعية عند مقارنتها بمجموعة السيطرة. وتم التوصل الى أن النقص في عملية البلعمة في مرضى الالتهاب الجلدي المتماحل الشديد قد يكون مرتبط بالإصابات المتكررة الموجودة في هؤلاء المرضى.

ABSTRACT

Comparative study has been made of the phagocytic capacity of leucocytes in patients with mild and severe atopic dermatitis (AD). For this screening study, the yeast particle method has been used. Neutrophil leucocytes in patients with severe atopic dermatitis show impairment in their phagocytic function, whereas phagocytosis in those with mild atopic dermatitis did not differ from that in healthy controls. The decreased phagocytosis might be associated with frequent infections in severe atopic dermatitis.

Keywords : atopic dermatitis, phagocytosis, PMN

INTRODUCTION

Defects in the phagocytic function of the neutrophil leucocytes have been demonstrated in several disorders^(1,2,3). Dermatological disorders found to be associated with defective phagocytosis are discoid lupus erythematosus, palmo-

plantar pustulosis are recurrent erysipelas⁽⁴⁾. Impaired phagocytosis has also been described in patients with alopecia areata and with accelerated hair loss⁽⁵⁾.

The main clinical symptoms hitherto known to be connected with impaired phagocytosis are recurring or chronic infections. Since patients with severe atopic dermatitis (AD) often suffer from frequent staphylococcal and streptococcal skin infections, a study of the phagocytic capacity of the neutrophils in patients with severe AD compared with that of those patients having mild to moderate dermatitis seemed to be of interest.

The ability of neutrophils to ingest yeast particles is the criterion commonly applied in studies of their phagocytic capacity and this paper presents the results of a screening where this method has been used for evaluation of the phagocytic capacity in AD.

Materials and Methods

Patients

This study involved (101) patients (59 females and 24 males; mean age 16.34 years, range 3-65).

The diagnosis of AD patients was confirmed by clinical basis. None of the patients received corticosteroids, antihistamines, or other systemic therapy at the time of study, or one month earlier.

The patients were divided into two groups according to the severity of their dermatitis.

- Mild group (< 10% surface area involvement which included 36 patients (23 females and 13 males). Their ages ranged between 3-65 years.
- Severe group (> 10% surface area involvement) which included 65 patients (36 females and 20 males). Their ages ranged between 3-65 years.

Control

The control group consisted of (50) healthy volunteers, (26) females and (24) males. Their ages ranged between (4-58) years, with no symptoms and history of atopic diseases.

The method for study of phagocytic activity is a modification of that described by⁽⁶⁾.

Preparation of polymorphonuclear cells suspension

Heparinized blood in test tube was incubated at (37°C) for (1 hr). After erythrocytes sedimentation, the leucocyte-rich plasma was removed, then washed twice with RPMI-1640 and resuspended in the same medium to give a final concentration of (1×10^6 cells / ml).

Heat killed *Candida albicans* Suspension

The suspension was prepared as described by^(7,8).

The yeast culture is (30ml) of subouroude dextrose broth (SDB) in (50 ml) of flask with screw cap, incubated at (37^oc) for (24 hr), then centrifuged at (300 rpm) for (15 min).

The pellet was suspended with Hank's balanced salt solution (HBSS); then placed in boiling water bath for (30 min). The yeast was counted by haemocytometer, diluted to (5× 10⁶ cells/ml).

The assay was done as follows

- (0.25) ml of PMNLs suspension was mixed with 0.25 ml of yeast suspension, 0.25ml of HBSS and 0.25 ml of normal human (AB) serum in sterilizer test tube.
- The mixture was incubated at (37^oc) for (30 min) and the pellet was gently resuspended, so that one drop was placed on a slide and smeared, then left to dry, fixed by methyl alcohol for (10 min) and stained for (20 min) with geimsa stain. Hence, examined under oil immersion to find that at least 200 cells of PMNLs were counted, then the percentage of phagocytosis were found as follows:

$$\text{Phagocytosis Factor} = \frac{\text{No.of phagocytic PMNLs}}{\text{Total no.}} \times 100$$

Statistical Analysis

Statistical analysis involves calculation of mean values (M), standard deviation (SD) and student t-test to find the significance of probability level (P) for the different groups.

Level of significance were established at (P < 0.05) and (P < 0.01).

RESULTS

Blood samples were collected from (101) AD patients and (50) healthy control subjects. Patients were divided into two groups according to the severity of their dermatitis. Furthermore a group "TG" was included which represent the total number of patients (See table 1).

Table 1: Number and age of subjects per each group

Group	Diagnosis	No.	Age in Years	
			Mean	Range
TG	Total AD pat.	101	16.34	3-65
SG	Severe AD	65	14.12	3-65
MG	Mild AD	36	19.25	3-65
CG	Control	50	15.68	4-58

The mean percentage of the phagocytosis was found in the various groups as shown in (tables -2).

The mean percentage of the phagocytosis for the total group of the patients (TG) with AD was lower than that found in the controls ($p < 0.01$). When the patients are divided into two groups according to the severity of the dermatitis it is obvious that the mean percentage of the phagocytosis in patients with mild AD (MG) does not differ significantly from that of the healthy volunteers ($p < 0.05$), but the patients with severe atopic dermatitis (SG) have lower percentage of the phagocytosis when a comparison with controls ($p < 0.01$) was made.

Table 2: The percentage of phagocytosis in AD patients and controls [M \pm SD]

Age Groups	No.	CG M \pm SD	No.	TG M \pm SD	No.	SG M \pm SD	No.	MG M \pm SD
<10	18	84.4 \pm 4.41	56	75.5 \pm 7.67**	40	71.8 \pm 5.4**	16	84.0 \pm 3.93
10-19	13	83.4 \pm 4.27	23	74.5 \pm 10.5**	13	70.3 \pm 11.3**	10	80.9 \pm 5.45*
20-29	6	83.1 \pm 4.03	6	74.8 \pm 5.70**	3	69.3 \pm 1.25**	3	80.3 \pm 1.70
30-39	7	82.4 \pm 3.06	6	70.8 \pm 5.24**	4	67.2 \pm 1.48**	2	78.0 \pm 1.00
40-49	3	80.3 \pm 4.50	6	72.3 \pm 5.85**	3	66.6 \pm 1.25**	3	78.0 \pm 1.63
>50	3	78.3 \pm 0.94	4	69.5 \pm 7.79*	2	62.0 \pm 4.00**	2	76.5 \pm 0.5
Total No.	50		101		65		36	

** Significant differences ($p < 0.01$)

* Significant differences ($p < 0.05$).

DISCUSSION

The increased interest in the phagocytic function of neutrophil leucocytes seemed to have been stimulated by the description in 1957 of the clinical entity termed chronic granulomatous disease (CGD) of childhood⁽⁹⁾.

This inborn disorder characterized by recurrent, suppurative, bacterial infections with organisms of low-grade pathogenicity. The patients frequently develop eczema, lymphadenopathy, and hepatosplenomegaly.

Although the neutrophils ingest the bacteria normally, the neutrophils from these patients have, in vitro, an impaired ability to kill various pathogenic as well as non-pathogenic bacteria⁽¹⁰⁾. This defect seemed to be associated with impaired H₂O₂ production by the neutrophils. In another syndrome characterized by severe exczematoid dermatitis and repeated local and systemic infections which was described by Miller⁽¹¹⁾.

In the present study the decrease in phagocytosis of yeast by neutrophils may be associated with frequent infections in severe AD⁽¹²⁾.

The impairment in phagocytosis and chemotactic defect of polymorphonuclear and monocytes associated with presence of chemotactic inhibitors, may lead to increased susceptibility to staphylococcal infections⁽¹³⁾. Ward and Schlegel investigated a child with frequent, severe and prolonged respiratory and cutaneous infections. As concerned for that child, the neutrophil leucotaxis was found to be decreased and attributable, at least in part, to the presence of an inhibitor of the leucotactic function of neutrophils in the patient's serum⁽¹⁴⁾.

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Study The Specialization of The Fungus *Verticillium Albo-Atrum*

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الخلاصة

لغرض دراسة تخصص الفطر *Verticillium albo-atrum* الذي أكدت التقارير والبحوث بأنه مسبب لمرض الذبول للكثير من النباتات، أخذت عزلتين من النوع *Verticillium albo-atrum* - العزلة V1 و V2 وأخذت عزلتين من النوع *Verticillium dahlaie* العزلة D1 و D2 وكذلك الانواع *Verticillium nigrecens* و *Verticillium mublium* والنوع *Verticillium tricoprus* ولقح بهذه الانواع والعزلات ضربين من نبات الجت، أحدهما مقاوم للفطر *Verticillium albo-atrum* والاخر حساس، وضرب واحد من الطمامه. وقد دلت النتائج على ان الانواع او الضروب التي عزلت من الجت تصيب الجت مرة اخرى والانواع التي عزلت من الطمامة تصيب الطمامة مرة اخرى فقط وهذا يؤكد وجود خاصية التخصص في الفطر *Verticillium albo-atrum*.

ABSTRACT

For investigation the specialization of the fungus *Verticillium* species and isolates which were reported to be causing vascular wilt to plants. *V. albo-atrum* two isolates V1 & V2, *V. dahlaie*, two isolates D1 & D2, *V. mublium* *V. nigrecens* and *V. tricoprus* were used to inoculate two cultivars of Alfalfa plants and one cultivars of Tomato. The results showed that V1 isolates of *V. albo atrum* which was isolated from alfalfa can infect alfalfa and cause wilt disease and the isolate V2 of the species *V. albo-atrum* and the two isolates of *Verticillium dahlaie* D1 and D2 can infect tomato only this result has proved the specialization of the fungus *Verticillium* species.

INTRODUCTION

The genus *Verticillium* belongs to the Class, Fungi Imperfecti (Duetromycotina), Order Moniliales (Hyphomycetes), (Barnett, 1958). This genus was first recognised by (Nees Von Esenbeck in 1816), based on the morphology of the conidiophores. Species of the *Verticillium* genus, form a very wide range with extremely diverse life styles, their only morphological link resting somewhat tenuously upon the production of more or less *Verticillately* – branched

conidiophore. The genus *Verticillium* comprises 40 species to date (Mace et al 1981). Only five of them are considered to be pathogenic to plants, causing vascular wilt disease. They are, *V.albo-atrum*, *V. dahliae*, *V. nubillum*, *V. nigrescens* and *V. tricorpus*. The first two species are considered to be more economical importance than the others. (Hastie & Heale, 1984) *V.albo-atrum* was first described by (Reinke & Berthold, 1879) as the causal pathogen of vascular wilt of potatoes in Germany. This fungus formed resting mycelia which were thick and dark. They called this fungus *V.albo-atrum*.

Specialization is the ability of one isolate of the pathogen to infect only one host of plants. (Isaac, 1957) reported that the isolates of *Verticillium albo-atrum*, and *V. dahliae*, exhibit specialised pathogenicity. They tested a large number of isolates of *V. albo - atrum* and *V.dahliae* against lucerne only those isolated from the lucerne are capable of infect lucerne and cause wilt disease, this result has been confirmed by many workers such as, (Cornell et al., 1988). The wilt disease caused by *Verticillium* species has been a destructive disease and a major limiting factor in many plants particularly lucerne, (Page et al., 1994). Therefore it became urgent and very important to establish an effective method to control this disease. The lucerne *Verticillium* wilt disease is the interaction between the host, lucerne plants (very important forage cultivar), and the pathogen, *Verticillium* species. To establish an effective method to control this disease it is very worthwhile to study the relationship between the lucerne and the fungus. The most important step in studying any plant disease is to establish the pathogenicity of the organism towards the plant, therefore, this has been the starting point of this study. Following isolation and identification of *Verticillium albo-atrum* from infected lucerne and tomato plants. The study has been involved with investigations concerning development of the wilt disease and the reaction of two cultivars of lucerne (resistant and susceptible) inoculated with *V. albo - atrum*, isolates V1 (lucerne - isolate) and V2 (tomato - isolate). Pathogenicity was investigated. After all of that the Specialisation of *Verticillium* species and isolates has also been examined to determine whether the phenomenon exists in the interaction involving lucerne.

MATERIALS AND METHODS

Isolation of the fungus

Lengths (2 cm) of stem from infected plants of lucerne or tomato were surface sterilized separately by washing with 0.5% W/V mercuric chloride for 1 minute. Segments were washed with sterile distilled water, (3 washes) and sections were sliced, using a sterile blade, directly onto plates of Dox's agar medium or moistened filter paper held in sterile petri-dishes. Plates were incubated at 23°C at the dark in incubator for 3-4 days following which pure

cultures of the fungus were obtained from the resulting growth of *Verticillium albo-drum* by one of two methods. "Streaking" or "Serial dilution".

Identification of *Verticillium*

For identification of the fungus the following procedure was used. Slides of the pure cultures were prepared and examined under a binocular light microscope to observe the growth of the fungus. The morphology of the conidia and conidiophore was used to identify *Verticillium* species, according to, (Barnet, 1958); (Isaac, 1949); (Heale, 1962).

Growth of fungi

Sources of the fungi that were used in this study are shown in table (1). The isolates were maintained on Potato dextrose agar (PDA) at 23°C in the dark. The cultures were renewed by subculturing monthly.

Growth of plants

Two cultivars of lucerne namely, Euver, Vela, and one cultivar of tomato, Ailsa Craig, were used in experiments.

Cultivar Euver were reported to be susceptible to *Verticillium* wilt, Cultivar Vela was reported to be resistant to wilt disease that caused by *Verticillium* species, (Plant Breeding Institute, Cambridge). The tomato cultivar, Ailsa Craig was known to be susceptible to wilt disease which cause by *Verticillium* species, (Flood, 1980). The Seed of Cultivar Euver, and cultivar Vela were obtained from the United Kingdom Seed Executive, (White House Lane, Huntingdon Rd, Cambridge, CB3 0L.). The seeds of the tomato cultivar were obtained from Sutton's seed Plymouth UK.

Seeds of lucerne were sown in trays containing unsterilised "All purpose compost", (Arthur Bowers) and maintained in the greenhouse. When the seedlings were 2 weeks old they were transplanted into plastics pots (9 cm diameter) containing the same compost and were maintained in the greenhouse at a temperature of 23°C with a photo-period of 16 hr and a light intensity of 9000 Lux, until required.

Preparation of Inoculum

Spore suspensions of fungi were prepared from 12 day old cultures, grown on Potato dextrose agar plates (9.5 cm, diameter, 1.5 cm, depth) at 23°C in the dark. Cultures were flooded with sterile distilled water (10ml per plate) and the surface of the culture was scraped gently with a sterile glass rod to free the spores. The spore suspensions were collected and filtered through a double layer of

muslin cloth. The concentration of spores was measured using a haemocytometer and sterile distilled water added to adjust the required concentration.

Method of Inoculation

A root-dip inoculation method was used to inoculate the plants in all experiments. 5-6 week old plants were carefully uprooted from the pots and the roots were washed with distilled water to remove the soil. The roots were then dipped in a beaker containing a spore suspension (100 ml of 10^7 spore ml^{-1}) of the fungus, for 10 minutes. Control plants were dipped into sterile distilled water for the same period of time. Inoculated plants were replanted either directly in to a field plot in the Botanical garden, Swansea University, or into plastic pots (15 cm diameter) containing unsterilized "all purpose compost" (Arthur Bowers) and maintained in the greenhouse at 23°C with a photo-period of 16hr and light intensity of 9000 Lux.

Pathogenicity and Specialization of *Verticillium* isolates & species

Lucerne Cultivar

6 week-old seedling of two cultivar of lucerne, Euver susceptible to *V. albo-atrum*, isolate V1 and resistant to isolate V2, and Vela with a degree of resistant to both isolates V1 and V2, (the result of the pathogenicity experiment), were inoculated by the roots dipping method as described in Materials and Methods for 10 minutes in spore suspensions (10^7 spore ml^{-1}) of one of two isolates of *Verticillium albo-atrum*, V1 & V2, or one of two isolates of *Verticillium dahliae*, D1 & D2 or one isolate of *Verticillium nigrescens*, or *Verticillium nubilum*, or *Verticillium tricorpus*. Control plants were dipped in sterile distilled water. The inoculated plants were replanted in pots (15 cm diameter) containing "all purpose compost" (Arthur Bowers), and maintained in the greenhouse. Measurement of the height of the main stem of the plants and recording of the development of wilt symptoms were made at weekly intervals over an 10-week period following inoculation. Six replicates were used for each treatment.

Longitudinal section from the basal part of the stem of the test plants and re-isolation (general Materials and Methods, "Isolation of the fungus") of the fungus were made from test plants.

Tomato cultivar

Plants of tomato cultivar Ailsa Craig, were inoculated by the same method and with same isolates and species of *Verticillium*, which were mentioned in lucerne above. Control plants were inoculated with sterile distilled water. Inoculated plants were maintained in the greenhouse.

Assessment of wilt disease

Progress of the severity of wilt disease as determined by visual assessment.

Plants were scored individually by observing growth (measuring the height of the main stem) and recording development of wilt disease symptoms at weekly intervals over a 10-week period following inoculation. The following rating, adapted from (Dixon and Doodson 1971) and used by; (Latunde-Dada & Lucas 1982); (Sayigh, 1981); (Esyanti, 1993) was used. The keys were:

0 = The plants healthy, no wilt symptoms appeared.

1 = The lower leaves lost turgor and showed yellowing.

2 = Less than a quarter of the plant showed wilt symptoms

3 = Less than half of the plant showed wilt symptoms.

4 = Less than three quarters of the plant showed wilt symptoms such as, loss of turgor

and yellowing in leaves, necrosis, desiccation, epinasty or leaves defoliated

5 = The plants were dying or dead

The following equation that was used by (Sayigh, 1981) and (Esyanti, 1993) was used to obtain the disease symptom index.

* Sum of individual rating x 100

% Disease symptom Index = -----

** 5 x the number of plants assessed

* Sum of each plant multiple by the particular value

** The maximum value of the symptoms.

Statistical Analysis

All the results were analysed statistically using a SWANSTAT program which has been written by, Dr. S.J. Wainwright, School of Biological Sciences, University College of Swansea. From "A computer program to perform a Joint Regression Analysis and to make simultaneous comparison of the slopes and means of Regression lines". International Labmate (1993) XVIII 25-25"

Table 1: Designation of *Verticillium* species and isolates which were used in the experiments

Desi	Species	Source of culture
V1	<i>V. albo-atrum</i>	Isolated from lucerne, from the field in Swansea
V2	<i>V. albo-atrum</i>	Gift from Dr. J.M. Milton (Swansea Uni.), isolated from tomato, in Swansea
D1	<i>V. dahliae</i>	Gift from Dr. J. Flood (Bath Uni.), isolated from tomato in Swansea
D2	<i>V. dahliae</i>	Gift from Dr. Flood (Bath Uni.), isolated from tomato in USA
Vi	<i>V. nigrescens</i>	International Mycological Institute, England. (44575). Isolated from tomato
Vu	<i>V. nubilum</i>	International Mycological Institute, England. (62467), Isolated from tomato
Vt	<i>V. tricorpus</i>	International Mycological Institute, England. (71799). Isolated from tomato

RESULTS

Specialization of *Verticillium* isolates & species to lucerne and tomato

The results (table 2 & 4) demonstrate that the susceptible cultivar of lucerne, Euver was affected by *V. albo-atrum* isolates V1, only. The wilt symptoms appeared on the plants at 3 weeks following inoculation. The plants that were inoculated with *V. dahliae*, isolate D1 showed stunting in growth, and no wilt symptoms were observed on the plants that were inoculated with the other species or isolates of *Verticillium* used in this experiment. These results lead to the conclusion, that only *V. albo-atrum* isolate V1 can infect lucerne plants and cause wilt disease but *V. dahliae*, isolate D1 can infect lucerne plants but without causes wilt disease symptoms.

Table 2: The mean heights of the main stem, of lucerne, cv. Euver (susceptible) inoculated with different species and isolates of *Verticillium*

Treatment								
W	C	V1	V2	D1	D2	Vi	Vu	Vt
1	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)
2	10.0(±0.0)	8.0(±0.0)	11.0(±0.0)	10.0(±0.0)	10.0(±0.0)	11.0(±0.0)	10.0(±0.0)	10.0(±0.0)
3	14.1(±1.2)	9.2(±2.2)	17.1(±2.2)	15.1(±2.2)	16.1(±6.2)	18.2(±2.2)	15.2(±1.1)	18.2(±4.4)
4	19.2(±2.2)	10.1(±1.1)	23.2(±2.1)	20.2(±3.3)	22.2(±5.5)	22.1(±7.8)	21.4(±8.3)	20.0(±1.9)
5	25.6(±3.5)	12.1(±1.1)	28.9(±9.0)	25.5(±9.1)	27.8(±0.8)	27.8(±0.7)	25.6(±1.0)	26.7(±3.0)
6	35.4(±4.0)	13.1(±2.0)	35.5(±1.0)	30.4(±6.0)	34.2(±3.0)	35.6(±8.9)	35.4(±2.0)	34.3(±3.9)
7	42.4(±4.0)	15.2(±5.2)	41.3(±2.2)	34.3(±2.2)	40.3(±3.3)	41.5(±5.0)	40.3(±2.2)	40.2(±4.8)
8	47.8(±8.0)	16.3(±0.8)	46.7(±3.2)	40.2(±3.2)	46.7(±2.2)	47.8(±0.8)	46.7(±5.9)	45.6(±5.4)
9	55.6(±6.0)	17.8(±7.0)	53.3(±2.2)	45.2(±2.5)	53.5(±5.0)	55.3(±2.2)	54.2(±7.0)	53.3(±6.1)
10	61.1(±2.0)	20.3(±3.2)	57.8(±1.3)	51.2(±9.0)	58.9(±2.7)	61.1(±3.3)	60.0(±2.2)	60.1(±7.0)

Figures in brackets represents the standard deviation

The key

W	Week following inoculation	Statistical analysis of the inoculated
*	Heights in cm the means of 5 plants	and control plants as follows
C	the control	
V1	<i>V. albo-atrum</i> , isolated from lucerne	S.d.
V2	<i>V. albo-atrum</i> , isolated from tomato	Not s.d.
D1	<i>V. dahliae</i> , isolated from tomato (Swansea)	S.d.
D2	<i>V. dahliae</i> , isolated from tomato (USA)	Not s.d.
Vi	<i>V. nigrescens</i>	not s.d.
Vu	<i>V. nubilum</i>	Not s.d.
Vt	<i>V. tricorpus</i>	Not s.d.

On the other hand the resistant cultivar Vela, plants tables, (3 and 4) that were inoculated with *Verticillium* species & isolates remained as healthy as the control, except the plants which were inoculated with *V. albo-atrum* isolate V1, these showed stunting of growth. These results lead to the suggestion that cultivar Vela is resistant to all species and isolates of *Verticillium* which were used in this experiment except *V. albo-atrum*, isolate V1 but without showing wilt symptoms.

Reisolation table 7 of the fungus from the test plants was successful with *V. albo-atrum* isolate V1 and V2 and *V. dahliae* isolates D1 & D2 only and failed with the other fungi including *V. nigrescence*, *V. nubilum* and *V. tricorpus*.

Statistical analysis (table 2 and 3) showed significant differences in growth between the isolate V1 inoculated plants and control plants. Therefore, it can be concluded that the only isolate of *Verticillium* that had been isolated from the lucerne can infect the lucerne plants again.

Table 3: The mean heights of the main stem of lucerne, cultivar Vela (resistant) inoculated with different species and isolates of *Verticillium*

W	C	V1	V2	D1	D2	Vi	Vu	Vt
1	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)
2	12.0(±0.0)	10.0(±0.0)	10.0(±0.0)	10.0(±0.0)	11.0(±0.0)	10.0(±0.0)	10.0(±0.0)	11.0(±0.0)
3	17.8(±0.1)	16.2(±2.5)	16.1(±6.0)	16.0(±9.0)	15.3(±3.0)	15.3(±7.0)	15.2(±5.2)	15.5(±0.6)
4	22.2(±5.0)	21.1(±1.2)	21.4(±6.0)	21.4(±8.0)	20.0(±3.2)	22.2(±3.3)	21.5(±0.8)	22.5(±9.0)
5	30.1(±9.0)	25.5(±5.1)	28.8(±3.2)	26.7(±6.4)	27.0(±6.0)	28.9(±9.0)	27.8(±2.9)	29.1(±7.0)
6	38.9(±1.1)	31.2(±2.4)	36.7(±6.7)	35.6(±0.6)	36.7(±6.0)	37.0(±0.0)	35.5(±0.5)	36.1(±0.6)
7	47.8(±7.7)	35.6(±7.9)	45.6(±6.7)	46.1(±1.9)	45.5(±0.9)	46.1(±7.7)	45.4(±9.0)	44.5(±4.5)
8	54.4(±4.4)	42.3(±7.8)	52.2(±3.3)	52.1(±3.4)	53.0(±0.2)	54.2(±2.3)	53.2(±2.2)	53.1(±1.1)
9	61.1(±1.0)	48.0(±0.2)	63.3(±3.0)	61.3(±0.3)	60.1(±1.1)	61.0(±2.8)	61.1(±2.2)	60.3(±0.4)
10	70.0(±2.0)	54.2(±9.0)	66.7(±6.7)	65.6(±0.9)	67.8(±4.5)	70.1(±2.2)	68.9(±1.1)	67.8(±0.6)

Figures in bracket represent the standard deviation

The Key

* Heights in cm, the means of 5 plants

C Control plants

V1 *V. albo-atrum*, isolated from lucerne

V2 *V. albo-atrum*, isolated from tomato

D1 *V. dahliae*, isolated from tomato (Swansea)

D2 *V. dahliae*, isolated from tomato (USA)

Vi *V. nigrescens*

Vu *V. nubilum*

Vt *V. tricorpus*

Statistical analysis of inoculated

and control plants as follows

S.d.

S.d.

Not s.d.

Not s.d.

Not s.d.

not s.d.

Not s.d.

Not s.d.

Table 4: The disease symptom index, of lucerne, cv. Euber (susceptible) and cv. Vela (resistant) inoculated with different species and isolates of *Verticillium*

Cv. EUBER (susceptible)

Week	C	V1	V2	D1	D2	Vi	Vu	Vt
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	5	0	0	0	0	0	0
4	0	10	0	0	0	0	0	0
5	0	25	0	0	0	0	0	0
6	0	35	0	0	0	0	0	0
7	0	55	0	0	0	0	0	0
8	0	65	0	0	0	0	0	0
9	0	75	0	0	0	0	0	0
10	0	75	0	0	0	0	0	0

Cv. VELA (resistant)

Week	C	V1	V2	D1	D2	Vi	Vu	Vt
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0

The reaction of the tomato cultivar is shown in the table (5 and 6) wilt symptoms appeared on all the tomato plants that were inoculated with *Verticillium albo-atrum* isolate V2 and *Verticillium dahliae* isolates D1 and D2, 3 weeks following inoculation. On the other hand plants that were inoculated with *Verticillium albo-atrum* isolate V1, and *Verticillium* species, *V. nigrescens*, *V. nubilum*, and *V. tricorpus* were as healthy as the controls.

Reisolation of the test fungus (table 7) was successful with isolates V1, V2, D1 & D2 only and failed with the other fungi, *V. nigrescens*, *V. nubilum* and *V. tricorpus*.

Statistical analysis (table 5) indicated that only plants that were inoculated with *V. albo-atrum* isolate V2 and *V. dahliae* isolates D1 and D2 showed significant differences from the control plants. Therefore, it can be concluded that the tomato cultivar Ailsa Craig is susceptible to *V. albo-atrum*, isolate V2 and *V. dahliae* isolates D1 and D2. It is resistant to *V. albo-atrum*, isolate V1, *V. nigrescens*, *V. nubilum* and *V. tricorpus*.

Table 5: The mean heights, of tomato cv. Ailsa Craig inoculated with different isolates and species of *Verticillium*

W	C	V1	V2	D1	D2	Vi	Vu	Vt
1	15.0 (+0.0)	15.0 (+0.0)	15.0 (+0.0)	15.0 (+0.0)	15.0 (+0.0)	15.0 (+0.0)	15.0 (+0.0)	15.0 (+0.0)
2	16.7 (+1.0)	17.0 (+1.0)	17.1 (+2.0)	17.0 (+0.0)	17.0 (+0.0)	17.0 (+0.0)	17.0 (+0.0)	17.0 (+0.0)
3	20.1 (+4.1)	20.3 (+3.0)	18.9 (+2.4)	18.4 (+2.3)	20.0 (+4.0)	20.1 (+4.0)	18.9 (+2.2)	20.2 (+1.2)
4	27.8 (+2.2)	27.8 (+1.1)	21.2 (+1.5)	20.2 (+1.0)	24.1 (+1.1)	27.8 (+3.3)	25.6 (+4.4)	26.7 (+7.6)
5	37.8 (+2.2)	37.8 (+2.5)	23.3 (+2.5)	22.1 (+1.5)	27.8 (+2.1)	35.6 (+5.6)	35.6 (+2.2)	36.7 (+1.1)
6	46.6 (+1.2)	45.6 (+2.5)	25.2 (+4.5)	23.3 (+1.5)	33.0 (+1.0)	46.1 (+2.4)	46.7 (+2.2)	47.8 (+1.5)
7	54.1 (+1.1)	54.1 (+3.5)	26.7 (+3.5)	25.2 (+2.5)	37.3 (+2.2)	55.1 (+0.0)	54.1 (+0.0)	55.6 (+1.1)
8	67.8 (+1.0)	66.7 (+5.5)	28.9 (+5.5)	26.7 (+4.5)	40.1 (+1.1)	67.8 (+2.2)	68.9 (+3.3)	68.1 (+2.2)
9	78.9 (+2.2)	79.1 (+3.3)	31.2 (+1.2)	28.9 (+1.1)	45.5 (+4.4)	78.9 (+1.1)	80.0 (+1.1)	78.9 (+1.1)
10	92.1 (+1.0)	92.2 (+7.5)	33.1 (+7.5)	31.2 (+5.5)	50.1 (+3.0)	91.2 (+1.3)	91.2 (+2.2)	91.4 (+3.3)

Figure in brackets represent the standard deviation

The key

Statistical analysis of inoculated and control plants as follows

W	Weeks following inoculation	
V1	<i>V. albo-atrum</i> , isolated from lucerne	Not s.d.
V2	<i>V. albo-atrum</i> , isolated from tomato	Not s.d.
D1	<i>V. dahliae</i> , isolated from tomato (Swansea)	s.d.
D2	<i>V. dahliae</i> , isolated from tomato (USA)	s.d.
Vi	<i>V. nigrescens</i>	not s.d.
Vu	<i>V. nubilum</i>	Not s.d.
Vt	<i>V. tricorpus</i>	Not s.d.

Table 6: The disease symptoms index, of tomato, cv. Ailsa Criag inoculated with different species and isolates of *Verticillium*, in the green house

The treatment

Week	C	V1	V2	D1	D2	Vi	Vu	Vt
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	15	15	10	0	0	0
5	0	0	25	25	15	0	0	0
6	0	0	25	25	15	0	0	0
7	0	0	35	35	25	0	0	0
8	0	0	55	55	45	0	0	0
9	0	0	75	75	45	0	0	0
10	0	0	75	75	55	0	0	0

Table 7: Reisolation the fungus from the inoculated lucerne plants in the specialization experiment

Treatment

Cultivar	V1	V2	D1	D2	Vi	Vu	Vt
Euver	<u>5</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>0</u>	<u>0</u>	<u>0</u>
	5	5	5	5	5	5	5
Vela	<u>1</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
	5	5	5	5	5	5	5
Tomato	<u>2</u>	<u>5</u>	<u>5</u>	<u>4</u>	<u>0</u>	<u>0</u>	<u>0</u>
	5	5	5	5	5	5	5

Upper & higher figure represents number of positive reisolation made
lower figure represents number of plants in treatment

DISCUSSION

Previous workers in the field of Plantpathology have studied wilt disease of lucerne, or the interaction between *Verticillium* species and lucerne, simply as an interesting phenomenon. They have focused, therefore, on describing the disease or the fungus which causes the disease, or they have studied one particular aspect of the phenomenon, such as, the defense response involved or, hypersensitivity, or phytoalexin production or the influence of the environmental conditions on development of disease symptoms or control of wilt disease. The present study has been attempted in order to study wilt disease as the central theme and has tried to establish a solution. Its aims has included evaluation the

best method of controlling the disease (meaning cheapest, easiest, safest and most effective) taking into account environmental safety.

There are many methods to evaluate the severity of the wilt disease caused by *Verticillium* species or the resistance of the plant to this disease, for example, (Frank et al., 1975) stated that foliar symptoms can be used to evaluate accurately resistance to vascular wilt in potato plants. But, (Papadopolous et al., 1991) reported that absence of symptom is not reliable indicator of resistance to *Verticillium* wilt disease. However, using the foliar symptoms to evaluate resistance of the plants was used by other workers, for example (Jorge, 1990); Esyanty, 1993); (Pennypacker & Leath, 1993). (Newcombe & Robb 1988) used symptoms evaluation and reisolation of the pathogen from test plants to determine disease resistance.

In this study more than one aspect of plant resistance evaluation was considered in evaluating pathogenicity and the development of wilt disease in lucerne cultivars in response to *Verticillium* isolates and species. These included; growth of plants (by weekly measurements the heights of the main stem, combined with statistical analysis of the results); disease symptoms index, and reisolation of the fungus from the test plants.

Pathogenicity is ability of a pathogen to produce disease, (Strobel & Mathre, 1970). But Agrios (1979) defined pathogenicity as a relative capability of pathogen to cause disease. In present study testing the pathogenicity of different species and isolates of *Verticillium* (two isolates of *V. albo-atrum*, V1 & V2; two isolates of *V. dahliae*, D1 & D2; three species of *Verticillium*, *V. nigrescens*, *V. nubilum* and *V. tricorpus*) served two purpose. The results show that the first investigation of the specialization of *Verticillium albo-atrum*, lucerne-isolates, towards the cultivars of lucerne established, that only isolate V1 could infect the lucerne cultivars and cause wilt disease. This result agreed with that of Isaac, (1957); Syigh, (1981) and Cornell et al. (1988). However, Smith et al., (1988) observed that there were few reports of host specialization in *Verticillium*, although it is well documented that isolates from one host can cause disease in same crops, but infect others without causing symptoms.

The second purpose of the specialization experiment was to determine which was the weakest pathogen of *Verticillium*, to lucerne in order to use it in subsequent experiments to induced resistance in lucerne. The results confirmed that isolate V1 was virulent pathogen towards lucerne and *V. nigrescens* was the weakest avirulent of the species of *Verticillium* which were used in this experiment and so these particular isolates and species were used in the experiment induced resistance in lucerne.

To confirm the pathogenicity and specialization, of the *Verticillium* isolates and species which were used in the previous experiments. Same isolates and species were used to inoculate tomato, cultivar Ailsa Craig (susceptible to *V.*

albo-atrum isolate V2 and resistant to V1) as described in Materials and Methods. The results show that those isolates or species of *Verticillium* which were isolated from tomato infected the tomato, *V. albo-atrum* isolate V2, *V. dahliae* isolates D1 and D2, and caused wilt diseasesymptoms on the tomato plants within 3-4 weeks following the inoculation. The other isolates and species including, *V. albo-atrum* isolate V1, *V. nigrescens*, *V. nubilum* and *V. tricorpus* failed to infect tomato an the plants remained as healthy as the control plants, (plants which were inoculated with sterile distilled water). These results agree with those of Isaac, (1957) and Cornell et al., (1988).

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Synthesis Of Some Substituted Benzo [b] Thiophenes

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الخلاصة

تؤدي تفاعلات حامض السيناميك المعوض وغير المعوض مع كلوريد الثايونيل بوجود البيريدين الى تكوين مشتقات 3 - كلورو - 2 - كلورو كاربونيل بـترو [b] ثايونين. وللخواص الكيميائية التي تتمتع بها هذه المشتقات باحتوائها على مجموعة كلورو كاربونيل في موقع (2) فقد تم من خلالها تحضير عدد من معوضاتها (الاميدات) من خلال التفاعلات النيوكلوфильية مع بعض الامينات الاولى والثانوية

ABSTRACT

Reactions of substituted and unsubstituted cinnamic acids with thionyl chloride in the presence of pyridine lead to the formation of 3- chloro -2- chlorocarbonyl benzo [b] thiophene derivatives. These derivatives subsequently react with secondary amines and hydrazine hydrate through nucleophilic reactions at position (2) (-COCl).

INTRODUCTION

A wide variety of compounds containing the benzo [b] thiophene skeleton are found in nature⁽¹⁻⁴⁾ and many displayed the marked applications in industry⁽⁵⁻¹¹⁾, agriculture⁽¹²⁻¹³⁾ and their pharmacological activities⁽¹⁴⁻²⁰⁾. With a review of these activities, there has been a surge of interest in the synthesis of benzo [b] thiophene derivatives especially those contain various groups at position 2⁽²¹⁻²⁴⁾. However, few synthetic methods for the preparation of these compounds are available⁽²⁵⁻³²⁾, more convenient and widely applicable methods are desired.

In the present work, four cinnamic acid derivatives are elected as a model of synthetic entry to the such type of derivatives.

EXPERIMENTAL

All melting points were determined with Gallen kamp apparatus and uncorrected, IR spectra were recorded on pye Unicam SP3-100 spectrophotometer in 600-400 cm^{-1} range using KBr and Nujil. UV spectra were measured in ethanol

using Hitachi-V-2000 spectro-photo-meter. Elemental analysis were performed on a CHN analyzer, type Perkin Elmer 240b. The ¹HNMR spectra for some compounds were taken on JOEL EX 90 (Basra Univ.).

Preparation of Cinnamic Acid Derivatives (1-4)

These acids are prepared according the reported procedures^(33,34), (Table 1).

Preparation of 3-chloro -2- chloro carbonyl benzo [b] thiophenes (5-8).

A 6 ml of thionyl chloride is gradually added to a mixture of appropriate cinnamic acid (0.023 mol.) and pyridine (0.5 ml). A mixture was refluxed for time shown in Table (2), then poured into n-hexane (100 ml), filtered an oily residue thus formed and the yellow solution is left at room temperature for several hours to give the required products (5-8) as precipitate (Table 2).

Reaction of Compounds (5-8) With Secondary Amines

An appropriate 3- chloro -2- chlorocarbonyl benzo [b] thiophene in 100 ml round bottom flask and the appropriate secondary amine (0.005 mol) (morpholine, piperidine, diethylamine) in dry dioxane (5ml) was added. The reaction mixture was refluxed for 4hrs. Evaporation of solvent under vaccum to give products (9-20) after recrystallization from benzene (Table 3).

Reaction of benzo [b] thiophenes (5-8) with hydrazine

An appropriate benzo [b] thiophene (5-8) (0.001 mol) dissolved in dry chloroform (5ml) and excess hydrazine hydrate (0.018 mol) was added. The mixture was refluxed for ½ hr, then evaporated under reduced pressure. The solid products formed were recrystallized from cyclohexane to produce the corresponding acid hydrazides (21-24) (Table 4).

Table (1) Physical properties of cinnamic acids (1-4)

.No	Yield %	M.P C ^o		Micro Analytical Data			
		Found	Lit	Found %		Calcd%	
1	86	131-132	131-132 ⁽³³⁾	71.47	7.10	71.52	7.28
2	79	163-164	163-164 ⁽³⁴⁾	67.36	5.60	67.41	5.61
3	60	158-159	-	91.60	8.30	91.66	8.33
4	63	120-121	-	91.07	8.83	91.14	8.86

Table (2) Physical properties of 3- chloro -2- clorocarbonyl benzo [b] thiophenes (5-8)

Comp No.	Time (min)	Temp. C	Yield %	M.P C ^o Found	Micro Analytical Data			
					Found %		Calcd %	
					C	H	C	H
5	60	140	51	112-114	46.73	1.73	46.75	1.73
6	30	140	57	116-117	45.97	2.30	45.97	2.30
7	15	120	33	116-118	45.29	2.72	45.36	2.77
8	10	120	41	150-153	44.89	3.00	44.84	3.11

Table (3) Physical properties of compounds (9-20)

No	R	Secondary amine	Yield %	M.P C	Micro Analytical Data				
					Found %				
					C	H	N	S	Cl
9	H	Morphiline	80	80-82	60.27	4.98	4.42	11.95	12.57
10	Para-OMe	Morphiline	87	130-132	53.81	4.52	4.53	10.07	11.23
11	5,6-di-OMe	Morphiline	62	126-128	38.38	5.30	4.00	9.53	10.65
12	5,6,7-tri-OMe	Morphiline	73	102-104	55.08	5.48	3.90	8.83	9.56
13	H	Piperidine	76	218-221	55.70	4.28	4.52	11.86	12.86
14	Para-OMe	Piperidine	85	59-60	58.20	5.12	4.30	10.14	11.19
15	5,6-di-OMe	Piperidine	49	105-108	52.69	4.75	4.30	9.56	10.50
16	5,6,7-tri-OMe	Piperidine	67	84-86	51.36	4.66	3.34	8.49	9.52
17	H	Diethyl amine	67	212-214	58.42	5.29	5.00	11.60	13.03
18	Para-OMe	Diethyl amine	79	125-127	56.67	5.39	4.61	10.86	11.83
19	Para-OMe	Diethyl amine	-	Oily	54.63	5.55	4.59	9.46	10.80
20	5,6,7-tri-OMe	Diethyl amine	35	132-134	53.44	5.61	3.63	8.84	9.80

Continued table 3

No	Calcd %				
	C	H	N	S	Cl
9	60.0	5.0	4.0	11.45	12.70
10	53.93	4.49	4.49	10.27	11.39
11	38.88	5.30	4.12	9.42	10.46
12	55.20	5.41	3.79	8.66	9.60
13	55.41	4.26	4.97	11.36	12.61
14	58.15	5.16	4.52	10.34	11.47
15	52.71	4.69	4.09	9.37	10.39
16	51.68	4.84	3.77	8.61	9.55
17	58.2	5.23	5.20	11.96	13.27
18	56.45	5.35	4.74	10.78	11.90
19	54.96	5.49	4.27	9.77	10.84
20	53.70	5.59	3.91	8.95	9.93

Table (4) Physical properties of compounds (21-24)

No	R	Primary Amine	Yield %	M.P °C	Micro Analytical Data				
					Found %				
					C	H	N	S	Cl
21	H	Hydrazine	69	162-165	47.60	3.18	12.14	14.00	15.72
22	Para-OMe	Hydrazine	72	226-229	46.65	3.59	10.71	12.25	13.62
23	5,6-di-OMe	Hydrazine	62	Oily	46.00	3.79	9.69	13.68	12.19
24	5,6,7-tri-Me	Hydrazine	-	Oily	45.02	4.67	8.81	10.18	11.13

Continued table 4

No	Calcd %				
	C	H	N	S	Cl
21	47.68	3.09	12.36	14.12	15.67
22	46.78	3.51	10.90	12.47	13.84
23	46.67	3.84	9.77	13.53	12.39
24	45.21	4.70	8.79	10.04	11.14

RESULTS AND DISCUSSION

Benzo [b] thiophene derivatives (5-8) were prepared from the cyclocondensation of corresponding cinnamic acids⁽¹⁻⁴⁾ with thionyl chloride promoted by pyridine as shown in scheme 1.

The following mechanism was suggested, which implies, the electrophilic addition of thionyl chloride through the double bond of cinnamyl chlorides to give sulfanyl chlorides.

These chlorides may be followed one of the following path ways: Pummerer reaction to give derivative (5) scheme -2 a or ring cyclization with loss of HCl to produce derivatives (6-8) as shown in Scheme -2b.

The structures of these compounds (5-8) were elucidated by IR, UV and CHN analysis. The IR of compounds (5-8) showed strong band in the range of 1740-1760 cm^{-1} which was characterized the C=O group at position (2) with the absence of bands at 3200-3500 cm^{-1} related to OH group.

All these compounds gave other characteristic band in the region 680-685 cm^{-1} due to the presence of δ C-S-C grouping. UV. Spectra gave two intense bands near 263-287 nm and 290-340 nm and were identical with reported values for benzo [b] thiophene derivative^(35,36).

¹HNMR spectrum data gave some additional information about the compound (6) which confirmed the proposed structure of (6). The signal of δ 3.75 ppm (3H) due to OCH₃; another broad signal at δ 7.55-6.85 (3H) due to phenyl protons.

The chemical reactivity of the chloro carbonyl group of position (2) in compounds (5-8) plays an important role as synthons in a way to study their reactions with various nucleophiles e.g. amines, hydrazine. This may be dependent on the nucleophilicity group and polarity of C=O group. In this study we examined the reaction of compounds (5-8) with secondary amines (morpholine, piperidine and diethyl amine); and hydrazine hydrate (see experimental section) where by the respective products (9-24) were obtained.

The nucleophilic substitution reaction involving the amino group occurs favorably at the C=O to yield compounds (9-24) as revealed from the decrease in frequency of characteristic bands in IR spectra tables (7-8) (due to the C=O group of amide) and appearance of new bands in the region 3380-3420 cm^{-1} which assigned for VNH of amide in compounds (21-24). UV spectra of compounds (9-24) didn't show much difference from compounds (5-8).

Further more, the structure's of these compounds (9-24) were confirmed by ¹HNMR. The ¹HNMR data of compound (10) as representative showed broad signals with chemical shifts δ 2.0-2.30 (4H) and δ 3.4-3.8 (4H) which were assigned to the protons CH₂-O-CH₂ and CH₂-N-CH₂ respectively. Also broad signals at 7.8-8.8 ppm (4H) was assigned to aromatic protons.

¹HNMR of compounds (14) and (22) were examined by similar method at shown below.

¹HNMR of compound (14) δ 0.95-1.2 (6H). CH₂-CH₂-CH₂
 δ 3.8-4.1 (4H)-CH₂N-CH₂-
 δ 3.7 (5) (3H) – OCH₃
 δ 7.00-7.96 (3H) aromatic protons

¹HNMR of compd (22) δ 3.5-4.0 (3H). OCH₃
 δ 8.0-8.3 (3H) – NHH₂
 δ 7.0-7.8 (3H) Aromatic protons.

Table (5) Infrared spectrum of Cinnamic acids (1-4)

No.	R	-OH	C-O	C=O	C=C	CH ₃ ,CH
1	H	2500-3200 (b)	1685	-	1610,1480	2860
2	Para-OMe	2400-3100 (b)	1660	1230,1050	1620,1450	2950,2860
3	5,6-di- OMe	2400-3200 (b)	1675	1260,1100	1620,1420	2960,2840
4	5,6,7-tri- OMe	2500-3200 (b)	1685	1200,1100	1620,1480	2970,2840

Table (6) Infrared and ultraviolet of compounds (5-8)

No	R	IR						UV EtOH λ Max,nm
		C=O	C-Cl	C-S-C	C-O	CH ₃	C=C	
5	H	1740	1080ar . 975al.	680	-	-	1600	294,304,263 ,278
6	Para- OMe	1760	1075ar 970al.	685	1240 1030	-	1600 1480	254,275,290 ,328
7	5,6,-di- OMe	1750	1050ar 975al.	685	1250 1020	2960	1600 1450	252,287,290 ,317
8	5,6,7-tir- OMe	1760	1080ar 970al.	680	1240 1030	2975	1610 1450	253,271,292 ,340

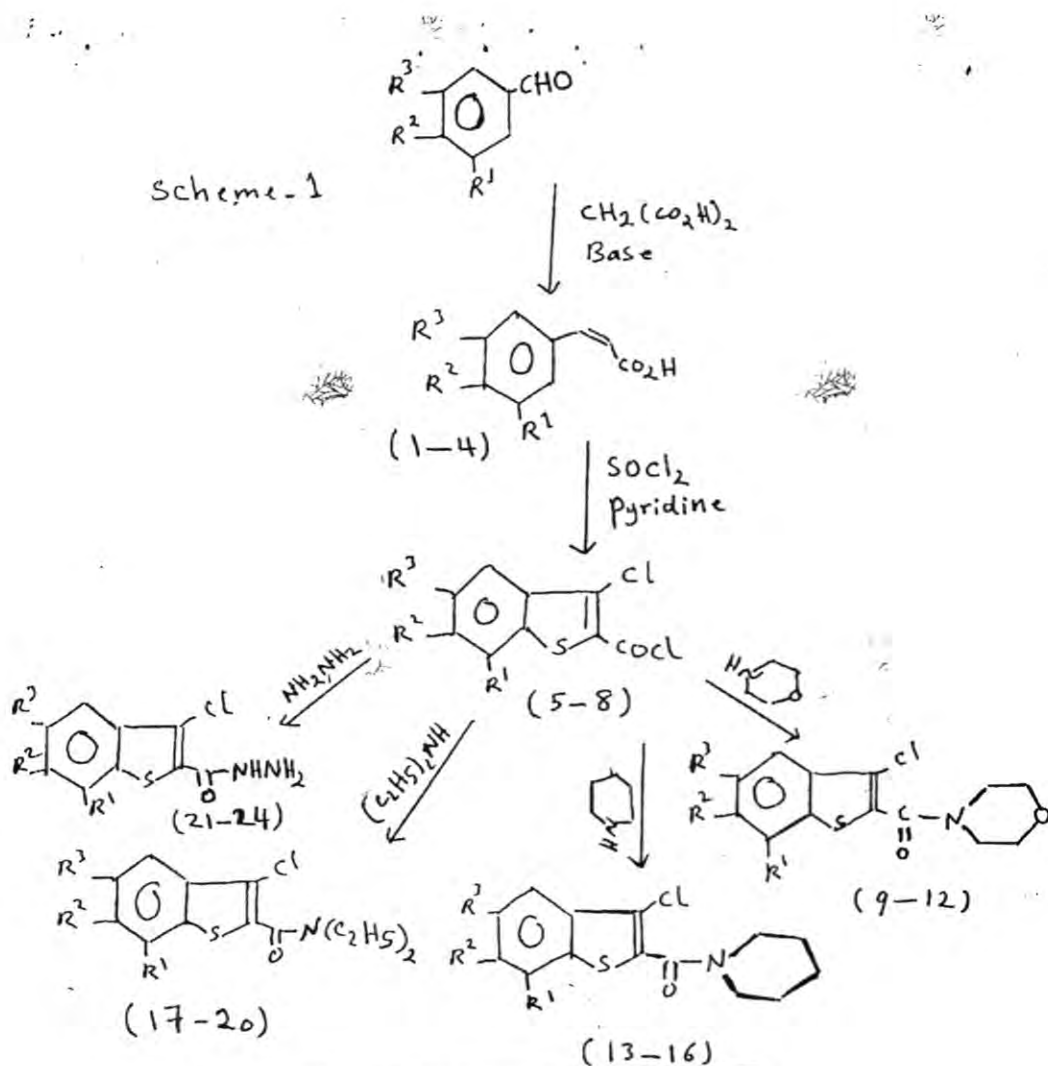
Table (7) IR and UV of compounds (9-20)

No	R	Secondary Amine	IR			UV EtOH Max,nm
			$\begin{array}{c} \text{O} \\ \parallel \\ \text{C-N} \end{array}$	C-O	C-N	
9	H	Morphiline	1640	1230 1050	1250	250,284,290
10	Para-OMe	Morphiline	1635	1230 1030	1240	252,294,317
11	5,6-di-OMe	Morphiline	1640	1270 1100	1230	253,286,319
12	5,6,7-tri-OMe	Morphiline	1640	1285 1100	1230	252,294,317
13	H	Piperidine	1680	...	1230	250,284,290
14	Para-OMe	Piperidine	1640	...	1250	252,294,317
15	5,6-di-OMe	Piperidine	1635	...	1210	253,286,319
16	5,6,7-tri-OMe	Piperidine	1670	...	1265	255,289,340
17	H	Diethylamine	1650	...	1280	250,284,290
18	Para-OMe	Diethylamine	1670	...	1250	252,294,317
19	5,6-di-OMe	Diethylamine	1670	...	1240	253,286,319
20	5,6,7-tri-OMe	Diethylamine	1675	...	1275	255,289,340

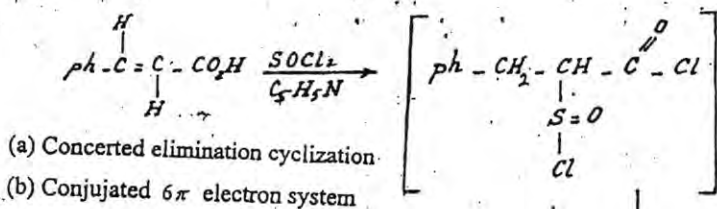
Table (8) IR and UV compounds (21-24)

No.	R	IR			UV EtOH λ Max,nm
		$\begin{array}{c} \text{O} \\ \parallel \\ \text{C-N} \end{array}$	C-N	N-H	
21	H	1646	1270	3380	249,264,284
22	Para-OCH ₃	1640	1270	3380	252,281,298
23	5,6-di-OCH ₃	1642	1250	3420	254,287,315
24	5,6,7-tri-OCH ₃	1655	1245	3398	251,290,329

Scheme - 1



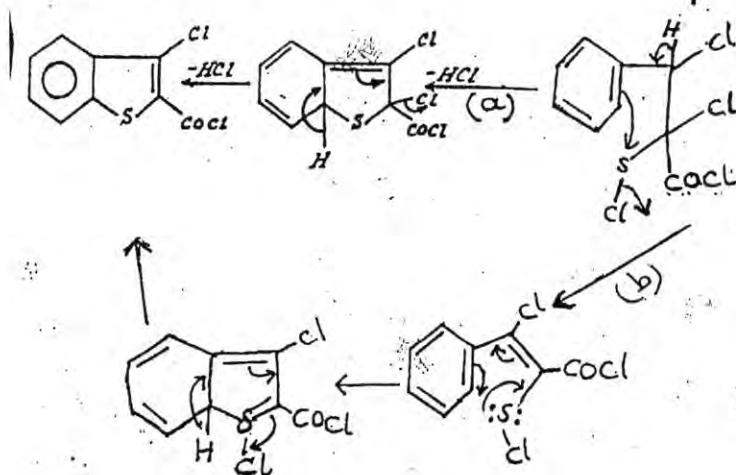
Comp.No.	R ¹	R ²	R ³
1, 5, 9, 13, 17, 21	H	H	H
2, 6, 10, 14, 18, 22	H	OCH ₃	H
3, 7, 11, 15, 19, 23	H	OCH ₃	OCH ₃
4, 8, 12, 16, 20, 24	OCH ₃	OCH ₃	OCH ₃



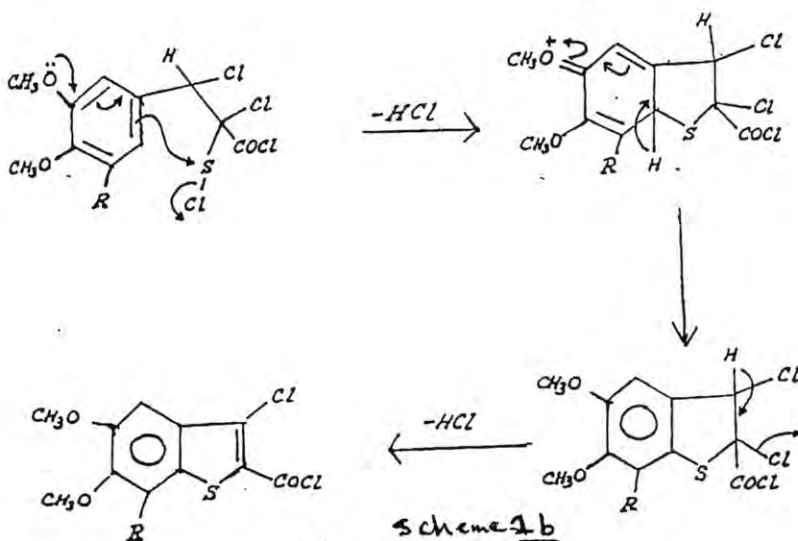
(a) Concerted elimination cyclization

(b) Conjugated 6π electron system

under goes a concerted cyclization.



Scheme 2a



Scheme 2b

(9)

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Synthesis Of Some 3- Chloro-4- Methyl and 4- Chloro Methyl Coumarins and Their Reactions With Primary and Secondary Amines

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الخلاصة

تم تحضير مشتق 3- كلورو - 4 - ميثيل كومارين ومشتقات 4 - كلورو ميثيل كومارين وتم استعمالها كمواد اولية لتحضير المشتقات الامينية للكومارين وذلك بتفاعل التعويض النيوكلوفيلي مع الامينات الاولى والثانوية. تم اثبات التراكيب المقترحة عن طريق التحليل الدقيق للعناصر (CHN) وبعض الخواص الطيفية (UV, IR).

ABSTRACT

3- Chloro - 4 - methyl and 4- chloro methyl coumarin derivatives have been synthesized and used as starting materials to synthesis other derivatives by their reaction with primary and secondary amines in nucleophilic substitution reaction. The chemical structure of all prepared compounds was confirmed on the basis of their elemental analysis and some spectral data (IR, UV spectroscopy).

INTRODUCTION

There are a large number of natural products derived from coumarin displaying the marked biological activities. The range of compounds includes antifungal⁽¹⁾, anticogulant⁽²⁾, photosensitizer⁽³⁾ and antibacterial activity⁽⁴⁾, especially with derviatives containing amino groups⁽⁵⁻⁸⁾.

However, few synthetic methods for the preparation of these compounds i.e. amino derivatives are available⁽⁹⁻¹⁰⁾ more convenient and widely applicable methods are desired, In the present work, we select 3-chloro 4- methyl and 4-

chloromethyl coumarin derivatives (III_{a-y}) as a model of synthetic entry to the such type of molecules.

EXPERIMENTAL

All melting points were determined with Gallen Kamp apparatus and are uncorrected. IR spectra were recorded on SP3 – 100 spectrophotometer using KBr discs. UV spectra were determined in methanol on a SP – 2000 spectrophotometer. Elemental analysis were performed on a CHN analyzer, type 1106 (Calro Erba). Ethyl 2- chloroacetic acetate (I_a) was prepared from ethyl aceto acetic and sulfonyl chloride according to the reported procedure⁽¹¹⁾.

Preparation of 3-chloro -4,7- dimethyl coumarin (III_a) and 4- chloro methyl Coumarin derivatives (III_{b-g}).

General Procedure

To a stirred mixture of ethyl 2- chloro (I_a) or 4- chloro acetoacetate (I_b) (0.05 mol) and appropriate phenol (0.05 mol) was added polyphosphoric acid (Ten fold excess weight of phenol used).

The mixture was heated at temperature (90-100 °C) for 1 hour, poured over crushed ice, the solid product collected, washed with cold water, dried and crystallised from Ethanol-water to afford coumarin derivatives (III_{a-g}) in excellent yields (Table 1). Preparation of Coumarin derivatives (III_{b-d}) was repeated using 75% sulfuric acid instead of (PPA). (table 1).

Reaction of Compounds (III_a) and (III b-g) with primary and secondary amines

General Procedure

A mixture of compound (III) (4 mmol) and appropriate amine (16 mmol) in dimethyl formamide (25ml) was refluxed for 30 minutes, cooled and poured over water (100 ml), the solid product collected, dried and crystallized from Hexane – Ethanol to give the desired compounds (IV_{a-d}) and (V_{a-q}) in good yields (table 3 and 4).

RESULTS AND DISCUSSION

Coumarin derivatives (III_{a-g}) were prepared from the reaction of ethyl 2- chloro (I_a) or 4- chloro aceto acetate (I_b) with substituted phenols (II_{a-r}) in the

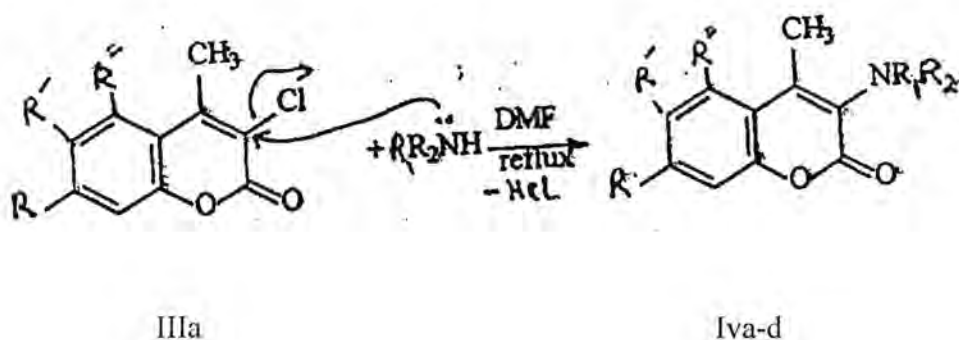
presence of polyphosphoric acid (PPA) or 75% sulfuric acid a condensing agent as shown in scheme -1-.

The structure of these compounds (III_{a-g}) was elucidated by spectral data (IR, UV) and CHN analysis. The IR spectral data for compounds (III_{a-g}) in general for the regions 1695-1730 cm^{-1} and 1610-1640 cm^{-1} were attentively attributed to VC=O and VC=C respectively.

Other characteristic strong band in region 720-760 cm^{-1} correlated to VC-CL (Table 2), the chemical reactivity of the halo group at position (3) and allylic halo group (i.e 4-CH₂X) in compounds (III_a) and (III_{b-g}) respectively plays an important role in using these compounds as good synthons in a way to study their reactions with nucleophiles. In order to give the comprehensive study of this type of nucleophilic reactions. We selected many primary amines depending on this for the basicity of amine used (Table 3). Scheme -1-.

The structure of these compounds (IV_a and V_{a-g}) were elucidated by IR, UV and CHN analysis, the IR spectral data showed strong absorption bands in the regions 1695-1738 cm^{-1} , 1600-1640 cm^{-1} and 1380-1390 cm^{-1} which correlated to VC=OVC=C and VC-N respectively (Table 3).

Furthermore, we have extended our study to secondary amines (Table 4). Compounds (IV_{b-d} and V_{b-q}) in IR showed a strong characteristic bands to C=O,C=C and C-N in the regions 1695-1750 cm^{-1} , 1610-1638 cm^{-1} and 1330-1390 cm^{-1} respectively.



Mechanism of the displacement of Cl of position 3 in compound (III_a) by R₁R₂NH.

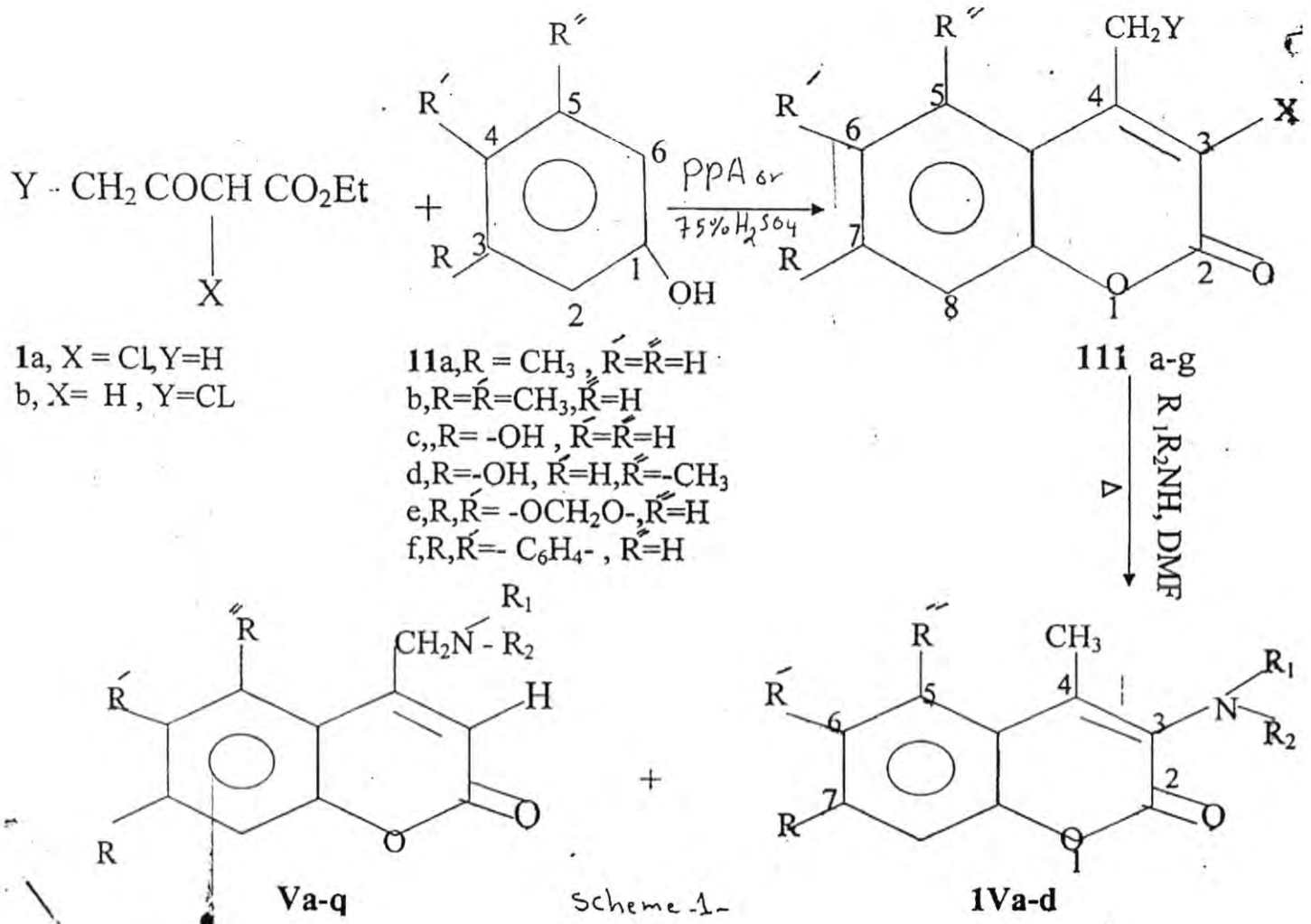


Table -1- Some physical properties for compound (IIIa-g)

Compound No.	Substituent					Yield %	Yield % 75% H ₂ SO ₄	m.p°C EtOH- water	C.H. Nanlysis Calc./found	
	R	R'	R''	X	Y				C	H
IIIa	CH ₃	H	H	Cl	H	85	-	134-136	68.37 69.02	4.32 4.62
b	CH ₃	H	H	H	Cl	94	58	213-215	63.31 63.96	4.32 4.28
c	CH ₃	CH ₃	H	H	Cl	94	55	226-228	64.72 65.04	4.94 5.30
d	OH	H	H	H	Cl	93	63	183-185	57.00 56.60	3.33 3.64
e	CH ₃	H	OH	H	Cl	93	-	176-179	- -	- -
f	-OCH ₂ O-		H	H	Cl	93	-	249-251	55.34 55.40	2.90 3.04
g	-C ₆ H ₄ -		H	H	Cl	77	-	158-160	68.71 69.02	3.68 3.34

Table 2 . IR and UV spectral data for compounds (III a-g)

Compound No.	IR vcm ⁻¹			UV
	C=O	C=C	C-Cl	(methanol λ _{max} (nm))
IIIa	1735	1630	730	217,282,319
b	1730	1620	725	215,277,312
cc	1730	1638	725	219,278,313
d	1695	1625	730	218,293,325
e	1695	1610	730	210,256,324
f	1710	1625	760	211,234,264 295,314
g	1740	1640	720	270,298,319

Table 3. Physical and speretal for compounds (Iva and Va-g)

Com. No.	Substituents				Yield %	M.P ^o C Hexane-Ethanol	CHN Calc. / Found		
	R	R'	R''	NR ₁ R ₂			C	H	N
Iva	CH ₃	H	H	Benzyl amine	48	115-117	74.42 77.82	6.09 6.96	5.02 4.96
Va	CH ₃	H	H	2-amino benzo thiozole	50	178-180	67.08 66.86	4.34 4.14	8.695 9.24
B	CH ₃	CH ₃	H	2-amino benzo thiozole	53	145-147	67.85 68.21	4.76 4.64	4.17 4.34
C	CH ₃	H	H	Benzyl amine	52	139-141	77.42 77.00	6.1 5.66	5.02 4.66
D	CH ₃	CH ₃	H	2-amino thiozole	48	92-94	62.93 63.15	4.89 4.69	9.76 9.52
E	OH	H	H	2-amino thiozole	40	219-221	56.93 57.20	3.65 3.44	10.22 10.26
F	CH ₃	CH ₃	H	2-amino pyridine	43	100-102	72.80 73.10	5.71 5.88	10.00 9.66
G	OH	H	H	2-amino pyridine	42	216-218	67.16 66.82	4.48 4.40	10.45 10.23

Continued table 3

Com. No.	IR cm ⁻¹	UV Methanol λ _{max} (nm)		
		C=O	C=C	C-N
Iva	1738	1625	1390	220,270,315
Va	1705	1618	1360	209,268,309
B	1708	1640	1385	212,273,311
C	1700	1620	1380	210,269,310
D	1710	1620	1390	215,271,312
E	1695	1620	1380	225,263,321
F	1710	1610	1380	226,264,290,320
G	1695	1600	1380	225,280,316

Table 4. Physical and spectral for compounds (IV b-d and Vh-q)

Compounds No.	Substituents				Yield %	M.P°C Hexane-Ethanol
	R	R'	R''	NR ₁ R ₂		
Ivb	CH ₃	H	H	Pipridine	60	103-105
c	CH ₃	H	H	Morpholine	60	149-151
d	CH ₃	H	H	Diethylamine	58	151-153
Vh	CH ₃	H	H	Morpholine	69	138-140
i	CH ₃	CH ₃	H	Morpholine	68	158-160
j	OH	H	H	Morpholine	52	140-142
k	OCH ₂ O		H	Morpholine	50	185-188
l	CH ₃	H	H	Piperidine	75	80-82
m	CH ₃	CH ₃	H	Piperidine	73	118-120
n	CH ₃	H	OH	Piperidine	50	191-192
o	OCH ₂ O		H	Piperidine	52	158-160
p	CH ₃	CH ₃	H	Diethylamine	50	98-100
g	C ₆ H ₄		H	Diethylamine	51	176-178

Continued table 4

Compounds No.	CHN Cal./Found	IR cm ⁻¹				UV Methanol λ _{max} (nm)	
		C	H	N	C=O	C=C	C-N
Ivb	74.71 74.34	7.40 6.88	5.45 6.02	1750	1620	1390	212,284, 317
c	69.49 69.84	6.56 6.04	4.45 5.84	1740	1618	1380	217,288, 317
d	73.46 73.22	7.76 7.34	5.71 5.30	1738	1620	1390	220,282, 318
Vh	69.50 70.12	6.56 6.74	5.41 5.28	1720	1630	1380	218,277, 307
i	70.33 69.69	6.96 7.03	5.13 5.25	1710	1620	1330	209,282, 311
j	64.37 67.50	5.75 5.26	5.36 5.84	1715	1620	1397	230,260, 294,304, 321
k	62.28 63.20	5.19 5.13	4.84 4.46	1717	1620	1380	210,280, 329
l	74.13 73.92	7.75 7.18	5.17 5.57	1710	1620	1370	220,279, 309

m	75.28	7.75	5.17	1718	1628	1390	215,281, 312
	75.10	7.95	5.54				
n	70.32	6.95	5.13	1795	1610	1390	209,282, 300
	70.82	7.28	5.34				
o	66.90	5.92	4.88	1700	1620	1370	211,233, 287,328
	67.20	5.86	4.36				
p	73.28	9.16	5.34	1720	1638	1397	210,279, 309
g	-	-	-	1710	1625	1385	209,280, 309

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Photooxidative Degradation of Fungicide "Benomyl" in Aqueous TiO₂ Suspension

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الخلاصة

تم خلال البحث الحالي دراسة الأكسدة الضوئية لمبيد الفطريات "البينوميل" في المحلول المائي المعلق لثاني أكسيد التيتانيوم. تم متابعة النواتج الأولية والنهائية لعملية التجزئة والأكسدة الضوئية بتقنيات كروماتوغرافيا العالي الكفاءة (HPLC)، مطيافية الأشعة فوق بنفسجية والمرئية، قياسات الحامضية و التوصيلية الكهربائية للمحلول. تم دراسة تأثير درجة الحرارة على نظام التفاعل وكانت طاقة التنشيط واطنة جداً (15.02 kJ/mol) مما يدل على أن التفاعل الضوئي لايعتمد على درجة الحرارة. ان رتبة تفاعل التجزئة الضوئية للبينوميل كانت من الدرجة الأولى وتم تعيين ثابت التجزئة الضوئية (K_d). كما وتم حساب قيم منتج الكم الظاهري لعملية الأكسدة الضوئية بتراكيز مختلفة من ثاني اوكسيد التيتانيوم و وجد بأن هذه القيم تتأثر بحامضية المحلول (pH). وعلى ضوء النتائج التجريبية تم إقتراح ميكانيكية التفاعل للأكسدة الضوئية لمادة البينوميل تحت ظروف التجارب التي أجريت خلال البحث.

ABSTRACT

The photooxidative degradation of fungicide "Benomyl" in aqueous TiO₂ (anatase) suspension is studied. The primary photolytic products were followed and identified by HPLC, solution pH, conductivity and UV-visible spectrophotometry. The temperature effect on the photodegradation process is also carried out. The activation energy deduced is very low (15.02 kJ mol⁻¹). It was found that the order of the benomyl photooxidation is first order. The apparent quantum yield is also determined with different TiO₂ loading and this is found affected by solution pH. According to the experimental results and kinetic analysis the reaction mechanism of the benomyl photodegradation os suggested.

INTRODUCTION

One of the most hazardous environmental pollutants (soil, water and sometimes air) are pesticides which are widely used in agriculture during the last 50 years. Both non-persistent and persistent pesticides create severe problems to environment since they are highly toxic human, animals and plants.

The first clear recognition and implementation of TiO₂ semiconductor sensitizer for pesticides (or generally organic compounds) pollutants oxidative degradation (mineralization) came with the work of Ollis and coworkers in 1983^(1,2). In 1980's and 1990's a great deal of research appeared in literature concerning the photooxidative degradation in homo- and heterogeneous catalysis system of pesticides including halogenated phenols, halogenated acetic acid, organic phosphorus compounds... etc. in aqueous system (for review see reference⁽³⁾ and references therein).

Recently, Aliwi and Abdul Kadir gave detailed kinetic and mechanistic studies of the photomineralization of insecticide carbaryl⁽⁴⁾ and herbicide propanil⁽⁵⁾ using TiO₂ (anatase) aqueous suspension, using UV or solar radiation.

Benomyl [methyl 1- (butyl carbamoyl) -2- benzimidazole carbamate] is widely used in Iraq as fungicide with systemic activity and effectiveness on a wide range of fungi affecting fruits, vegetables and field crops. Reports in literature reveal that benomyl is hydrolyzed in water to methyl -2- benzimidazole carbamate (MBC)⁽⁶⁾. No reports have been detected for the photochemical behaviour of benomyl in aqueous solution. However, Flecker and Lacy⁽⁷⁾ have reported in 1977 the photochemical activity of the benomyl hydrolysis intermediate (MBC) in aqueous solution in the presence of acetone or riboflavin as photosensitizer. Guanidine carbomethoxy urea and carbomethoxy guanidine are the main photolytic products.

In the present work it is intended to investigate the main parameters affecting the photodegradation of fungicide benomyl and photomineralization of this substance using UV-TiO₂ (anatase) aqueous suspension photolysis system.

Experimental

Techniques

Photolysis procedure and apparatus: the UV beam of light is generated from 150 Watt medium mercury lamp (PHYWE/ England) which gives main light intensity at wavelength 365 nm. The photolysis experiments were carried out in a photolytic Pyrex cell (capacity 35 cm³) with Pyrex window cell (capacity 35 cm³) with Pyrex window of 2cm in diameter. The cell is fitted with water jacket for temp. control. A magnetic stirrer was used to maintain the solution in homogeneous suspension from through the photolysis process. The photolytic cell

is located 20cm apart from the lamp and the solution temp. is controlled by circulation thermostat type Haake FE2.

Titanium dioxide load ranges from 0.5 to 4gm/l was added to the aqueous solution containing known conc. Of carbaryl (c.g. 4ppm). The solution is then saturated with oxygen gas during experiments with flow rate of 10cm³/min. Magnetically stirred mixtures in the photoreactor were irradiated and 2cm³ of the sample were taken at various irradiation time intervals and then centrifuged to separate TiO₂ solid. The samples were analyzed by high performance chromatographic technique, UV-visible spectrophotometry, pH and conductivity measurements. Phosphate buffer was used to control the solution pH in the range from 4-7.

High performance liquid chromatography (HPLC): benomyl conc. Was determined by HPLC using Shimatzu 6A instrument. This was equipped with UV-detector (wavelength = 254nm). The mobile phase used is methanol/water mixture (65:35 v/v). The analytical column type C18 used was 25cm in length and 4.6mm in diameter. Under these conditions the retention time of benomyl was 4.2min.

Other analysis techniques:

- a- The pH measurements: solution pH was measured before and after irradiation using Orien SA 752 pH meter.
- b- conductivity measurements: the solution conductivity was measured by WTW conductivity meter type LF191. The cell constant is 0.1cm⁻¹.
- c- Nitrate conc. Measurement: The NO₃⁻ conc. Was determined quantitatively by ion chromatographic technique using Dionex 16USA ion chromatographic instrument. 0.025M sodium carbonate and 0.025 M sodium bicarbonate solution mixture is used as diluent. Qualitative measurements of NO₃⁻ were also carried out using Orien ion selective electrode. NaNO₃ (AR grade) is used as standard solution for calibration.
- d- Spectrophotometry: the UV-visible spectral changes of benomyl before and after different time of irradiation was monitored by a double beam Hitachi 2000 spectrophotometer.
- e- Incident light intensity at wavelength 365 nm was measured by the usual ferrioxalate actinometry technique (Hatchard and Parker method⁽³⁾)

Chemicals

Titanium dioxide powder type anatase was supplied by Fluka AG. The surface area is 18.11m²g⁻¹. It was preheated for 12 hr. at 200°C before it was sieved through 400 mesh size sieve.

Benomyl [methyl 1-(butyl carbamoyl)-2-benzimidazole carbamate] (C₁₄H₁₈N₄O₃). This fungicide is supplied by E.I. Dupont de Nemours company of

purity 99.9% as a white crystalline solid. Benomyl is sparingly soluble in water (maximum solubility is 4mg/l, 4ppm at 25°C) soluble in chloroform and alcohols. Decomposes when treated with strong acids or alkalis and also decomposes on heating without melting.

Nitrogen and oxygen gases were supplied by Al-Mansour factory-Baghdad fo Purity ~99%. These gases were passed through bidistilled water before passing through benomyl-TiO₂ emulsion.

RESULTS AND DISCUSSION

It is now generally accepted that anatase photocatalyst, water and dissolved oxygen gas (or air) are the main parameters that bring about the photooxidative degradation of organic molecule on the surface on the photocatalyst. However, reports in literature⁽⁸⁾ reveal that there are several factors generally affect the rate of the photocatalytic degradation of organics. These factors are: TiO₂ loading, solution pH and the initial conc. Of the organic substrate. Therefore, several experiments were carried out in order to reach the optimum conditions for each of these parameters.

Many experiments were executed using different TiO₂ load (ranged from 0.5 to 4gm/l) keeping other parameters constant. Results show that the optimum TiO₂ load is 2 gm/l for the highest rate of photodegradation of benomyl (initial conc. Is 4ppm). This TiO₂ load is considered the optimum conc. Used for all other photolysis experiments. The effect of TiO₂ load in photooxidation process of organic compounds is extensively explored by Serpone⁽⁹⁾ in which the TiO₂ suspension becomes more opaque to the incident light and therefore the light absorption will be limited to the first layers of the photolytic mixture and the rest of the solution layers don't receive light.

Benomyl is slightly soluble in water with maximum solubility at 25°C is 4ppm. Therefore, initial conc. Of benomyl ranged from 1-4ppm is used to predict the optimum initial conc. That gives higher rate of decomposition. However, the experiments show that in very low conc. Of benomyl, i.e. less than 2ppm, any quantity of TiO₂ is enough for photodegradation of benomyl, i.e. there is no optimal conc. Value of TiO₂ is such experiments. It is generally found that higher initial conc. Of the organic substrate needs higher surface area of the catalyst and accordingly higher catalyst load^(3,5). It is known that benomyl is hydrolyzed in aqueous solution to 2-benzimidazole carbamate (MBC) at pH more than 7. Therefore, the rate of photodegradation is measured at pH range from 4 to 7. The optimum solution pH is found 6.45 using phosphate buffer. Therefore, all photodegradation experiments of benomyl were carried out using 4ppm initial

conc. (1.377×10^{-5} mol/l), 2gm/l as TiO_2 load, pH is 6.45 and oxygen flow rate is 10ml/min. at 25°C .

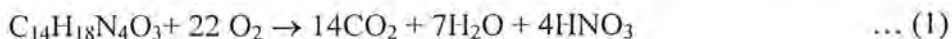
The identification of the photodegradation products have been carried out using similar techniques utilized in previous studies for the photodegradation of carbaryl⁽⁴⁾ and propanil⁽⁵⁾.

The UV-visible absorption spectrum of benomyl in water has shown several absorption peaks in the spectral range from 230 to 340nm. The highest absorption peak is located at 283nm ($\epsilon_{\text{max}}=40100 \text{ L mol}^{-1} \text{ cm}^{-1}$). Three absorption shoulders at 242, 270, 293nm were also appeared. Results shown in fig.1 illustrate the absorption spectrum of benomyl in water before and after several times of irradiation. The spectral changes show that the maximum absorption band for benomyl at 283 nm decreases in intensity with irradiation time. Two isosbestic points were appeared at 296 and 265nm and these are then disappeared after 30 min. irradiation. This might suggest that during photolytic degradation of benomyl, only one product could be produced and after 2hr. irradiation the spectrum of benomyl disappeared showing no characteristic bands in the UV-visible spectral region (250-350nm).

High performance chromatography (HPLC) technique is used to identify the photolytic product and also to monitor the change in benomyl conc. During the photolysis experiments. The benomyl concentrations were monitored by following the peak intensity at retention time of 4.1 min. (benomyl peak). After 10min. irradiation new peaks appeared at retention times 2.66 min. and 1.64min. Figure 2 shows the variation of the HPLC chromatogram during the photolysis process. It is clear that the peak belongs to benomyl disappeared after about 2 hr. of irradiation. The 2 peaks appeared at retention times 2.66 and 1.64min. are increased in intensity during the first 10 min. irradiation and then gradually disappeared after prolong irradiation (about 2hr.). This suggests that the primary photolytic product of benomyl decomposition are ultimately photodegraded on TiO_2 surface.

Acidity and conductivity were also measured during the photodegradation of benomyl on TiO_2 surface. It is found that the solution pH is decreased from 6.45 to 5.20 and the conductivity is increased from $11.2\mu\text{s}$ to $92\mu\text{s}$ at 25°C , this could be explained by the formation of highly conductive species such as H^+ and NO_3^- ions during the photocatalytic oxidative degradation process.

The stoichiometric amounts of nitrate ion that could be formed for 4ppm initial benomyl conc. Is 3.41ppm according to the stoichiometric equation (1):



The detection of nitrate ion generated during photolysis is carried out using ion-chromatography technique. At the end of irradiation period (about 6hr) the ion chromatogram show only one peak of the nitrate ion.

Small amounts of carbon dioxide were also detected using barium hydroxide solution and the white precipitate of barium carbonate is generally increased with irradiation time. No quantitative analyses for CO₂ liberation in solution were carried out.

Kinetic Analysis

The initial rate of the photocatalytic degradation of benomyl on TiO₂ surface was determined spectrophotometrically by following the change in the absorbance at $\lambda=280\text{nm}$. The logarithm plot of the change in absorbance (i.e. benomyl conc.) with irradiation time give a straight line which indicates the first order reaction. The slope of the straight line therefore gives the value of the specific rate constant (k_d) of benomyl which is equal to $3.33 \times 10^{-4} \text{ sec}^{-1}$ using the initial benomyl conc. 2ppm, TiO₂ load 2gm/l and pH=6.45 at 25°C. The same value of k_d was obtained when the benomyl initial conc. is 4ppm under similar experimental conditions. The results indicate the k_d is independent on the initial conc. Which is in contrast to that obtained for carbaryl⁽⁴⁾ and propanil⁽⁵⁾ pesticides on TiO₂ surface under similar conditions. However, addition of $2 \times 10^{-5} \text{ mol/l}$ of H₂O₂ enhances the photodegradation process which is in agreement with that found for the photodegradation of carbaryl and propanil^(4,5). This could suggest that hydroxyl radical, produced from the decomposition of H₂O₂ on TiO₂, is the initiator for the photodegradation of benomyl.

The photodegradation of benomyl on TiO₂ surface was also studied at different temperatures ranged from 289 to 338K. Figure 3 shows the Arrhenius plot between logarithm of specific rate constant of photodegradation ($\ln k_d$) and reciprocal of reaction temp. The straight line obtained in fig. 3 gives the value of the activation energy (15.02 kJ/mol or 3.59 kcal/mol). This very low activation energy value recalls the fact that the photocatalytic process is temp. independent.

The apparent quantum yield^(3,9,10) was also determined and found to be affected by TiO₂ load. Table 1 shows the variation of apparent quantum yield for the photooxidative degradation of benomyl is steadily increasing with increasing TiO₂ load up to 2.0gm/l and after that become constant.

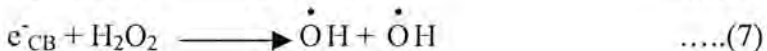
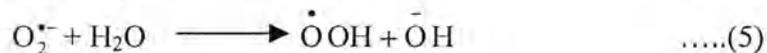
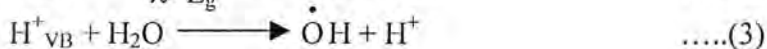
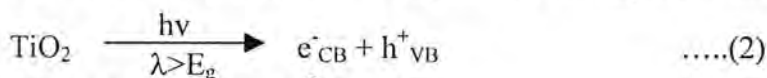
Table 1: Apparent quantum yield at different TiO₂ loading. Initial benomyl conc. Is 4ppm (1.377x10⁻⁵ mol/l) at 25°C and pH=6.45

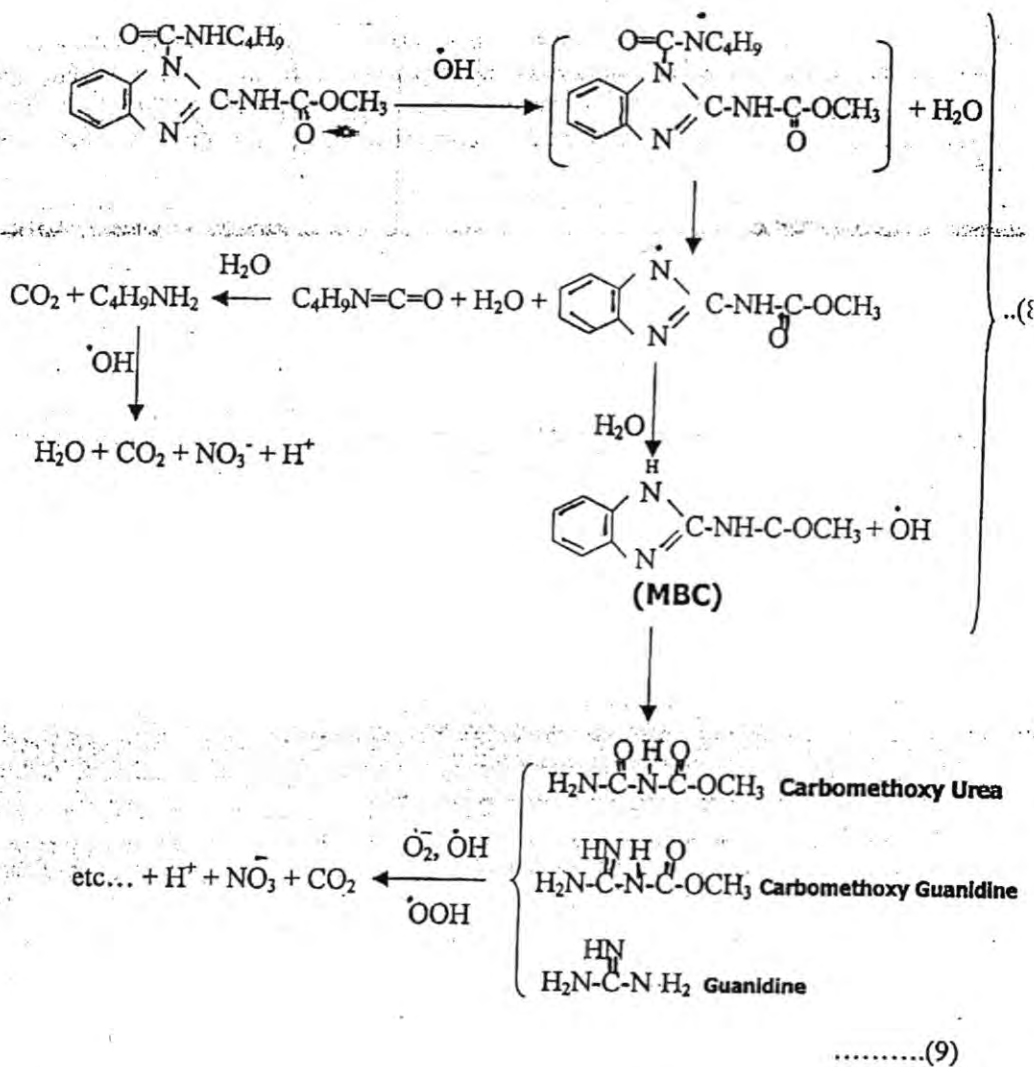
TiO ₂ load Gm/l	0.85	1.42	2.0	2.85	3.42	4.0
Φ	0.125	0.134	0.149	0.148	0.147	0.147

The apparent quantum yield was also found to be affected by solution pH. It is equal to 0.215 and 0.135 at pH 5 and 6 respectively. Therefore, the apparent quantum yield increases as pH value decreases.

The proposed reaction mechanism

Reports in literature reveal that the mechanism of photooxidative degradation of organic compounds, such as surfactant⁽¹¹⁾ and pesticides⁽¹²⁾ on TiO₂ surface is often very difficult task and a detailed pathways of the photoreaction can't be easily interpreted. According to the experimental results and the kinetic studies already discussed one might suggest the following reaction scheme for the photooxidative degradation of fungicide benomyl:





The products of photolytic decomposition of MBC shown in equation (9) is suggested according to Flecker and Lacy⁽⁷⁾ and these might completely mineralize on TiO₂ surface to CO₂, nitrate ion... etc.

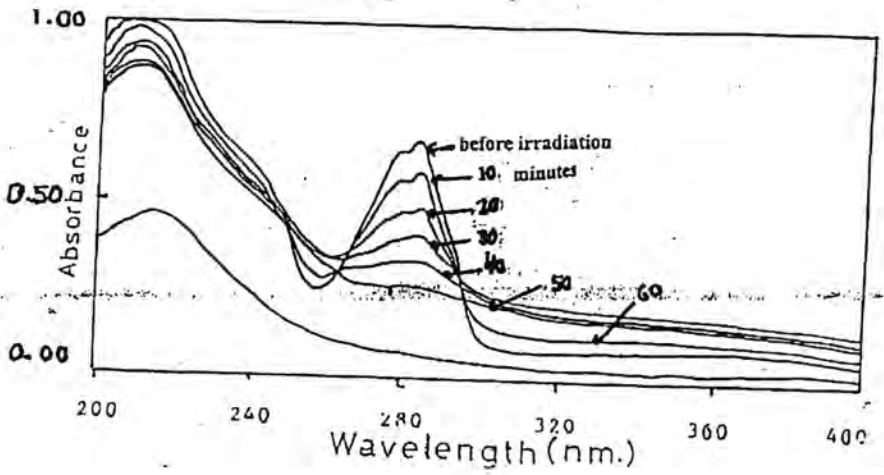


Figure (1)

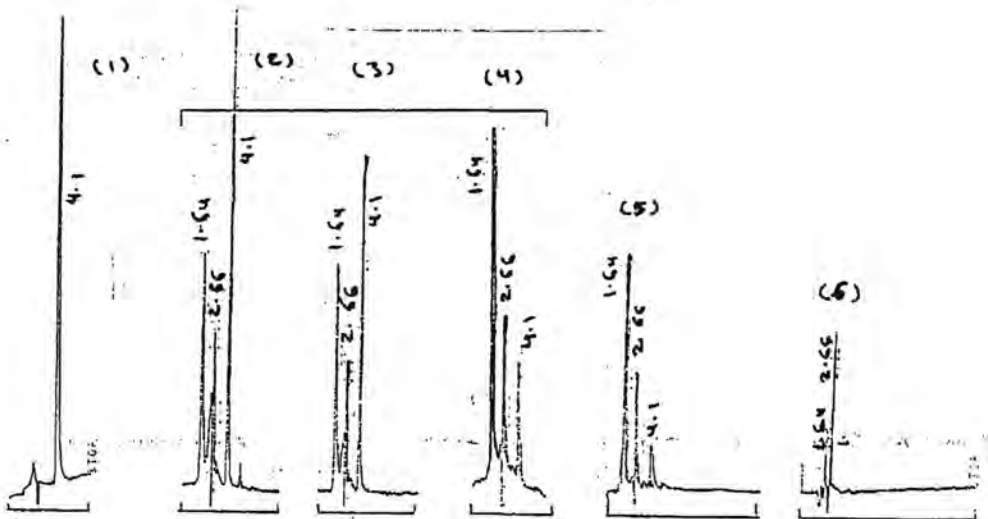


Figure (2)

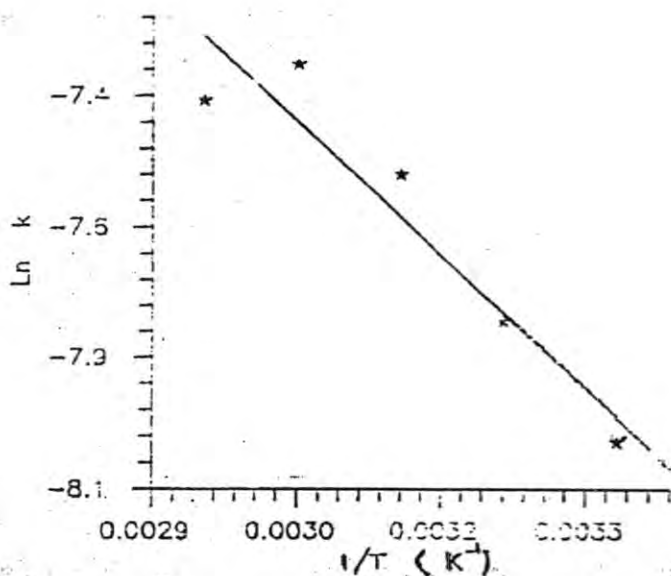


Figure (3): Arrhenius - plot for the degradation of benomyl (4ppm) on irradiated TiO₂ (2gm/l)

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A Study of The Extraction Beam From A Modified Bernas Ion Source

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الخلاصة

من خلال تطبيق جهد سالب نسبة الى جهد البلازما V_{EXT} بين شق المصدر وقطب الاستخراج، اجريت مجموعة من التجارب لدراسة تيار الاستخراج I_{EXT} وتيار حزمة الايونات I_{beam} لغاز الاركون من المصدر الايوني برناس المحور. أن حزمة الايونات المستخرجة عند طاقة 12 keV قد درست تحت تأثير الظروف التشغيلية الاساسية للمصدر الايوني وموقع قطب الاستخراج نسبة الى شق الانود. أظهرت النتائج ان أفضل مسافة لقطب الاستخراج من شق الانود هي 20mm إذ يكون التيار الايوني الكلي 1.25mA وجهد استخراج 12kV

ABSTRACT

Through the application of a negative potential with respect to the plasma potential V_{EXT} between the slit of the source and extraction electrode, a set of experiments were conducted to study the extraction current I_{EXT} and the extraction ion beam I_{beam} of Argon ions from a modified Bernas ion source. The extracted ion beam of 12 keV was studied under the effect of the main operational conditions of the ion source and the position of the extraction electrode with respect to the anode slit. The results of this study have shown that the best distance for the extraction electrode from the anode slit was 20 mm with a total ion extraction current of 1.25 mA and an extraction voltage of 12 kV .

INTRODUCTION

It is well known that, numerous parameters are involved in the operation and performance of the ion source. These parameters affect indirectly the beam divergence through the variation of the ion current density emitted from the ion source. The purpose of this paper is to present the results of an experimental study of the parameters related to the extraction system for a modified Bernas ion source, such as, extraction voltage, interelectrode distance, effects of the main operational conditions of the ion source and electrode geometry factor⁽¹⁾.

It is shown that the beam divergence is most conveniently controlled by the adjustment of the extraction electrode distance. This type of this source is utilized in various fields, e.g. electromagnetic isotope separation and ion implantation^(1,2,3).

Experimental investigation of extraction beam parameters

The present source and the extraction system, fig. (1) consist of

- A cathode (Filament) (1), made of Tungsten wire of 1mm diameter curved in a "V" shape.
- An arc chamber (anode pole) (2), made from Graphite of dimensions $(83 \times 34 \times 34) \text{mm}^3$ with rectangular aperture $(1 \times 40) \text{mm}^2$ for lateral extraction of ions.
- An extraction electrode (6), is made from Graphite material with rectangular aperture $(2 \times 40) \text{mm}^2$ for lateral extraction of ion.
- An Aluminum plate (7), used for measuring the ion beam current I_{Beam} , the dimensions of which are $(65 \times 85) \text{mm}^2$ at a distance of 15 mm from the extraction electrode. The operating characteristics for the ion source were investigated in ref⁽⁴⁾ and Argon gas was used as a feeding material.

Extraction voltage V_{EXT}

As the extraction V_{EXT} increases, the total ion current I_t increases where

$$I_t = I_{\text{EXT}} + I_{\text{Beam}} \quad \dots(1)$$

Where

I_{EXT} : The ion current measured on the extraction electrode

I_{Beam} : The ion current measured on the Aluminum plate.

Fig. (2) shows a family of curves of I_{EXT} and I_{Beam} as a function of V_{EXT} for different values of arc voltage V_{arc}

Extraction distance d

To determine the appropriate distance d between the extraction electrode and the slit of ion source, the effect of V_{EXT} as a function of I_{EXT} and I_{Beam} for various values of d (10, 15, 20, 25, 30 mm) was studied. Simultaneously, the influence of d on I_{EXT} and I_{Beam} was observed. Figs. (2 to 6) show that as f increases, the values of I_{EXT} decrease which is a normal behavior. However Fig (7) shows that at $V_{\text{EXT}} = 12\text{kV}$, the maximum value for I_{Beam} can be obtained at $d = 20$ mm which in agreement with Chavet results⁽¹⁾. The measured current on the extraction electrode and Aluminum plate must be corrected by applying eq. (2) and eq. (3)⁽⁵⁾.

$$I_{EXT} = \frac{I_X}{1 + \gamma_{sc(Gr)}} \quad \dots(2)$$

$$I_{Beam} = \frac{I_S}{1 + \gamma_{sc(Al)}} \quad \dots(3)$$

where

I_X : is the measured current on the extraction electrode.

I_S : is the measured current on the Aluminum plate.

$\gamma_{sc(Gr)}$: coefficient of secondary emission for Graphito (0.109 at $V_{EXT} = 12kV$)⁽⁶⁾

$\gamma_{sc(Al)}$: Coefficient of secondary emission for Aluminum (0.17 at $V_{EXT} = 12kV$)⁽⁷⁾.

For the best operational conditions of the system, the α value, which is the ratio of I_{EXT} to I_{Beam} , must be as small as possible without affecting the value of I_{Beam} . The values of I_{EXT} and I_{Beam} before and after the corrections and the values of (α) for different values of the distance d at $V_{EXT} = 12kV$ are shown in table (1)

Effect of the main operational condition of the source on the extraction ion beam

Effect of arc voltage V_{arc}

The values of the current I_{EXT} and I_{Beam} increases as the arc voltage V_{arc} increases as shown in Figs (2 to 6). However, it was found that there was no increase in the values of I_{EXT} and I_{Beam} when V_{arc} is higher than 55V, which is due to the fact that I_{arc} approaches its saturation region⁽⁴⁾.

Effect of gas pressure P_{Ar}

The gas pressure P_{Ar} is related to the chamber pressure P by the relation $P_{Ar} = 50.42 P$ ⁽⁴⁾. The extraction ion current I_{EXT} increases as P increases. However, when P reaches the value 7×10^{-4} mbar the value of I_{EXT} and I_{Beam} increases rapidly then begin to decreases as shown in Fig. (8). This is due to the low generation of ions at such values of pressure.

Effect of filament current I_f

The total extraction ion current increases as the filament current I_f increases as shown in Fig. (9) at various values of the pressure.

Effect of Magnetic Field B

The magnetic field B is measured at half distance between the poles of the electromagnet along the cathode – anode axis of the ion source. Maintaining all parameters (extraction and source) constant, the application of the magnetic field causes the total extraction ion current to increase till it reaches its maximum value at B = 15 mT and then begin to decrease as shown in Fig. (10) which is in agreement with Chavet⁽⁸⁾.

Effect of arc current I_{arc}

The influence of the arc current I_{arc} on the extraction current I_{EXT} is clearly demonstrated in Fig. (11-a). The values of I_{EXT} increase as the arc current increases. Similar behavior was found for I_{Beam} current as shown in Fig. (11-b).

Determination of geometry factor C_e

This factor is an important one to determine the design of the extraction electrode and the slit of the ion source to obtain a high quality beam optics. When the beam optics are optimized, the total extraction ion beam current I_t at the extraction voltage V_{EXT} can be approximately given by the following Child – Langmuir relation for space – charge limited extraction (slit apertures)^(9,10).

$$I_t = \frac{4}{9} A \epsilon_0 \left(\frac{2e}{M} \right)^{1/2} \frac{V_{EXT}^{3/2}}{d^2} \quad \dots(4)$$

Where

- A : area of ion source slit.
- ϵ_0 : permittivity of free space.
- e : the electron charge.
- M : The effective mass of ion
- d : extraction gap distance

The experimental results differ from the theoretical values calculated with the aid of eq. (4). Therefore, the geometry factor C_e can be defined as^(1,11)

$$C_e = \frac{I_{I(exp)}}{I_{I(the)}} \quad \dots(5)$$

- $I_{I(the)}$: The total extraction ion current calculated from eq. (4).
- $I_{I(exp)}$: The total extraction ion current obtained from experiments.

In table (2), the values of C_e for different values of d , at $V_{EXT} = 12$ kV were calculated. It can be seen that its value approaches unity at $d = 20$ mm. At this distance the experimental and theoretical values are of similar order as shown in Fig. (12).

CONCLUSION

Several experiments were carried out to study the parameters related to the extraction ion beam from a modified Bernas ion source. It was found that at the best operational conditions are.

$I_{arc} = 1.5$ A, $V_{arc} = 55$ V, $I_f = 55$ A, $P = 6 \times 10^{-4}$ mbar and $B = 15$ mT.

The total extraction Argon ion beam current reached 1.25 mA at $V_{EXT} = 12$ kV and $d = 20$ mm with stable operation for ion source.

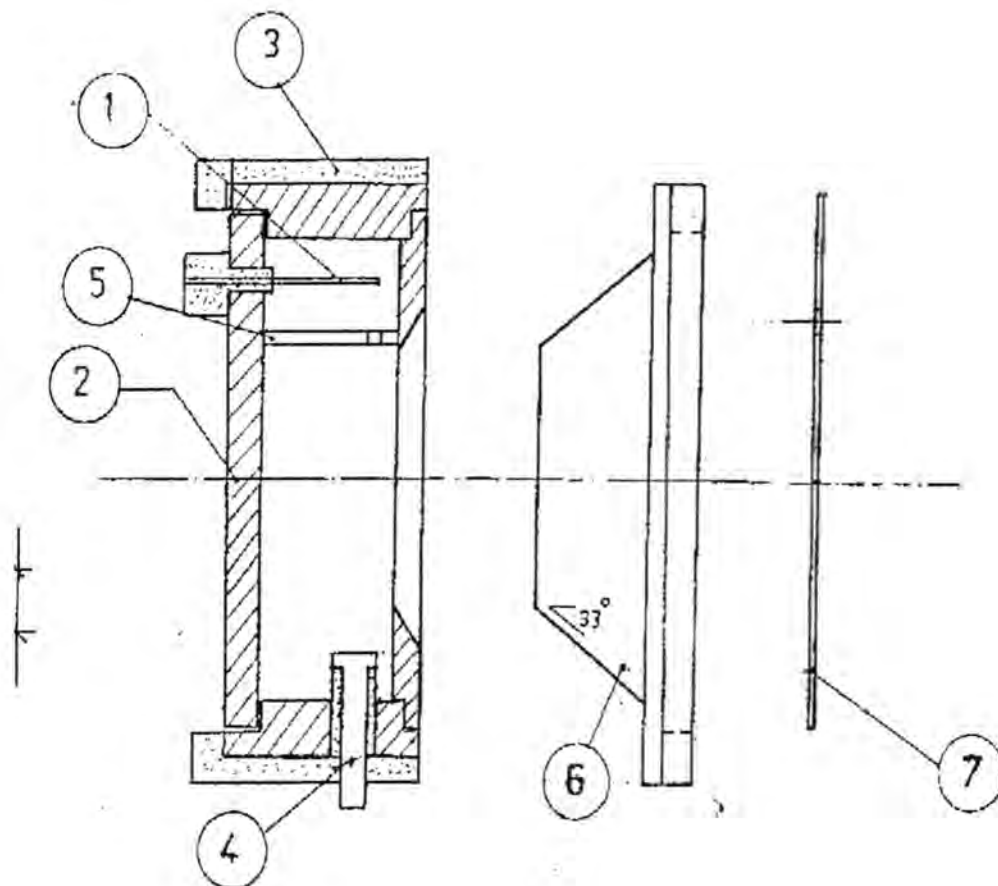
According to these results, the electrode geometry has been determined. It was found that $C_e \approx 1$ at $d = 20$ mm at which the values of the total extraction ion beam current was in agreement with the theoretical values.

Table (1) : The values of I_{EXT} , I_{Beam} before and after correction and α for different values of d at 12 kV

d (mm)	I_x (mA)	I_s (mA)	I_{EXT} (mA)	I_{Beam} (mA)	α
10	2.9	187×10^{-3}	2.615	160×10^{-3}	16.34
15	1.8	210.5×10^{-3}	1.63	180×10^{-3}	9
20	0.96	444.5×10^{-3}	0.87	380×10^{-3}	2.28
25	0.72	298.3×10^{-3}	0.65	255×10^{-3}	2.54
30	0.49	284.3×10^{-3}	0.45	243×10^{-3}	1.85

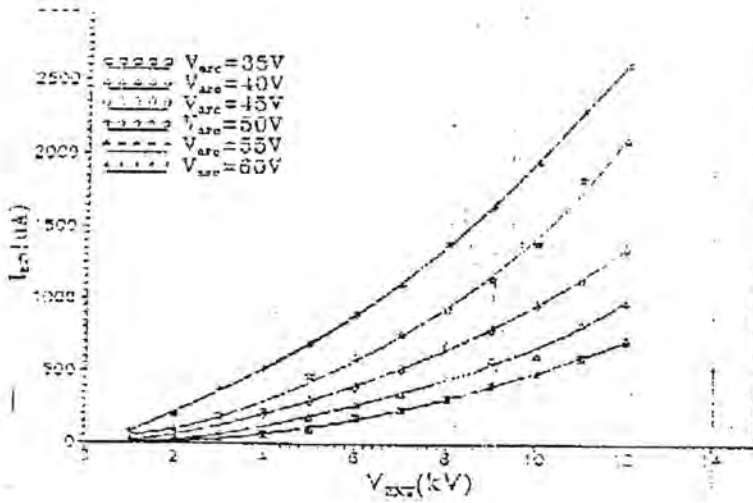
Table (2) : The values of C_e for different values of d at $V_{EXT} = 12$ kV

d (mm)	$I_{(the)}$ (mA)	I_{EXT} (mA)	I_{beam} (mA)	$I_{(exp)}$ (mA)	C_e
10	5.28	2.615	160×10^{-3}	2.77	0.52
15	2.41	1.63	180×10^{-3}	1.81	0.75
20	1.35	0.87	380×10^{-3}	1.25	0.91
25	0.86	0.65	255×10^{-3}	0.905	1.03
30	0.6	0.45	243×10^{-3}	0.7	1.166

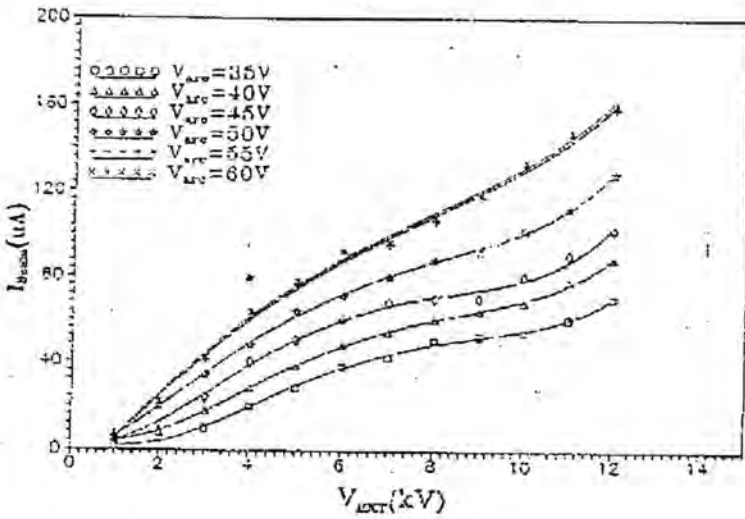


NO.	DESCRIPTION	MATERIAL
1	FILAMENT	TUNGSTEN
2	ARC CHAMBER	GRAPHITE
3	INSULATOR	BORON NITRIDE
4	PROBE	GRAPHITE
5	DEFINING SLOT	GRAPHITE
6	EXTRACTION ELECTRODE	GRAPHITE
7	ALUMINUM PLATE	ALUMINUM

Fig. (1) A schematic drawing of a modified Bernas ion source and extraction system .



-a-



-b-

Fig. (2) A graph of the ion current as a function of the extraction voltage for different values of the arc voltage .

$I_f = 55 A$, $P=6 \times 10^{-4} mbar$, $B=15mT$, $d = 10 mm$.

a - For extraction ion current.

b- For ion beam current .

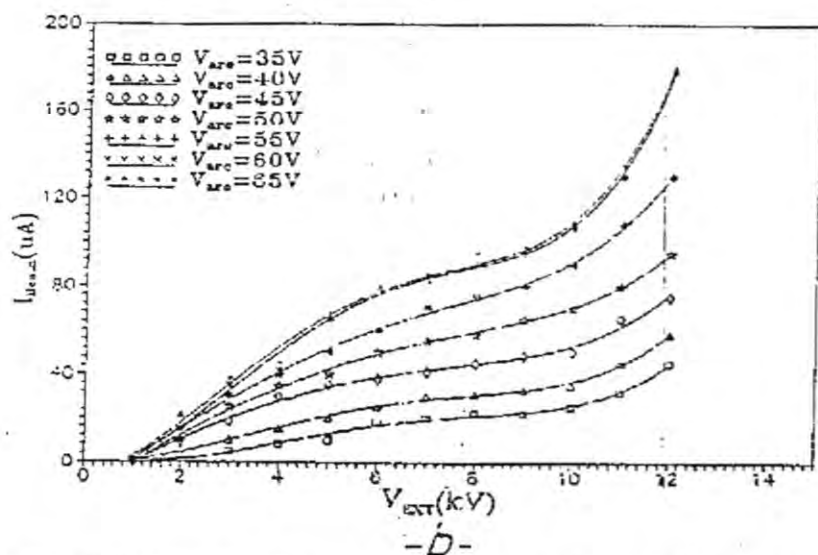
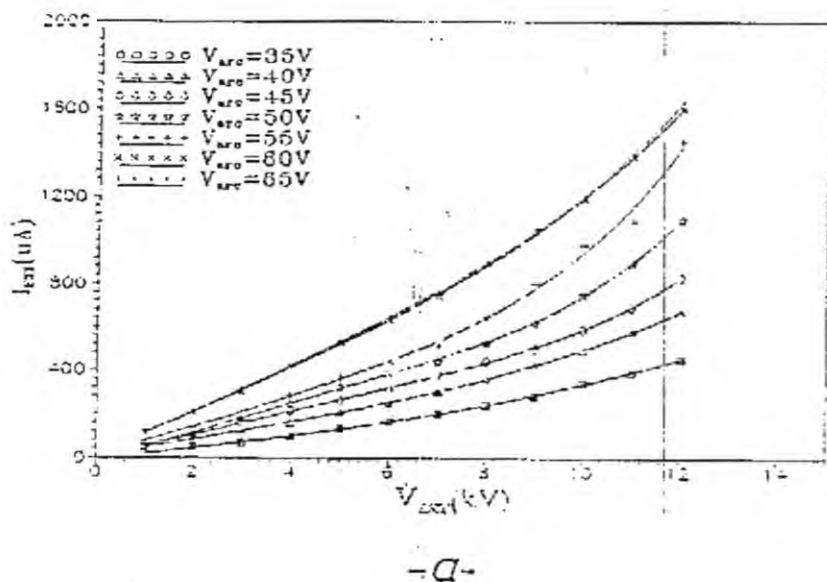


Fig. (3) A graph of the ion current as a function of the extraction voltage for different values of the arc voltage .

$I_f = 55 \text{ A}$, $P=6 \times 10^{-4} \text{ mbar}$, $B=15 \text{ mT}$, $d = 15 \text{ mm}$.

a - For extraction ion current.

b - For ion beam current .

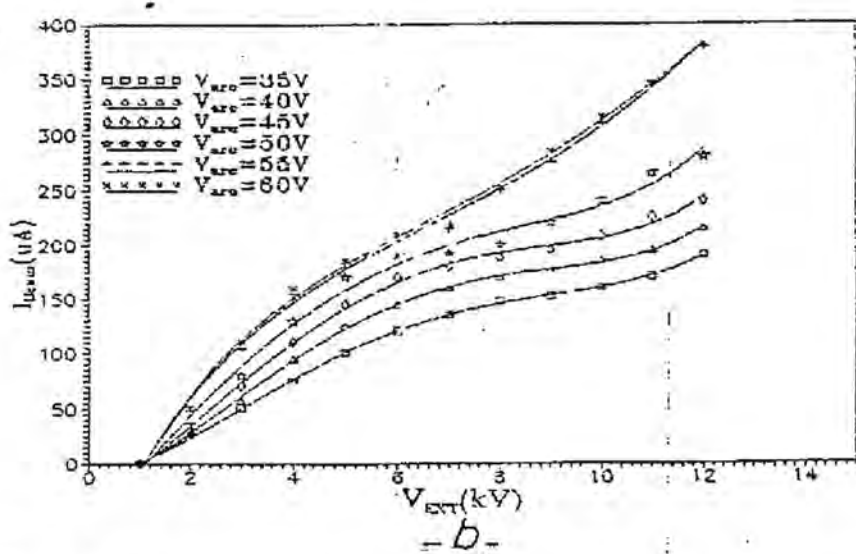
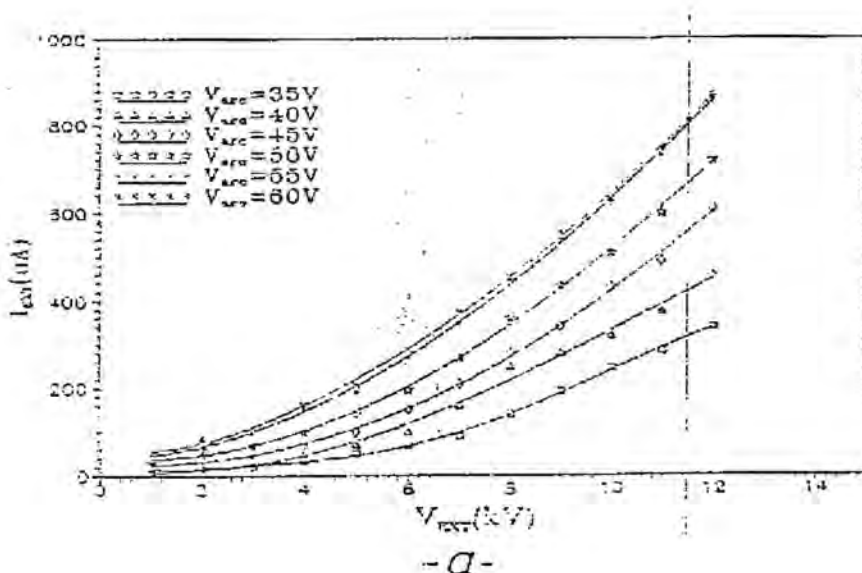


Fig. (4) A graph of the ion current as a function of the extraction voltage for different values of the arc voltage .

$I_t = 55$ A , $P = 8 \times 10^{-4}$ mbar , $B = 15$ mT , $d = 20$ mm .

a - For extraction ion current .

b - For ion beam current .

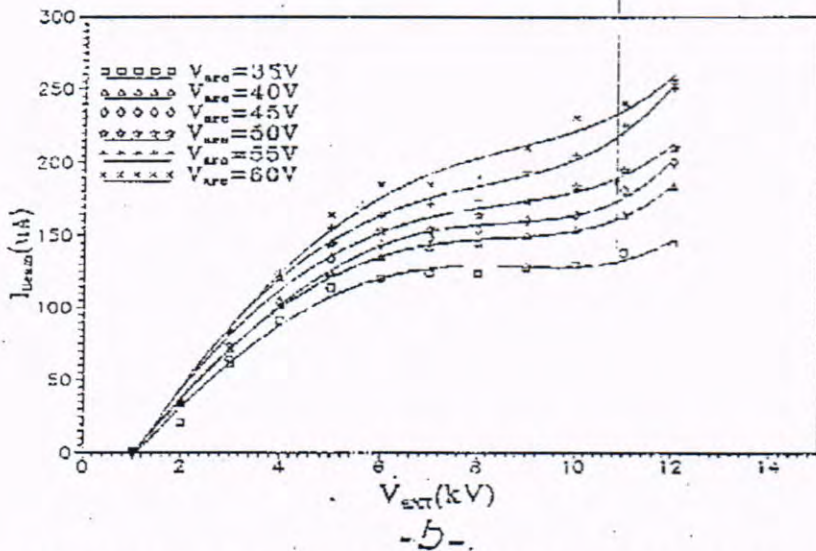
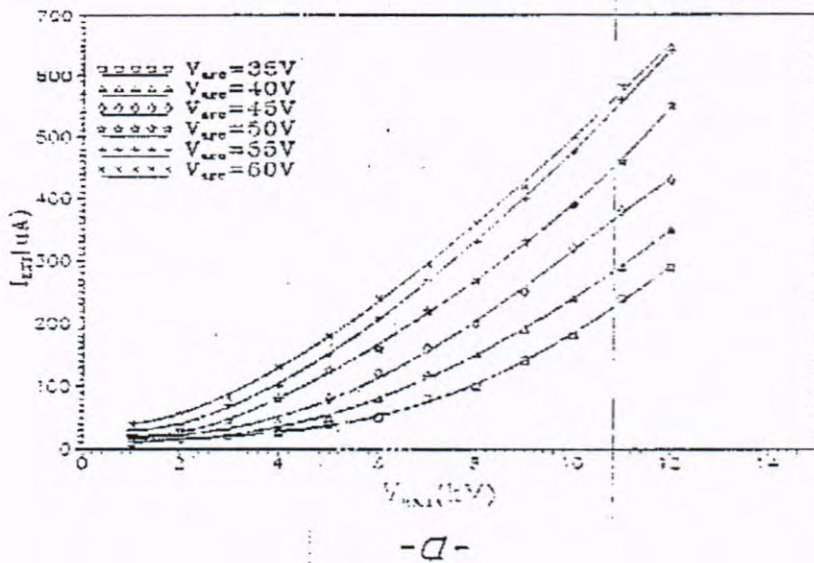


Fig. (5) A graph of the ion current as a function of the extraction voltage for different values of the arc voltage .

$I_f = 55 A$, $P = 6 \times 10^{-4}$ mbar , $B = 15 mT$, $d = 25$ mm .

a - For extraction ion current .

b - For ion beam current .

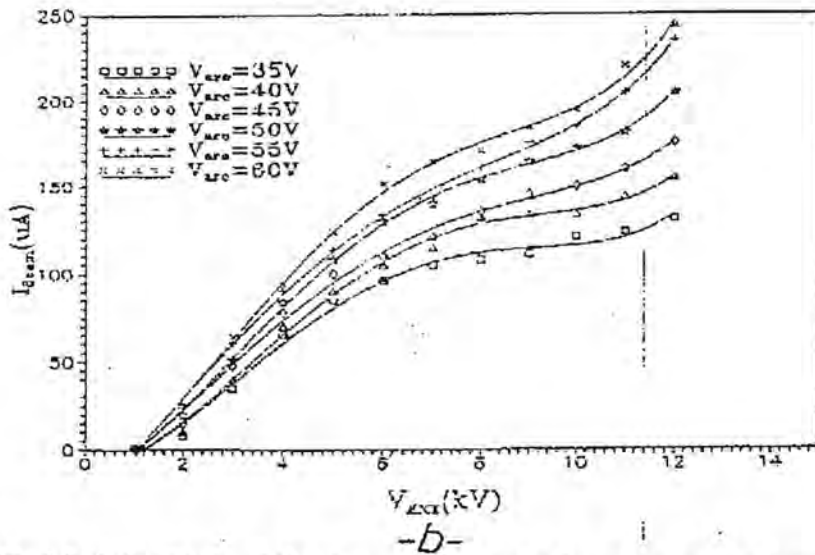
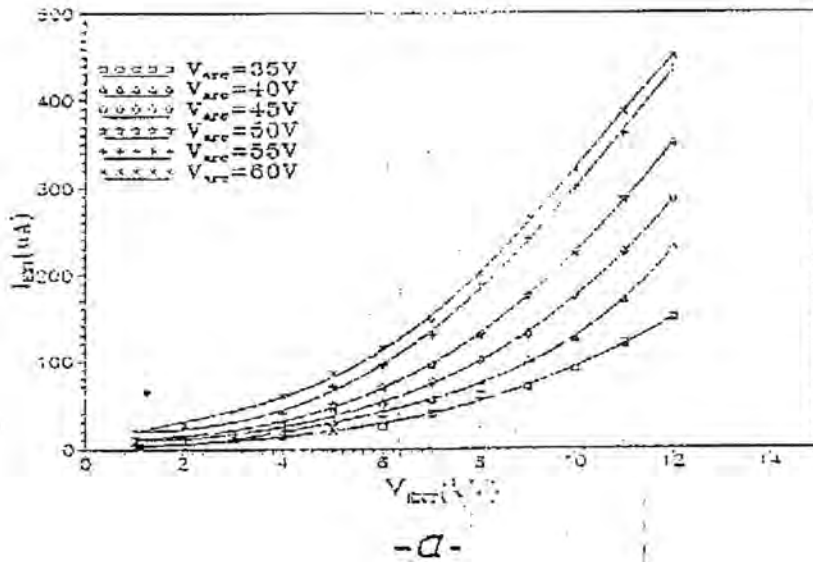


Fig. (6) A graph of the ion current as a function of the extraction voltage for different values of the arc voltage .

$I_r = 55 A$, $P=6 \times 10^{-4}$ mbar , $B=15mT$, $d = 30$ mm .

a - For extraction ion current .

b - For ion beam current .

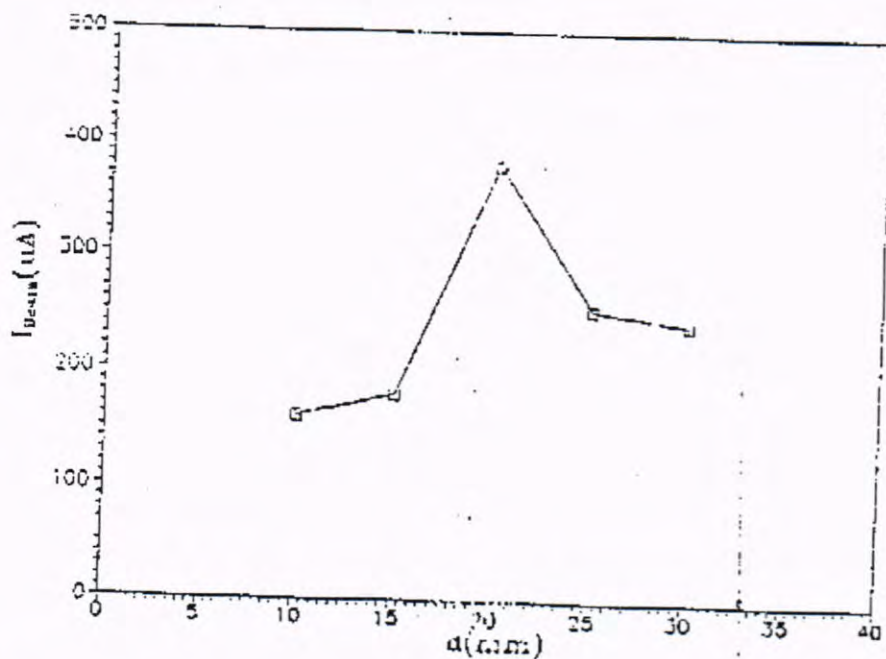


Fig. (7) A graph of the ion beam current as a function of the distance between the extraction electrode and the slit of ion source
 $I_1 = 55 \text{ A}$, $P=6 \times 10^{-4} \text{ mbar}$, $B=15 \text{ mT}$, $V_{\text{EXT}} = 12 \text{ kV}$.

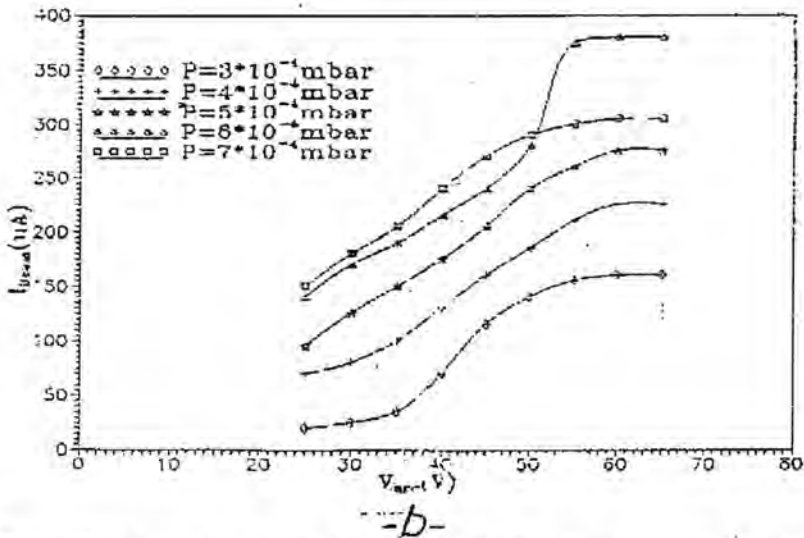
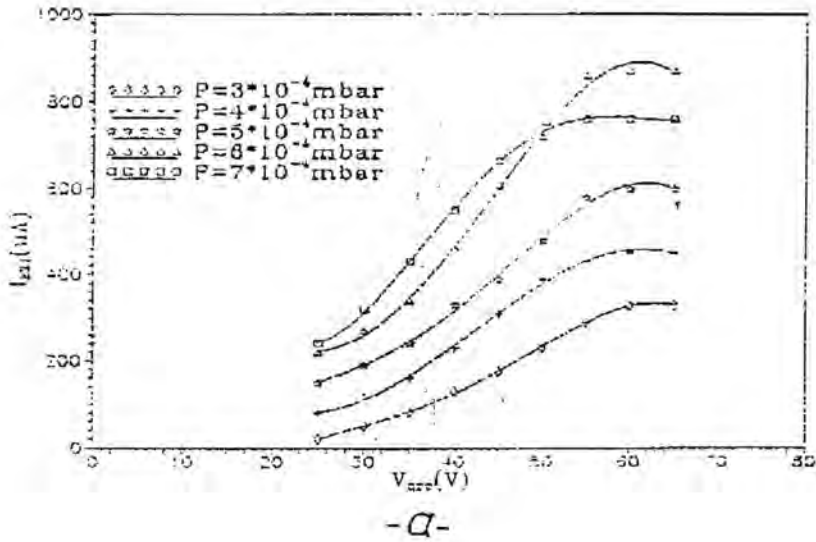
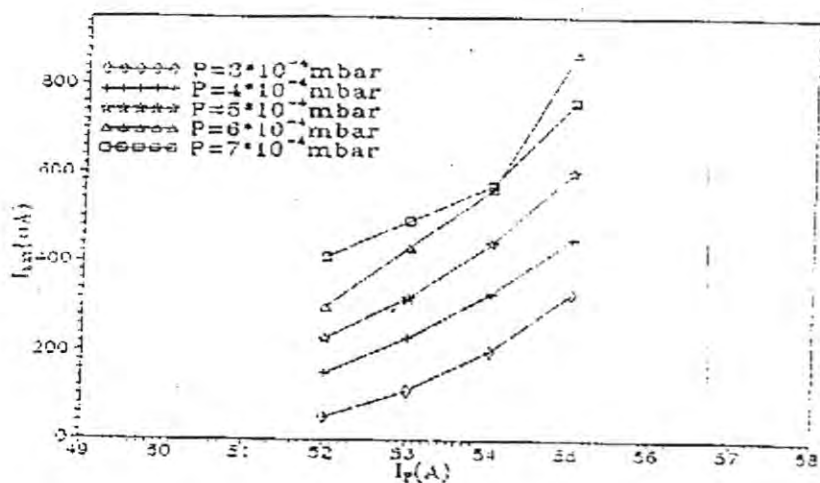


Fig. (8) A graph of the ion current as a function of the arc voltage for different values of the chamber pressure.

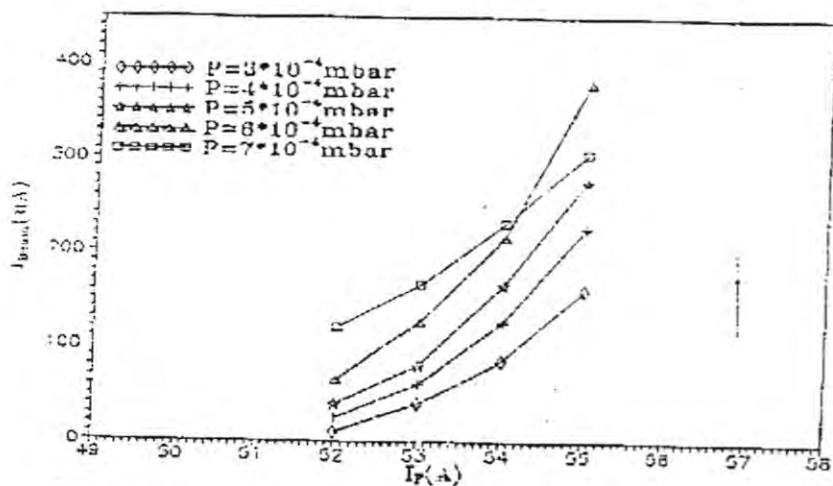
$I_f = 55$ A , $V_{arc} = 12$ kV , $B = 15$ mT , $d = 20$ mm .

a - For extraction ion current.

b- For ion beam current .



-a-



-b-

Fig. (9) A graph of the ion current as a function of the filament current for different values of the chamber pressure.

$V_{arc} = 55$ V , $V_{ext} = 1.2$ kV , $B = 15$ mT , $d = 20$ mm .

a - For extraction ion current .

b - For ion beam current .

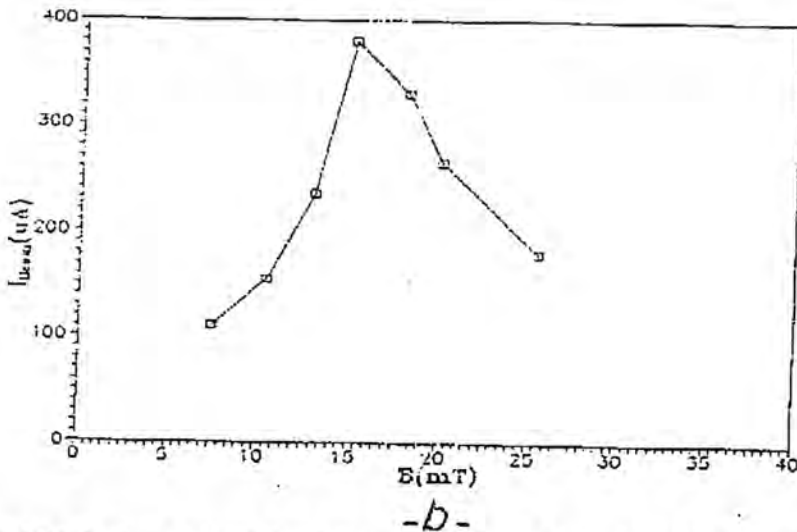
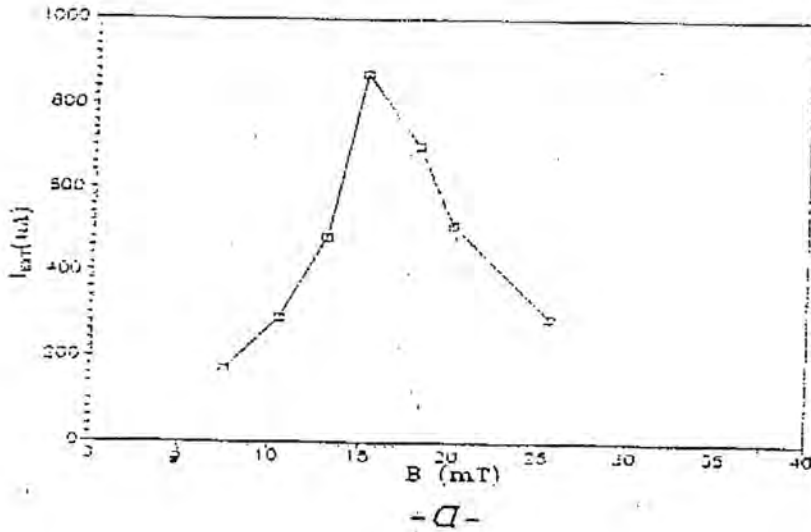
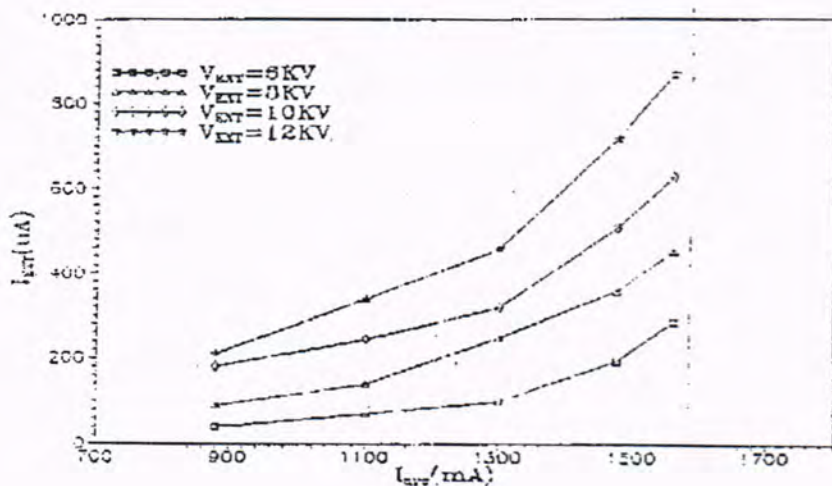


Fig.(10) A graph of the ion current as a function of the magnetic field .

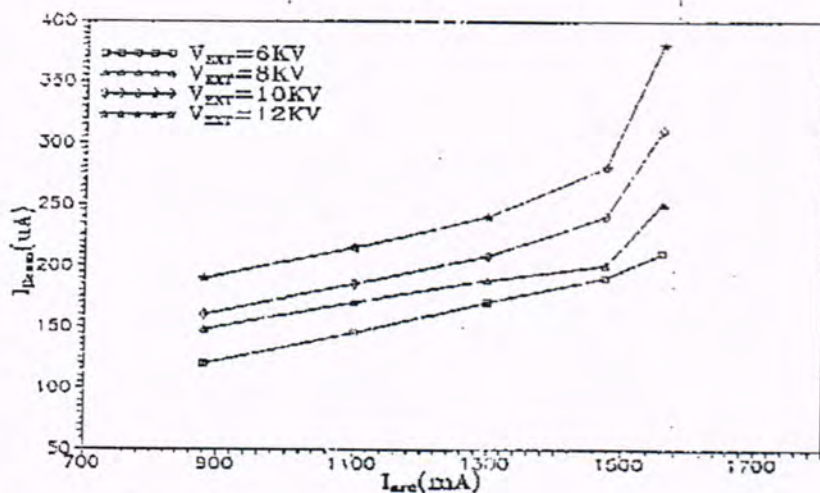
$I_f = 55 A$, $V_{arc} = 55 V$, $P = 6 \times 10^{-4} mbar$, $V_{sc} = 12 kV$, $d = 20 mm$.

a - For extraction ion current .

b- For ion beam current .



-a-



-b-

Fig. (11) A graph of the ion current as a function of the arc current for different values of the extraction voltage.

$I_r = 55 A$, $P = 6 \times 10^{-4}$ mbar, $B = 15$ mT, $d = 20$ mm.

a - For extraction ion current.

b - For ion beam current.

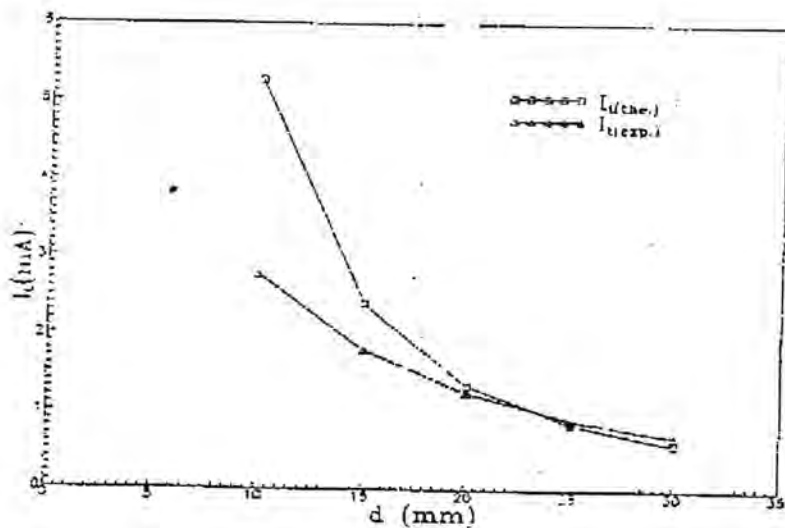


Fig.(12) A graph of the total ion current as a function of the distance between the extraction electrode and the slit of the ion source .

$I_f = 55 \text{ A}$, $P = 6 \times 10^{-4} \text{ mbar}$, $B = 15 \text{ mT}$, $V_{\text{ext}} = 12 \text{ kV}$.

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A Study Of Extraction Of The Electron Beam In A Low Voltage Electron Gun (0-500 eV) System

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الخلاصة

من خلال تطبيق جهد موجب نسبة الى جهد الشبكة (V_A) بين قطبي الشبكة والانود. أجريت مجموعة من التجارب لدراسة أستخراج الحزمة الالكترونية عند الجهد الواطئ لمنظومة القاذف الالكتروني (0-500)eV ولمرحلة واحدة. أن نتائج الدراسة قد أظهرت ان أفضل مسافة لقطب الانود عن قطب الشبكة هو 4 mm اذ يكون التيار الكلي المستخرج 18.24 mA عند جهد (480 V) وان عامل الشكل الهندسي لهذه الحالة هو 0.909.

ABSTRACT

Through the application of a positive potential, with respect to the grid potential (V_A), between the grid electrode and anode electrode a set of experiments were conducted to study the extraction of the electron beam in a low voltage electron gun (0-500 eV) system with one stage. The results of this study have shown that the best distance for the anode electrode from the grid electrode was 4mm with a total electron extraction current of 18.249 mA at an anode voltage of 480 V and the geometry factor for this case was 0.949.

INTRODUCTION

It is well known that numerous parameters are involved in the operation and performance of the electron gun, such as the operation condition and the distance between electrodes^(1,2,3). These parameters indirectly affect the qualities and optics of the beam. The present work aims to determine the best position for the anode electrode and the geometry factor of the electrode experimentally. This type of electron gun system is utilized in various fields, e.g. focusing the beam of electron stream and it is also used in many excitation experiments which require electron beam of low energies, only few electron volts, and of highest possible currents. This system has been also used for deposition technique^(2,4,5,6).

General Design and Construction

The present electron gun, shown in Fig. (1) consists of the following :

- (a) The cathode (filament) (1) which is made of tungsten wire of diameter 0.125 mm in a "U" – shape with a 10 mm spacing between legs.
- (b) The grid and anode electrode (2), (3) which are made of molybdenum plate with dimensions $(70 \times 35 \times 1)$ mm³ and circular aperture of diameter 4mm.
- (c) Molybdenum plate (4) of dimension (70×35) mm² used for measuring the electron beam currents I_E .
- (d) Insulators, such as Teflon, glass and ceramic.

Experimental Measurements

Thermionic Emission Current I_E

As the voltage of the V_g increases, the thermoionic emission current I_E increases, as shown in Fig (2), For all experiments, the distance between the filament and the grid electrode d_g is 2mm.

The filament current was adjusted at 5.2 A due to its configuration, diameter and operation stability.

Influence Of Extraction Distance d_a

To determine the best distance d_a between the grid and anode electrode, the effect of the anode voltage V_A as a function of anode current I_A (measuring is made at anode electrode) and the electron beam current I_{EC} (measuring is made at molybdenum palte) for various values of d_a (3,4,5,6)mm was studied. Figs. (3,5,7,9) show that as d_a increases, the values of I_A decrease which is a normal behavior. However, Figs (4,6,8,10) show different values of I_{EC} as a function of anode voltage for different values of d_a . It is found that the maximum value for I_{EC} is obtained at $V_A = 240$ V at $d_a = 4$ mm as shown in table (1). For these experiments the electron beam diameter was (3-4) mm.

Determination of the Best Electrode Position

The geometry factor C is an important factor which has to be taken into account when designing an extraction electrode. The total extraction electron current I_t is given by

$$I_t = I_A + I_{EC} \quad \dots (1)$$

This current at the extraction anode voltage V_A can be approximately given by the following Child – Langmuir relation for space – charge limited extraction^(7,8,9).

$$I_{t(\text{the})} = \frac{4}{9} A \epsilon_0 \left(\frac{2e}{M} \right)^{1/2} \frac{V_A^{1/2}}{d_a^2} \quad \dots (2)$$

where A , ϵ_0 , M , e are area of circular aperture, permittivity, mass of electron and electron charge respectively. The experimental results showed different values from these theoretically calculated from eq. (2). It was found that relation [3]

$$C = \frac{I_{t(\text{exp})}}{I_{t(\text{the})}} \quad \dots (3)$$

Correlated the two values. Table (II), shows the values of d_a at $V_A = 480$ V, from which it can be seen that the best position for the electrode is 4 mm at which C approaches unity.

CONCLUSION

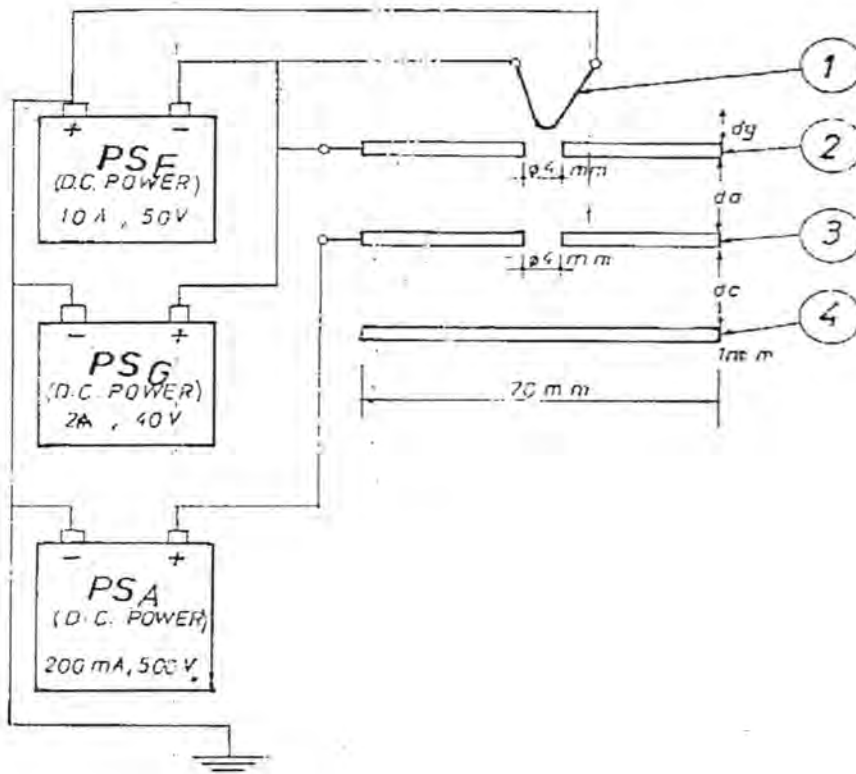
Several experiments were carried out to determine the best distance for anode electrode in a low voltage electron gun system. Setting the system at the operation conditions ($I_F = 5.2$ A, $V_F = 4.2$ V, $P = 1.7 \times 10^{-4}$ mbar and $V_g = 2$ V). For different distance d_a , of the electrode from the grid, it was found that the geometry factor C approaches unity at $I_t = 18.249$ mA, $V_A = 480$ V and $d_a = 4$ mm. Hence, the best electrode position is 4 mm. It was also noted that at this distance (4 mm) the maximum value of I_{EC} was obtained at $V_A = 240$ V, and vacuum pressure set at 1.7×10^{-4} mbar, which is in agreement with the results of Simpson et al⁽²⁾.

Table (1) : Shows the maximum values of electron beam current I_{EC} for different values of d_a

D_a (mm)	V_A (V)	I_{EC} (uA)
3	220	60.2
4	240	85.2
5	480	20.1
6	420	5.6

Table (II) : Shows the values of geometry factor C for different values of d_a at $V_A = 480$ V

d_a (mm)	$I_{l(the)}$ (mA)	I_A (mA)	I_{EC} (uA)	$I_{l(the)}$ (mA)	C
3	34.15	29.1	44.4	29.144	0.8534
4	19.21	18.2	49.1	18.249	0.9490
5	12.29	9.8	20.1	9.820	0.7990
9	8.53	4.4	2.1	4.402	0.5160



NO.	DESCRIPTION	MATERIAL
1	FILAMENT	TUNGSTEN
2	GRID ELECTRODE	MOLYBDENUM
3	ANODE ELECTRODE	MOLYBDENUM
4	MOLYBDENUM PLATE	MOLYBDENUM

Fig. (1) A schematic drawing of the electron gun system .

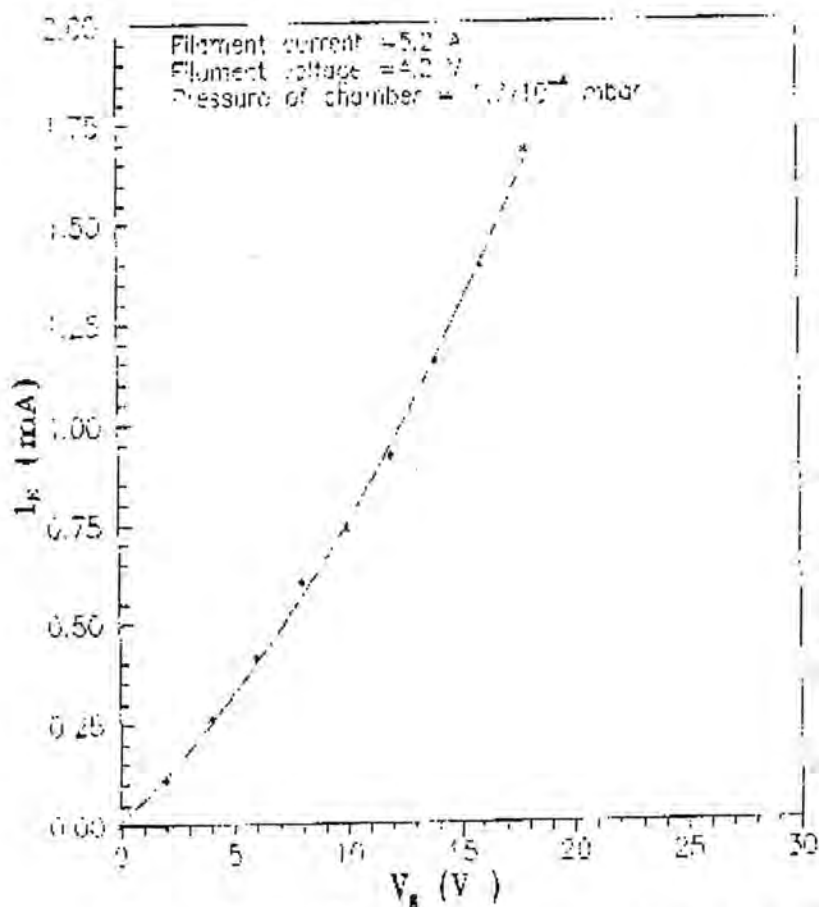


Fig. (2) A graph of the grid current as a function of the grid voltage at $d_g=2$ mm .

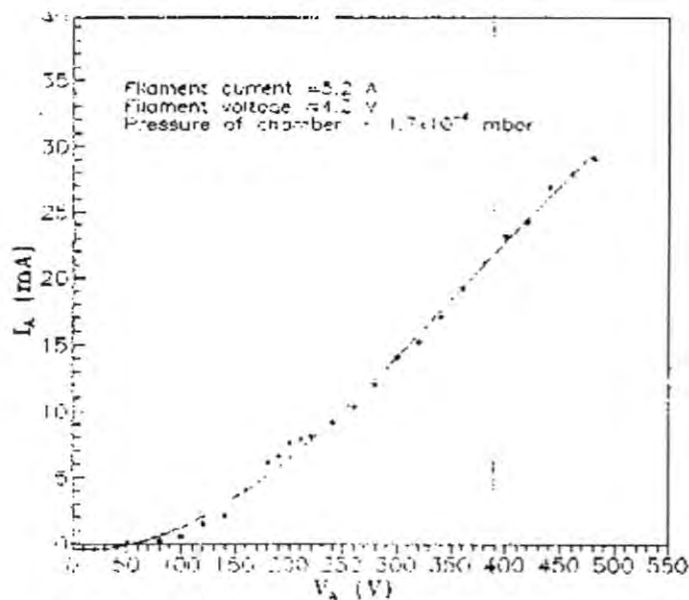


Fig. 13) A graph of the anode current as a function of the anode voltage at $d_0=2$ mm , $d_a=3$ mm .

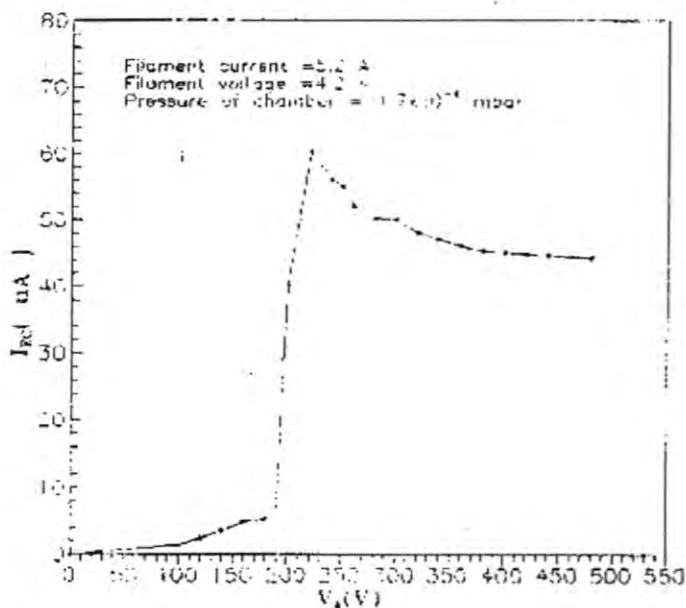


Fig.14) A graph of the electron beam current as a function of the anode voltage at $d_0=2$ mm , $d_a=3$ mm .

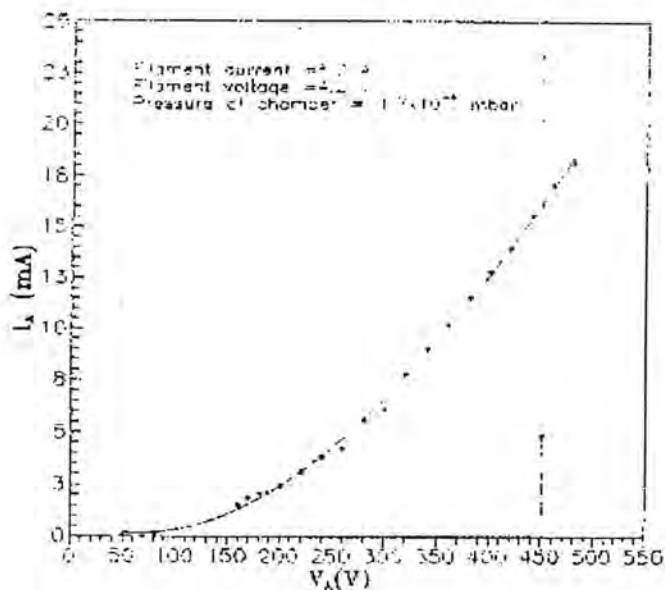


Fig.(5) A graph of the anode current as a function of the anode voltage at $d_1=2\text{mm}$, $d_2=4\text{mm}$.

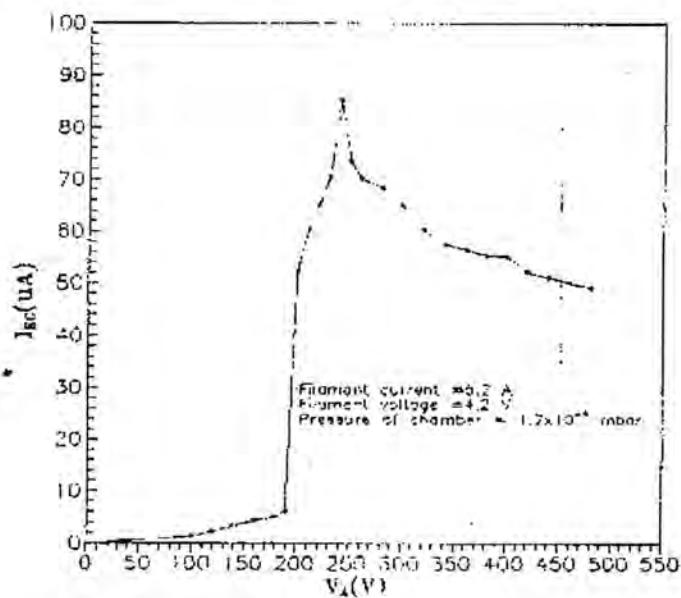


Fig .(6) A graph of the electron beam current as a function of the anode voltage at $d_1=2\text{mm}$, $d_2=4\text{mm}$.

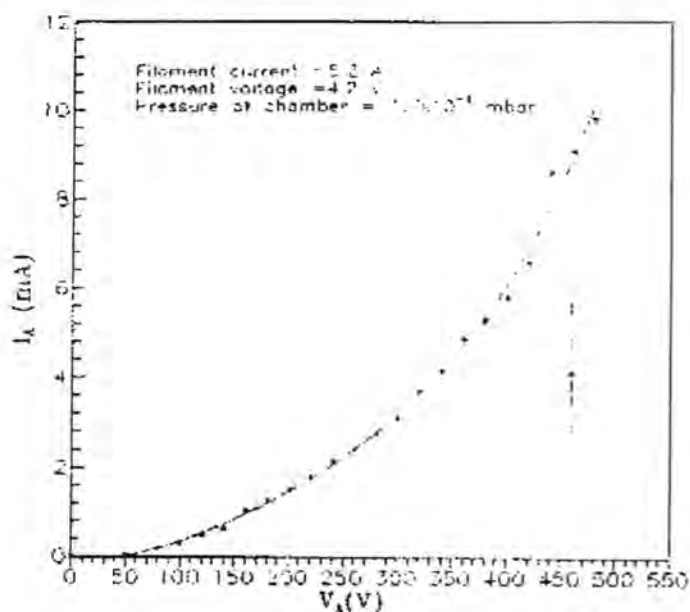


Fig.(7) A graph of the anode current as a function of the anode voltage at $d_1=2\text{mm}$, $d_2=5\text{mm}$.

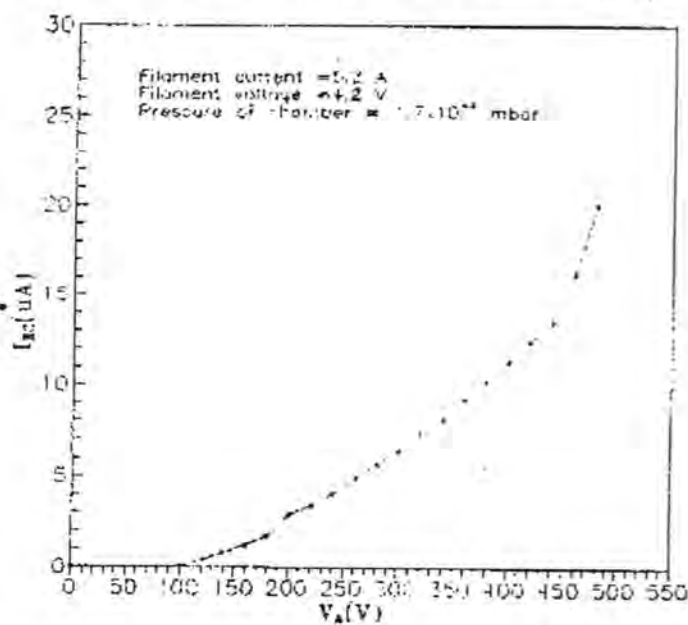


Fig . (8) A graph of the electron beam current as a function of the anode voltage at $d_1=2\text{mm}$, $d_2=5\text{mm}$.

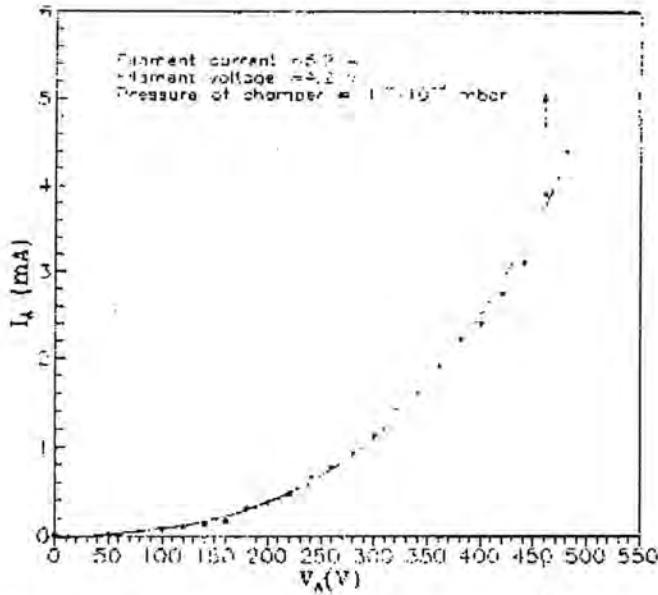


Fig.(9) A graph of the anode current as a function of the anode voltage at $d_g=2\text{mm}$, $d_a=6\text{mm}$.

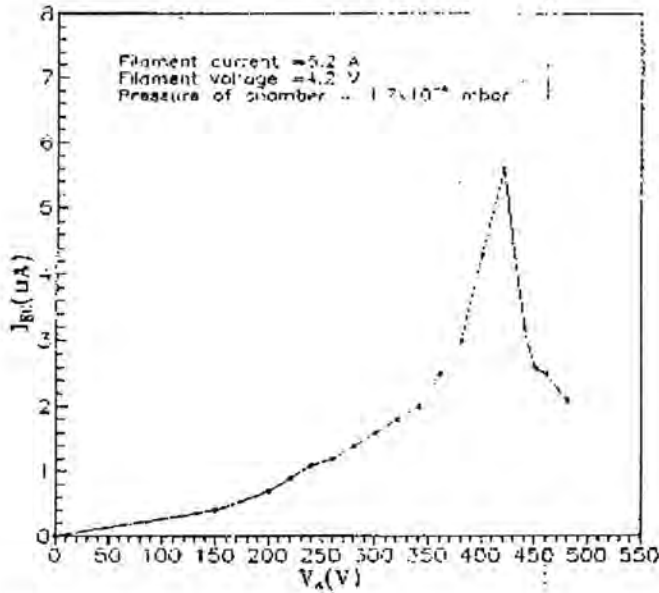


Fig.(10) A graph of the electron beam current as a function of the anode voltage at $d_g=2\text{mm}$, $d_a=6\text{mm}$.

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The Difference in The Determination of Normal Hearing Threshold Level Between Left and Right Ears In Different Age Groups of Both Sexes

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الخلاصة

تم تحديد مستويات عتبة السمع لكل من الأذن اليمنى واليسرى لـ (253) شخص (123 ذكور، 130 اناث) لاعمار مختلفة باستخدام جهاز فحص السمع (Audiometer) قسمت العينة حسب الأعمار الى خمسة مجاميع (10-19), (20-29), (30-39), (40-49), (50-59) سنة. عند تحليل النتائج احصائياً لوحظ انه لا توجد فروقات معنوية بين الاذن اليسرى واليمنى لكلا الجنسين ولكافة الاعمار. كما اظهرت النتائج احصائياً حصول انخفاض حاد في معدل مستوى عتبة السمع للتردد 6 kHz لكلا من الاذن اليسرى واليمنى ولكافة الاعمار.

ABSTRACT

A total of (235) normal individual (123) males and (130) females were divided into five age groups at 10 years intervals, were tested for normal threshold levels in both ears by using the conventional pure tone Audiometer. They had been exposed to a minimum noise of occupation. All the results were analyzed and discussed using (t-test). There was no statistically significant difference in the mean Threshold value of hearing acuity in both ears at the difference age groups in both sexes. The mean threshold value of hearing acuity for left & right ears exhibit a decrease in sensitivity at higher frequencies with advancing age in both sexes. However there was a marked dip in the audiogram at (6 kHz) for left & right ears in both sexes in the youngest age groups.

INTRODUCTION

It had been well known fact that impairment in pure tone threshold of hearing has always been associated with age such a fact had been documented by

several research workers⁽¹⁻⁴⁾. However with the discovery of electronic audiometer by Bunch 1929⁽¹⁾, it was possible to find out the importance of other factors such as age, sex, race, occupation and other environmental factors⁽⁵⁻⁷⁾.

It is of crucial importance to have enough systemic knowledge of the whole life history of the individual for the clinical diagnosis of minor pathological changes of hearing acuity by the conventional method of audiometry.

The aim of present study is to determine the normal hearing threshold level (HTL) for left & right ears in different age groups for both sexes in normal individuals and to find out if there is a difference between left & right ears in the normal threshold of hearing acuity.

MATERIAL AND METHOD

A total of (253) of (123) males (130) females were tested for normal value thresholds of hearing acuity in both left & right ears using the conventional laboratory audiometry (type Ollmann Company / Germany) at (0.25, 0.5, 1, 2, 3, 4, 6, 8) kHz frequencies respectively.

The majority of these individuals tested were Kufa university students and resident at Najaf City. They were carefully examined and questioned regarding previous ear diseases and abnormal exposure. Those abnormal were excluded from the study. Those above thirty years of age were carefully interrogated for a history of mid-ear infections and hypertension. All abnormal were excluded from the study. They were divided into five groups at (10) years interval.

The statistical analysis of the data was performed using t-test⁽⁸⁾ as well as the mean of hearing threshold levels (HTL), the standard deviation, the standard errors of the mean, the standard errors of the mean between left & right ears, the median and coefficient of variance were estimated.

RESULTS AND DISCUSSION

Figs.(1) & (2) showed the mean hearing threshold values of both ears in (dB) in males for five age groups.

We carried out a succession of t-test to find any significant differences in the different age groups. The results showed no statistically significant difference between both ears in males at different age groups, but both curves for left & right ears exhibit a marked decrease in the mean hearing threshold values at higher frequencies with the advancing of age.

Fig. (3) & (4) showed the mean hearing threshold values of both ears in (dB) in females for five age groups.

We carried out also a succession of t-test on both ears at different age groups. The results showed no statistically significant differences between left &

right ears. Again both curves for left & right ears exhibit a marked decrease in the mean of hearing threshold at higher frequencies with the advancing of age.

However Sulkowski⁽⁹⁾ reported statistically significant differences in the mean hearing threshold values at different age groups in wood cutter workers for both ears with a greater losses were observed in the left ears.

This might be due to the posture of wood cutter operator during his work besides to left side position of the saw motor.

L.H. Royster⁽¹⁰⁾ noted significant differences between left & right ears of the hearing threshold for the subgroups of the industrial noise exposed population.

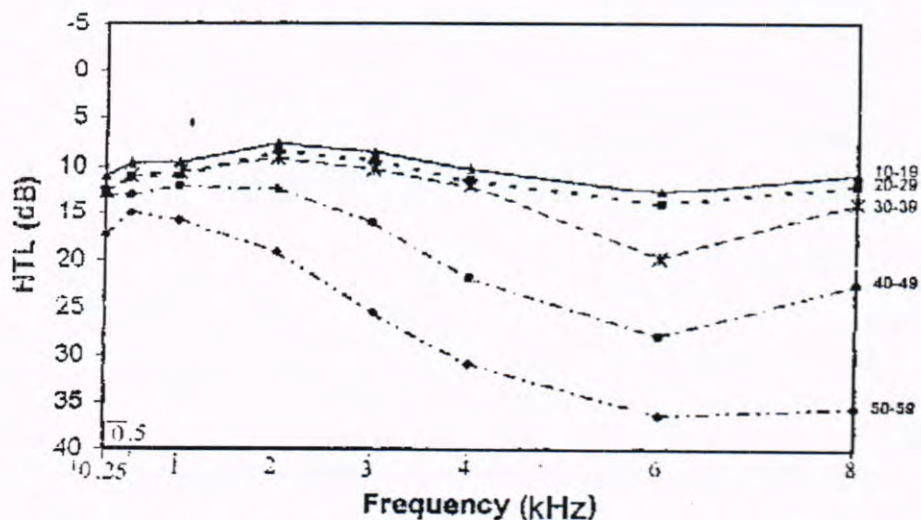
Tables (1) & (2) shows the mean of hearing threshold levels (HTL), the standard deviation, the standard errors of the mean, the standard errors of the mean between left & right ears, the median and coefficient of variance for male & female in the five age groups.

Both the left & right ears in both sexes exhibit decreases in the mean threshold values with the advancing age which might be due to aging effect which could be a physiological as well as environmental factor.

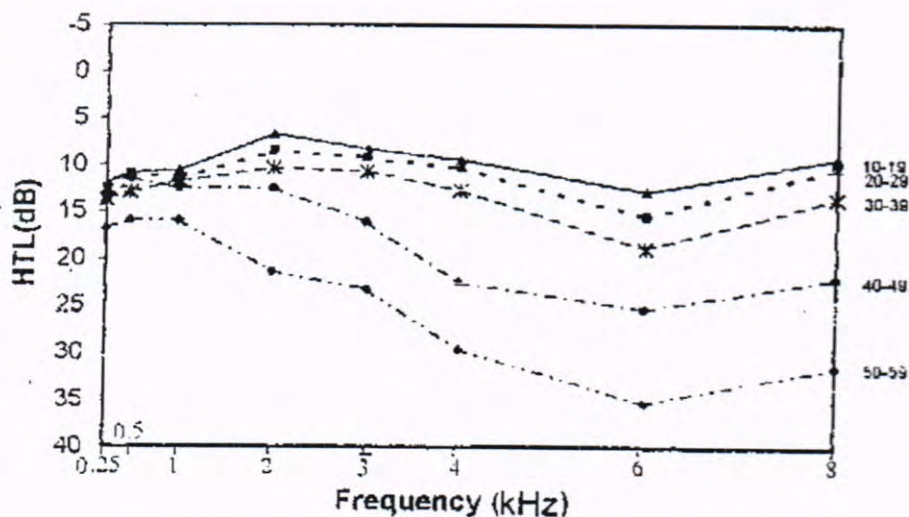
These two factors acts together in the aging process with advancing age^(11,12), simillar result had been reported by Ryostes and Robinson^(10,13).

There was a markable dip in the (HTL) at (6 kHz) in both ears & both sexes at the different age groups fig (1), (2) & fig (3), (4).

Such finding had been reported by Obsterhammal & Molvaer^(14,15) which seems that at the (6 kHz) frequency, the (HTL) is the lowest threshold values in all age groups for both sexes which might indicate that the normal hearing physiology of human ears is less sensitive at (6 kHz) frequency especially with advancing age.



Fig(1): Mean hearing threshold level (HTL) for right ear in male in five age groups



Fig(2): Mean hearing threshold level (HTL) for left ear in male in five age groups

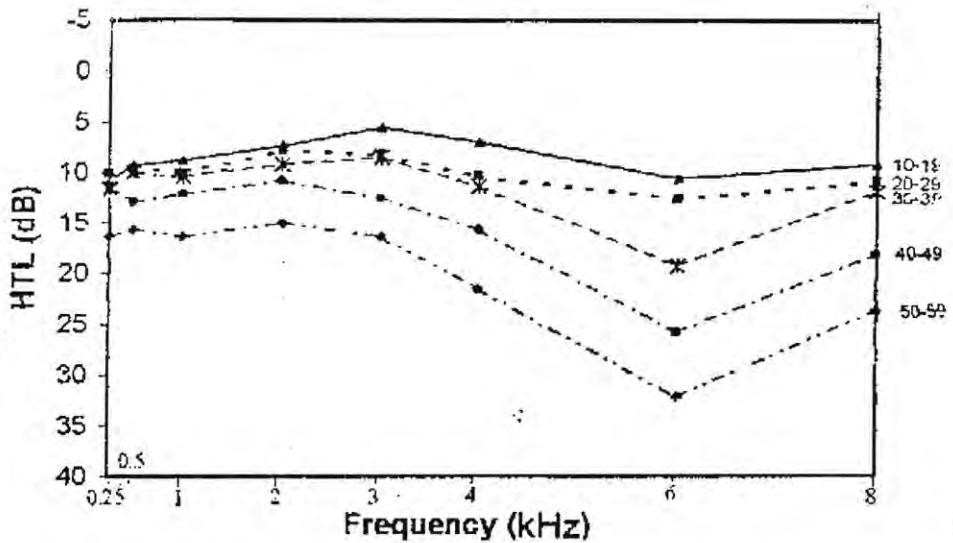
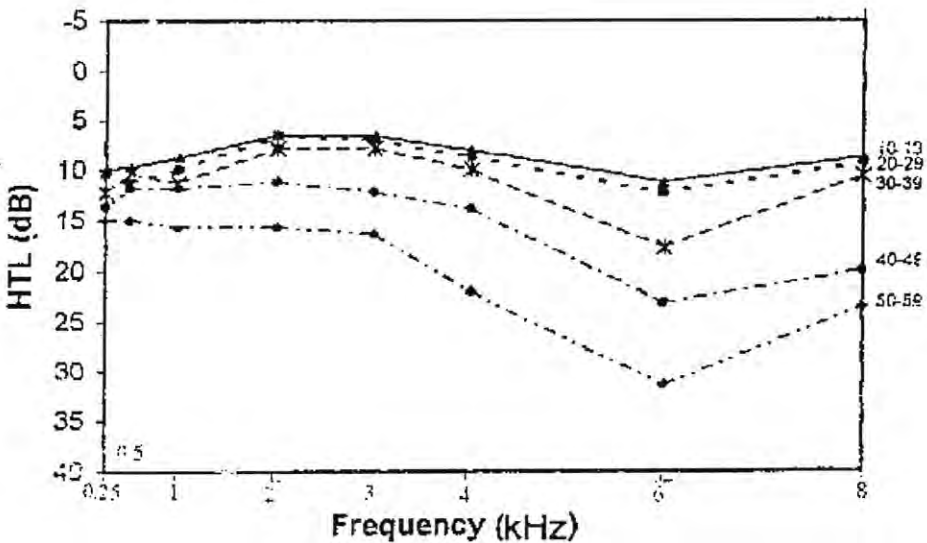


Fig (3):Mean hearing threshold level (HTL) for right ear in female in five age groups



Fig(4):Mean hearing threshold level (HTL) for left ear in female in five age groups

Table (1) mean hearing threshold levels (HTL) for males for different frequencies in five age groups, including standard deviation (S.D), standard error of the mean between left and right ear (S.E.M. L-R), Median & Coefficient of Variance (CV)

Age (Yr)	Parameter	0.25 kHz		0.5 kHz		1 kHz		2 kHz	
		L	R	L	R	L	R	L	R
10-19 No. 18	Mean	11.9	11.1	10.8	9.7	10.6	9.7	6.7	7.7
	S.D.	5.2	5.3	6.0	5.0	4.2	6.1	4.8	5.8
	S.E.M	1.23	1.25	1.41	1.18	0.99	1.44	1.13	1.37
	S.E.M.L-R	1.75		1.84		1.75		1.77	
	MEDIAN	10	10	10	10	10	7.5	5	7.5
	CV	43.7	47.7	55.6	51.5	39.6	62.09	71.6	75.3
20-29 44	MEAN	12.7	12.6	10.8	11.25	11.5	11.0	8.5	8.5
	S.D	7.9	5.2	8.3	7.5	4.4	5.8	5.9	5.7
	S.E.M.	1.91	0.89	1.25	1.13	0.66	0.87	0.89	0.86
	S.E.M.L-R	1.48		1.68		1.06		1.23	
	MEDIAN	15	15	12.5	12.5	10.0	10.0	10.0	5.0
	CV	62.2	46.8	76.9	66.7	38.3	52.7	69.4	67.1
30-39 36	MEAN	13.8	12.9	12.9	11.0	11.7	10.6	10.3	9.2
	S.D	5.8	6.0	6.4	7.0	2.9	5.7	4.8	8.7
	S.E.M	0.97	1.0	1.07	1.17	0.48	0.95	0.8	1.12
	S.E.M.L-R	1.4		1.065		1.5		1.9	
	MEDIAN	15	15	12.5	7.5	10.0	10.0	7.5	7.5
	CV	42.0	46.5	49.6	63.6	24.8	53.8	46.6	72.8
40-49 14	MEAN	13.9	13.2	11.4	13.2	12.5	12.1	12.5	12.5
	S.D	5.3	5.8	6.6	5.0	3.3	5.1	6.1	5.1
	S.E.M	1.42	1.55	1.76	1.34	0.88	1.36	1.63	1.36
	S.E.M. L-R	2.09		2.40		1.69		2.21	
	MEDIAN	15.0	10.0	12.5	12.5	10.0	12.5	12.5	15.0
	CV	38.1	43.9	57.9	37.9	26.4	42.2	48.8	40.8
50-59 11	MEAN	16.8	17.3	15.9	15.0	15.9	15.9	21.4	19.1
	S.D	6.8	17.2	5.8	3.9	4.4	5.4	6.4	8.3
	S.E.M	2.05	2.38	1.75	1.18	1.33	1.63	1.93	2.5
	S.E.M.L-R	1.77		2.12		2.10		3.16	
	MEDIAN	15.0	15.0	15.0	15.0	15.0	15.0	20.0	20.0
	CV	40.5	45.7	36.5	26.0	27.7	34.0	29.9	43.5

Continued table 1

Age (Yr)	3 kHz		4 kHz		6 kHz		8 kHz	
	L	R	L	R	L	R	L	R
10-19	8.3	8.6	9.4	10.3	12.8	12.8	9.2	10.8
No. 18	4.9	4.1	6.8	5.0	5.5	6.2	6.2	5.7
	1.16	0.97	1.6	1.18	1.3	1.46	1.46	1.34
	1.51		1.99		1.95		1.99	
	10	10	7.5	10	15.0	15.0	7.5	10
	59.0	47.7	72.3	48.5	43.0	48.0	67.4	52.8
20-29	9.2	9.4	10.3	11.5	15.5	14.1	10.2	11.9
44	5.2	5.7	5.5	4.8	8.3	7.0	6.8	6.6
	0.78	0.86	0.83	0.72	1.25	1.06	1.03	0.99
	1.34		1.2		1.63		1.42	
	10.0	10.0	10.0	10.0	15.0	15.0	10.0	10.0

	56.5	60.6	53.4	41.7	53.5	49.6	66.7	55.5
30-39	10.7	10.3	12.6	12.1	18.9	19.7	13.5	13.9
36	3.8	4.8	5.1	6.3	5.5	6.8	5.4	6.0
	0.63	0.8	0.85	1.05	0.92	1.13	0.9	1.0
	1.05		1.84		1.58		1.92	
	10.0	10.0	12.5	12.5	20.0	20.0	12.5	15.0
	35.5	46.6	40.5	52.1	29.1	34.5	40.0	43.2
40-49	16.1	16.1	22.5	21.8	25.4	27.9	22.1	22.5
14	6.6	5.9	7.3	7.7	9.7	10.7	6.1	8.9
	1.76	1.58	1.95	2.06	2.59	2.86	1.63	2.38
	2.46		2.95		4.02		2.27	
	15.0	17.5	25.0	22.5	25.0	30.0	22.5	25.0
	41.0	36.6	32.4	35.3	38.2	38.4	27.6	39.6
50-59	23.2	25.5	29.5	30.9	35.5	36.4	31.4	35.5
11	5.6	5.2	11.3	8.0	10.8	9.2	8.4	9.6
	1.69	1.57	3.4	2.41	3.26	2.77	2.53	2.89
	2.31		4.18		4.28		3.85	
	25.0	25.5	30.0	30.0	35.0	35.0	30.0	35.0
	24.1	20.4	38.8	25.9	30.4	25.3	26.8	27.0

Table (2) : Mean hearing threshold levels (HTL) for females for different frequencies in five age groups, including standard deviation (S.D), standard errors of the mean between left and right ear (S.E.M. L-R), Median & Coefficient of Variance (CV)

Age (Yr)	Parame-ter	0.25 kHz		0.5 kHz		1 kHz		2 kHz	
		L	R	L	R	L	R	L	R
10-19 No. 18	Mean	10.0	10.7	9.7	9.3	8.8	8.8	6.5	7.3
	S.D.	5.9	5.0	4.3	3.7	4.4	3.7	5.4	4.2
	S.E.M	0.77	0.65	0.56	0.48	0.57	0.48	0.70	0.55
	S.E.M.L-R	1.01		0.74		0.75		0.89	
	MEDIAN	12.5	12.5	10.0	10.0	10.0	10.0	5.0	5.0
	CV	59.0	46.7	44.3	39.8	50.0	42.0	83.1	57.5
20-29 44	MEAN	10.4	10.1	11.3	9.6	10.0	10.0	6.6	7.8
	S.D	8.1	7.6	9.4	7.3	4.8	4.1	4.9	5.0
	S.E.M.	1.39	1.30	1.61	1.25	0.82	0.70	0.83	0.86
	S.E.M.L-R	1.91		2.04		1.08		1.20	
	MEDIAN	10	10	10	10	7.5	7.5	5	10
	CV	77.9	75.2	83.2	76.0	48.0	41.0	74.2	64.1
30-39 36	MEAN	12.2	11.6	10.3	10.0	11.3	10.0	7.8	9.1
	S.D	6.6	7.2	4.3	5.8	5.3	4.6	5.8	5.8
	S.E.M	1.65	1.8	1.08	1.45	1.33	1.15	1.45	1.45
	S.E.M.L-R	2.44		1.80		1.76		2.05	
	MEDIAN	10.0	10.0	10.0	10.0	8.0	10.0	5.0	10.0
	CV	54.1	52.1	41.7	58.0	46.9	44.7	74.4	63.7
40-49 14	MEAN	13.6	11.4	11.8	12.9	11.8	12.1	11.1	10.7
	S.D	6.0	6.0	4.6	6.4	5.4	4.7	4.5	4.3
	S.E.M	1.60	1.60	1.23	1.71	1.44	1.26	1.20	1.15
	S.E.M.L-R	2.29		2.11		1.91		1.66	
	MEDIAN	15.0	10.0	12.5	10.0	12.5	12.5	10.0	10.0
	CV	44.1	52.6	39.0	49.6	45.8	38.8	40.5	40.8
50-59 11	MEAN	15.0	16.4	15	15.7	15.7	16.4	15.7	15
	S.D	4.1	4.8	5.0	5.3	3.4	4.8	4.5	6.5
	S.E.M	1.55	1.81	1.9	2	1.29	1.81	1.70	2.46
	S.E.M.L-R	2.39		2.76		2.22		2.99	
	MEDIAN	15.0	15.0	15.0	15.0	15.0	15.0	15.0	10.0

CV	27.3	29.3	33.3	33.8	21.7	29.3	28.7	43.3
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Continued table 2

Age (Yr)	3 kHz		4 kHz		6 kHz		8 kHz	
	L	R	L	R	L	R	L	R
10-19	6.6	5.5	8.1	7.0	11.2	10.6	8.8	9.2
No. 18	8.4	6.3	7.2	6.2	8.3	8.0	6.2	6.7
	1.07	0.82	0.94	0.81	1.08	1.04	0.80	0.87
	1.35		1.24		1.5		1.19	
	5.0	5.0	5.0	5.0	10.0	10.0	10.0	10.0
	124.2	114.5	88.9	88.6	74.1	75.5	70.5	72.8
20-29	6.9	8.2	8.8	10.3	12.2	12.6	9.3	10.9
44	4.6	5.8	5.7	5.7	5.9	7.2	6.6	6.3
	0.14	0.99	0.98	0.98	1.01	1.23	1.13	1.08
	1.27		1.39		1.6		1.57	
	5	7.5	5.0	5.0	15.0	10.0	10.0	10.0
	66.7	70.7	64.8	55.3	48.4	57.1	71.0	57.8
30-39	7.8	8.4	10.0	11.3	17.8	19.3	10.6	11.9
36	7.3	5.7	4.1	5.0	6.0	7.0	5.4	4.9
	1.83	1.43	1.03	1.25	1.5	1.75	1.35	1.23
	2.32		1.58		2.31		1.74	
	5.0	7.5	10.0	10.0	17.5	20.0	10.0	10.0
	93.6	67.9	41.0	44.2	33.7	36.3	50.9	37.0
40-49	12.1	12.5	13.9	15.7	23.3	25.7	20.0	18.2
14	4.7	5.5	6.6	7.0	7.0	5.0	6.5	6.7
	1.26	1.47	1.76	1.87	1.87	1.34	1.74	1.79
	1.94		2.57		2.32		2.50	
	12.5	15.0	15.0	17.5	25.0	25.0	20.0	17.5
	38.8	44.0	47.5	44.6	30.2	19.5	32.5	36.8
50-59	16.4	16.4	22.1	21.4	31.4	32.1	23.6	23.6
11	4.8	3.8	6.4	5.6	9.5	5.7	4.8	8.0
	1.81	1.44	2.42	2.12	3.6	0.38	1.81	3.02
	2.14		3.22		4.19		3.53	
	15.0	15.0	20.0	20.0	30.0	35.0	25.0	20.0
	29.3	23.2	29.0	26.2	30.3	17.8	20.3	33.9

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Inverse of a patterned Covariance Matrix By Urquaharts Technique, With Applications

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الخلاصة

في هذه الدراسة تم شرح ايجاد معكوس مصفوفة تبين مشترك ذات قالب باستخدام اسلوب اوركوهارت كما تم مناقشة تطبيقات نتائج هذه الدراسة لاجاد معكوس مصفوفة التباين المشترك لبعض نماذج التأثيرات العشوائية والمختلطة ذي ثلاثة أطراف وتم تبسيط الحسابات بشكل ملحوظ.

ABSTRACT

This paper demonstrate finding the inversion of a patterned covariance matrix, by using Urquhart's technique. Application of the results to the covariance matrices of certain 3-way mixed and / or random effects model will also be discussed, the calculations are considerably simplified.

KEY WORDS: Correlation matrices; covariance matrices; Kronecker product; matrix inversion; mixed models; patterned matrices, random models; Urquhart's technique.

INTRODUCTION

Elements of the information matrix for the variance components of a linear model involve, under normality, the inverse of the covariance matrix of the vector of observation. A typical element of the information matrix is given in Searle⁽¹⁾. This element is $\text{tr}(V^{-1}V_iV^{-1}V_j)$, where V is the covariance matrix of the vector of observations and V_i is the partial derivative of V with respect to σ_i^2 , some variance components of the model. It is clear that V^{-1} essential to finding the information matrix from which come the sampling variances of the large sample maximum likelihood estimatos of the components.

Experssions for V^{-1} for neseted classifications with unbalanced data are given in Searle⁽¹⁾ for the 2-way case, in Rudan and Searle⁽²⁾ for the 3-way case, and in LaMotte⁽³⁾ for the general p-way nested classifications. Searle and Rudan⁽⁴⁾

indicate attempts to obtain the inverse of the covariance matrix for 2-way crossed classifications random model with interaction.

Wansbeek⁽⁵⁾ used the method of tearing to derive an alternative to the method of Searle and Rundan⁽⁴⁾ to invert certain covariance matrix arising from the analysis of variance (ANOVA) of unbalanced data. Bonny and Kissling⁽⁶⁾ Gabbara and Naji^(7,8) and Gabbara⁽⁹⁾, used the method of tearing also, to find matrix inversion to certain patterned matrices.

Al-Abdullah⁽¹⁰⁾ and Gabbara and Al-Abdullah⁽¹¹⁾ have discussed the inversion of certain patterned matrices using Urquhart's technique, Urquhart⁽¹²⁾ and have given certain applications to each case.

In this paper, we demonstrate finding the inversion of a patterned covariance matrix by using Urquhart's technique, where this matrix has a structure which is different than any of the matrices studied by any of the authors mentioned above. Results of this work can be used to find the inverse of the covariance matrix of certain 3-way mixed and / or random effects models.

Notations

If A is an $m \times p$ matrix and $B = (b_{ij})$ is an $n \times q$ matrix, then the Kronecker product of A and B , written as $A \otimes B$, is the $mnpq$ matrix $C = (C_{ij})$, where $C_{ij} = b_{ij}A$, $i = 1, \dots, n$; $j = 1, \dots, q$.

Let a^i be the reciprocal of a_i ; the matrix $\text{diag}(a_i)$ be diagonal over the subscript i whose i th element is a_i and (a^{ij}) be the inverse of the matrix (a_{ij}) .

The following vector and matrices will be used frequently in this paper. Let $1_s = (1, \dots, 1)' \in R^s$, ($s > 0$); let I_s be the $s \times s$ identity matrix, and let J_{rxs} be the rxs matrix with 1 in every position.

The Covariance Matrix

Suppose that Σ is an $n \times n$ covariance to be inverted. Σ can be decomposed as

$$\Sigma = \text{diag}(a_j) \quad \dots (1)$$

where R is the $n \times n$ matrix and $\text{diag}(\sigma_i)$ is the $n \times n$ diagonal matrix of standard deviations. Then

$$\Sigma^{-1} = \text{diag}(\sigma^i) R^{-1} \text{diag}(\sigma^i) \quad \dots (2)$$

Now, suppose that R can be partitioned into b^2 blocks and each block can be partitioned into c^2 subblocks, where each subblock is an $n_{jk} \times n_{j'k'}$, matrix with $j = 1, \dots, b; k = 1, \dots, c$. Let us suppose that the matrix R can be written as

$$R = (R_{jj'}), \quad \text{for } j, j' = 1, \dots, b \quad \dots(3)$$

where

$$R_{jj'} = \delta_{jj'} a_j I_{n_j} + b_{jj'} \text{diag} (J_{n_{j_1} \times n_{j_1}}) + c_{jj'} J_{n_{j_1} \times n_{j_1}} \quad \dots(4)$$

Where $\delta_{jj'}$ is equal to 0 or 1 if $j \neq j'$ or $j = j'$ respectively, and n_j is the sum of n_{jk} over the subscript k. Then

$$R = (\delta_{jj'} a_j I_{n_j}) + (b_{jj'} \text{diag}(J_{n_{j_1} \times n_{j_1}})) + (c_{jj'} J_{n_{j_1} \times n_{j_1}}) \quad \dots(5)$$

In order to see R in an explicit form, let $b=2, c=3$ and n_{jk} is given in the following table :

		n_{jk}			n_j
		1	2	3	
j	1	1	3	2	6
	2	2	4	2	8
	n_k	3	7	4	$n_{..}=14$

According to the table, R is an 14×14 matrix having the form:

$$R = \begin{pmatrix} \alpha_{11} & c_{11} & c_{11} & c_{11} & c_{11} & c_{11} & \beta_{12} & \beta_{12} & c_{12} & c_{12} & c_{12} & c_{12} & c_{12} & c_{12} \\ c_{11} & \alpha_{11} & \beta_{11} & \beta_{11} & c_{11} & c_{11} & c_{12} & c_{12} & \beta_{12} & \beta_{12} & \beta_{12} & \beta_{12} & c_{12} & c_{12} \\ c_{11} & \beta_{11} & \alpha_{11} & \beta_{11} & c_{11} & c_{11} & c_{12} & c_{12} & \beta_{12} & \beta_{12} & \beta_{12} & \beta_{12} & c_{12} & c_{12} \\ c_{11} & \beta_{11} & \beta_{11} & \alpha_{11} & c_{11} & c_{11} & c_{12} & c_{12} & \beta_{12} & \beta_{12} & \beta_{12} & \beta_{12} & c_{12} & c_{12} \\ c_{11} & c_{11} & c_{11} & c_{11} & \alpha_{11} & \beta_{11} & c_{12} & c_{12} & c_{12} & c_{12} & c_{12} & c_{12} & \beta_{12} & \beta_{12} \\ c_{11} & c_{11} & c_{11} & c_{11} & \beta_{11} & \alpha_{11} & c_{12} & c_{12} & c_{12} & c_{12} & c_{12} & c_{12} & \beta_{12} & \beta_{12} \\ \beta_{12} & c_{21} & c_{21} & c_{21} & c_{21} & c_{21} & \alpha_{22} & \beta_{22} & c_{22} & c_{22} & c_{22} & c_{22} & c_{22} & c_{22} \\ \beta_{21} & c_{21} & c_{21} & c_{21} & c_{21} & c_{21} & \beta_{22} & \alpha_{22} & c_{22} & c_{22} & c_{22} & c_{22} & c_{22} & c_{22} \\ c_{21} & \beta_{21} & \beta_{21} & \beta_{21} & c_{21} & c_{21} & c_{22} & c_{22} & \alpha_{22} & \beta_{22} & \beta_{22} & \beta_{22} & c_{22} & c_{22} \\ c_{21} & \beta_{21} & \beta_{21} & \beta_{21} & c_{21} & c_{21} & c_{22} & c_{22} & \beta_{22} & \alpha_{22} & \beta_{22} & \beta_{22} & c_{22} & c_{22} \\ c_{21} & \beta_{21} & \beta_{21} & \beta_{21} & c_{21} & c_{21} & c_{22} & c_{22} & \beta_{22} & \beta_{22} & \alpha_{22} & \beta_{22} & c_{22} & c_{22} \\ c_{21} & \beta_{21} & \beta_{21} & \beta_{21} & c_{21} & c_{21} & c_{22} & c_{22} & \beta_{22} & \beta_{22} & \beta_{22} & \alpha_{22} & c_{22} & c_{22} \\ c_{21} & c_{21} & c_{21} & c_{21} & \beta_{21} & c\beta_{21} & c_{22} & c_{22} & c_{22} & c_{22} & c_{22} & c_{22} & \alpha_{22} & \beta_{22} \\ c_{21} & c_{21} & c_{21} & c_{21} & \beta_{21} & \beta_{21} & c_{22} & c_{22} & c_{22} & c_{22} & c_{22} & c_{22} & \beta_{22} & \alpha_{22} \end{pmatrix} \quad (6)$$

Where

$$\alpha_{jj} = a_j + b_{jj} + c_{jj} \quad , \quad \beta_{jj'} = b_{jj'} + c_{jj'} \quad , \quad j, j' = 1, 2 \quad \dots(7)$$

Since R is a correlation matrix, then

$$a_j = 1 - b_{jj} - c_{jj} \quad , \quad b_{jj'} = b_{j'j} \quad , \quad c_{jj'} = c_{j'j} \quad \dots(8)$$

Inverting R

To invert R of (5) we use lemma 2 of Urquhart⁽¹²⁾ which is stated in Appendix A. The terms of that lemma are by associating (5) with (A1), (A2), (A3), (A7) and (A8),

$$p, q = jk, j'k' \quad , \quad j, j' = 1, \dots, b \quad , \quad k, k' = 1, \dots, c \quad \dots(9)$$

$$b_{jk} = a_j \quad \forall k \quad \dots(10)$$

$$g_{pq} = g_{jk, j'k'} \quad \left\{ \begin{array}{ll} b_{jj'} + c_{jj} & \text{if } j = j', k = k' \\ c_{jj} & \text{if } j = j', k \neq k' \\ b_{jj'} + c_{jj'} & \text{if } j \neq j', k = k' \\ c_{jj'} & \text{if } j \neq j', k \neq k' \end{array} \right. \quad \dots(11)$$

$$G_{jj'} = (g_{jk, j'k'}) = b_{jj'} I_c + c_{jj'} J_{c \times c} \quad , \quad \text{for } j, j' = 1, \dots, b \quad \dots(12)$$

Then

$$G = (G_{jj'}) = I_c \otimes (b_{jj'}) + J_{cc} \otimes (c_{jj'}) \quad \dots(13)$$

$$B = I_c \otimes \underset{j}{\text{diag}}(a_j) \quad , \quad D = \underset{j, k}{\text{diag}}(n_{jk}) \quad \dots(14)$$

According to (A4), (A5), (A7) and (A8), then we get R^{-1} as follows :

$$R^{-1} = ((R^{-1})_{jk, j'k'}) \quad , \quad \text{of order } n_{jk} \times n_{j'k'} \quad \dots(15)$$

where

$$(R^{-1})_{jk, j'k'} = \delta_{jk, j'k'} a^j I_{n_{jk} \times n_{j'k'}} + h_{jk, j'k'} J_{n_{jk} \times n_{j'k'}} \quad \dots(16)$$

where for

$$H = (h_{jk, j'k'}) \quad \text{for } j, j' = 1, \dots, b \quad , \quad k, k' = 1, \dots, c \quad \dots(17)$$

$$H = D^{-1} [G + BD^{-1}]^{-1} - B^{-1} D^{-1} \quad \dots(18)$$

In (18), the difficult term is F where

$$F=G+BD^{-1} = I_c \otimes (b_{jj'}) + J_{exc} \otimes (c_{jj'}) + [I_c \otimes \text{diag} (a_j)] \text{diag} (n^{jk}) = M+N$$

.....(19)

with

$$M = \text{diag} (a_j n^{jk}) + I_c \otimes (b_{jj'}), \quad N = J_{exc} \otimes (c_{jj'})$$

.....(20)

and we don't know any way to overcome this difficulty to find F^{-1} unless we relax the condition on the number of observations n_{jk} per cell, see Al-Abdullah⁽¹⁰⁾. For this reason we assume that we have n_j observations in the (j,k)th cell for each k. In this case R of (5) become

$$R = \text{diag} (a_j I_{c n_j}) + (b_{jj'} J_{n_j \times n_j} \otimes I_c) + (c_{jj'} J_{c n_j \times c n_j})$$

.....(21)

and D of (14) and its inverse become

$$D = I_c \otimes \text{diag} (n_j), \quad D^{-1} = I_c \otimes \text{diag} (n_j^{-1})$$

.....(22)

and F of (19) becomes

$$F = I_c \otimes (b_{jj'}^*) + J_{exc} \otimes (c_{jj'})$$

.....(23)

where

$$b_{jj'}^* = \delta_{jj'} a_j n_j^{-1} + b_{jj'}$$

.....(24)

Then F of (23) has the same structure of the correlation matrix of Gabbara and Naji⁽⁷⁾ and consequently

$$F^{-1} = I_c \otimes (b^{*jj'}) + (1/c) J_{exc} \otimes (d^{jj'})$$

.....(25)

where

$$d^{jj'} = f^{jj'} - b^{*jj'} \quad , \quad f_{jj'} = cc_{jj'} + b^{*jj'} \quad \dots(26)$$

Substituting (14), (22) and (25) in (18), we get

$$\begin{aligned} H &= \left[I_c \otimes \text{diag}(n^j) \right] \left[I_c \otimes (b^{*jj'}) + (1/c)J_{cxc} \otimes (d^{jj'}) \right] \left[I_c \otimes \text{diag}(n^j) \right] \\ &\quad - \left[I_c \otimes \text{diag}(a^j) \right] \left[I_c \otimes \text{diag}(n^j) \right] \\ &= I_c \otimes (n^j n^j b^{*jj'}) + (1/c)J_{cxc} \otimes (n^j n^j d^{jj'}) - I_c \otimes (\delta_{jj'} a^j n^j) \\ &= I_c \otimes (n^j n^j b^{*jj'} - \delta_{jj'} a^j n^j) + (1/c)J_{cxc} \otimes (n^j n^j d^{jj'}) \quad \dots (27) \end{aligned}$$

Now

$$H = (H_{jj'}) \quad \dots(28)$$

where

$$H_{jj'} = \phi_{1jj'} I_c + \phi_{2jj'} J_{cxc} \quad \text{for } j, j' = 1, \dots, b \quad \dots(29)$$

With

$$\phi_{1jj'} = n^j n^j b^{*jj'} - \delta_{jj'} a^j n^j \quad \dots(30a)$$

$$\phi_{2jj'} = (1/c) n^j n^j d^{jj'} \quad \dots(30b)$$

Also, $H_{jj'}$ can be partitioned as follows :

$$H_{jj'} = (h_{jk, j'k'}) = \begin{bmatrix} h_{j1, j'1} & h_{j1, j'2} & \cdot & \cdot & \cdot & h_{j1, j'c} \\ h_{j2, j'1} & h_{j2, j'2} & \cdot & \cdot & \cdot & h_{j2, j'c} \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ h_{jc, j'1} & h_{jc, j'2} & \cdot & \cdot & \cdot & h_{jc, j'c} \end{bmatrix} \quad (31)$$

where

$$h_{jk, j'k'} = \begin{cases} \phi_{1jj'} + \phi_{2jj'} & \text{if } j = j', k = k' \\ \phi_{2jj'} & \text{if } j = j', k \neq k' \\ \phi_{1jj'} + \phi_{2jj'} & \text{if } j \neq j', k = k' \\ \phi_{2jj'} & \text{if } j \neq j', k \neq k' \end{cases} \quad \dots(32)$$

Using (16) and (32), $(R^{-1})_{jk,j'k'}$ has the following form

$$(R^{-1})_{jk,j'k'} = \begin{cases} \alpha^j I_{n_j} + (\phi_{1jj} + \phi_{2jj}) J_{n_j \times n_j}, & \text{if } j = j', k = k' \\ \phi_{2jj} J_{n_j \times n_j}, & \text{if } j = j', k \neq k' \\ (\phi_{1jj} + \phi_{2jj'}) J_{n_j \times n_j}, & \text{if } j \neq j', k = k' \\ \phi_{2jj'} J_{n_j \times n_j}, & \text{if } j \neq j', k \neq k' \end{cases} \quad (33)$$

Therefore

$$\begin{aligned} (R^{-1})_{jj'} &= ((R^{-1})_{jk,j'k'}) \\ &= \delta_{jj'} \alpha^j I_{cn_j} + \phi_{1jj'} J_{n_j \times n_j} \otimes I_c + \phi_{2jj'} J_{cn_j \times cn_j} \end{aligned} \quad (34)$$

Now, using (15) and (34), then we get R^{-1} in the following form :

$$\begin{aligned} R^{-1} &= ((R^{-1})_{jj'}) , \text{ for } j, j' = 1, \dots, b \\ &= (\delta_{jj'} \alpha^j I_{cn_j}) + (\phi_{1jj'} J_{n_j \times n_j} \otimes I_c) + (\phi_{2jj'} J_{cn_j \times cn_j}) \end{aligned} \quad (35)$$

Applications

In this section, we consider a cattle breeding experiment of Gabbara and Al-Abdulla⁽¹¹⁾ when $n_j = n$ for each j and certain 3-way random and / or mixed effects models as applications to illustrate the importance of this paper results.

The Cattle Breeding Experiment

Let Y_{ijkl} be the weight at nine months of the l th calf from the k th cow and j th bull in the i th herd with $i = 1, \dots, m, j = 1, \dots, b, k = 1, \dots, c, l = 1, \dots, n$. We assume that all measurements have the same variance σ^2 and every pair of measurements has certain covariance which is given as follows :

$$\text{Cov}(Y_{ijkl}, Y_{i'j'k'l'}) = \begin{cases} \sigma^2 & \text{if } i = i', j = j', k = k', l = l' \\ \sigma^2 \rho_4 & \text{if } i = i', j = j', k = k', l \neq l' \\ \sigma^2 \rho_3 & \text{if } i = i', j = j', k \neq k' \\ \sigma^2 \rho_2 & \text{if } i = i', j \neq j', k = k' \\ \sigma^2 \rho_1 & \text{if } i = i', j \neq j', k \neq k' \\ 0 & \text{if } i \neq i' \end{cases} \quad (36)$$

Let $Y_i = (Y_{i1}, \dots, Y_{iben})'$ be the vector of observations of the i th herd and let $y = (y'_1, \dots, y'_m)'$ be the vector of all observations of the experiment. In terms of (6), with $n_{jk} = n_j$ for each k , then

$$a_j = 1 - \rho_4, \quad b_{jj'} = \begin{cases} \rho_4 - \rho_3 & \text{if } j=j' \\ \rho_2 - \rho_1 & \text{if } j \neq j' \end{cases} \quad c_{jj'} = \begin{cases} \rho_3 & \text{if } j=j' \\ \rho_1 & \text{if } j \neq j' \end{cases} \quad (37)$$

Therefore, R of (12) becomes

$$R = (1 - \rho_4) I_{bcn} + (\rho_4 - \rho_3 - \rho_2 + \rho_1) J_{n \times n} \otimes I_{bc} + (\rho_3 - \rho_1) J_{cn \times cn} \otimes I_b + (\rho_2 - \rho_1) J_{n \times n} \otimes I_c \otimes J_{b \times b} + J_{bcn \times bcn} \quad (38)$$

Now to invert R we only need to invert F of (23) and according to (25), we only need to invert.

$$(b^*_{jj'}) = (\delta_{jj'} a_j n_j + b_{jj'}) = [\rho_4 - \rho_3 - \rho_2 + \rho_1 + ((1 - \rho_4) / n)] I_b + (\rho_2 - \rho_1) J_{b \times b} \quad (39)$$

$$(f^*_{jj'}) = (c c_{jj'} + b^*_{jj'}) = [\rho_4 - \rho_3 - \rho_2 + \rho_1 + ((1 - \rho_4) / n) + c(\rho_3 - \rho_1)] I_b + [\rho_2 + (c + 1)\rho_1] J_{b \times b} \quad (40)$$

and both matrices have the well-known structure of a patterned matrix

$$(a - b) I_s + b J_{s \times s} \quad (41)$$

where its inverse is known to be

$$(a - b)^{-1} [I_s - (b / (a + (s - 1) b)) J_{s \times s}] \quad (42)$$

see Graybill⁽¹³⁾, p 191. Then

$$(b^*_{jj'}) = (1 / \theta_1) [I_b - (\theta_2 / \theta_3) J_{b \times b}] \quad (43a)$$

$$(f^*_{jj'}) = (1 / \theta_4) [I_b - (\theta_5 / \theta_6) J_{b \times b}] \quad (43b)$$

where

$$\begin{aligned} \theta_1 &= \rho_4 - \rho_3 - \rho_2 + \rho_1 + (1 + \rho_4) / n, & \theta_2 &= \rho_2 - \rho_1, & \theta_3 &= \theta_1 + b\theta_2 \\ \theta_4 &= \theta_1 + c(\rho_3 - \rho_1), & \theta_5 &= \theta_2 + c\rho_1, & \theta_6 &= \theta_4 + b\theta_5 \end{aligned} \quad (44)$$

Substituting (43a) and (43b) in (26), we get

$$d^{jj'} = \begin{cases} \frac{\theta_6 - \theta_5}{\theta_4 \theta_6} - \frac{\theta_3 - \theta_2}{\theta_1 \theta_3} = \frac{\theta_1 \theta_3 \theta_6 - \theta_1 \theta_3 \theta_5 - \theta_3 \theta_4 \theta_6 + \theta_2 \theta_4 \theta_6}{\theta_1 \theta_3 \theta_4 \theta_6}, & j = j' \\ -\frac{\theta_5}{\theta_4 \theta_6} + \frac{\theta_2}{\theta_1 \theta_3} = \frac{\theta_2 \theta_4 \theta_6 - \theta_1 \theta_3 \theta_5}{\theta_1 \theta_3 \theta_4 \theta_6}, & j \neq j' \end{cases} \quad (45)$$

Now, substituting (37), (43a) and (45) in (30a) and (30b), we get

$$\phi_{ijj'} = \begin{cases} \frac{\theta_3 - \theta_2}{n^2 \theta_{16} \theta_3} - \frac{1}{n(1 - \rho_4)} = \frac{(1 - \rho_4)(\theta_3 - \theta_2) - n\theta_1 \theta_3}{n^2 (1 - \rho_4) \theta_1 \theta_3} = \lambda_1, & j = j' \\ \frac{\theta_3 - \theta_2}{n^2 \theta_1 \theta_3} = \lambda_2, & j \neq j' \end{cases} \quad (46a)$$

$$\phi_{2ij'} = \begin{cases} \frac{\theta_1 \theta_3 \theta_6 - \theta_1 \theta_3 \theta_5 - \theta_3 \theta_4 \theta_6 + \theta_2 \theta_4 \theta_6}{cn^2 \theta_1 \theta_3 \theta_4 \theta_6} = \lambda_3, & j = j' \\ \frac{\theta_2 \theta_4 \theta_6 - \theta_1 \theta_3 \theta_5}{cn^2 \theta_1 \theta_3 \theta_4 \theta_6} = \lambda_4, & j \neq j' \end{cases} \quad (46b)$$

Substituting (46) in (35), we get R^{-1} in the following formula

$$R^{-1} [1 / (1 - \rho_4)] I_{bcn} + (\lambda_1 - \lambda_2) J_{n \times n} \otimes I_{bc} + (\lambda_3 - \lambda_4) J_{cn \times cn} \otimes I_b + \lambda_2 J_{n \times n} \otimes I_c \otimes J_{b \times b} + \lambda_4 J_{bcn \times bcn} \quad (47)$$

3-Way Random Effects Model,

We consider the 3-way random effects model which has the form

$$Y_{ijkl} = \theta + a_i + b_{ij} + c_{ik} + (bc)_{ijk} + e_{ijkl} \quad (48)$$

with $i=1, \dots, m; j=1, \dots, b; k=1, \dots, c; l=1, \dots, n;$ and θ is unknown parameter.

The $a_i, b_{ij}, c_{ik},$ and e_{ijkl} are independent random variables with zero expectations and respective variances $\sigma_a^2, \sigma_b^2, \sigma_c^2, \sigma_{bc}^2$ ad σ_e^2 . We note that Y_{ijkl} and $Y_{i'j'k'l'}$, are not independent for the 3-way random model of (48) and $cov(Y_{ijkl}, Y_{i'j'k'l'})$ has the same form given in (36) with

$$\sigma^2 = \sigma_a^2 + \sigma_b^2 + \sigma_c^2 + \sigma_{bc}^2 + \sigma_e^2, \quad \rho_4 = (\sigma_a^2 + \sigma_b^2 + \sigma_c^2 + \sigma_{bc}^2) / \sigma^2, \\ \rho_3 = (\sigma_a^2 + \sigma_b^2) / \sigma^2, \quad \rho_2 = (\sigma_a^2 + \sigma_c^2) / \sigma^2, \quad \rho_1 = \sigma_a^2 / \sigma^2 \quad (49)$$

Hence, its inverse is the same of that given in (47)

3-Way Mixed Effects Models

Consider 3-way mixed effects models which have the form

$$(i) Y_{ijkl} = \theta + a_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (a\beta\gamma)_{ijk} + e_{ijkl} \quad (50)$$

$$(ii) Y_{ijkl} = \theta + a_i + b_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (a\beta\gamma)_{ijk} + e_{ijkl} \quad (51)$$

where the Greek letters $(\theta, \alpha, \beta, \dots)$ and the Roman letters (a, b, c, \dots) have the classical meaning of that used in ANOVA models. And both models can be worked out by the same manner of 3-way random effect model.

CONCLUSIONS

1. The method adopted in this work for matrix inversion is useful and convenient for many types of matrices that have the structure of (A1), and it reduces the problem to the inversion of a smaller order than the original matrix.
2. We can also use the result of this work to any of the ANOVA models used or mentioned in this paper after adding any number of fixed effects to the models as long as those added effects do not interact with any of the random effects already in the model, since those added effects do not affect the structure of the covariance matrix.
3. The result of this work can be used to assist in finding elements of the information matrix for the variance components of the linear models under normality given above and any other linear model that has covariance matrix of the form (5).
4. For a more general model of (5), when we replaced b and c by b_i and c_i , respectively for each i , the result will not be affected since this assumption will not change the structure of the covariance matrix, but only its order.

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Appendix

The following is lemma 2 of Urquhart (1962).

Lemma : For the matrix A partitioned as

$$A = (A_{pq} \text{ of order } n_p \times n_q), \quad \text{for } p, q = 1, \dots, N \quad (A1)$$

where

$$A_{pq} = \delta_{pq} b_p I_{n_p \times n_q} + g_{pq} J_{n_p \times n_q} \quad (A2)$$

with

$$G = (g_{pq}) \text{ and } \delta_{pp} = 1, \delta_{pq} = 0 \text{ for } p \neq q \quad (A3)$$

then

$$A^{-1} = ((A^{-1})_{pq} \text{ of order } n_p \times n_q) \quad (A4)$$

with

$$(A^{-1})_{pq} = \delta_{pq} (1/b_p) I_{n_p \times n_q} + h_{pq} J_{n_p \times n_q} \quad (A5)$$

$$H = (h_{pq}) = [(GD+B)^{-1} - B^{-1}] D^{-1} \quad (A6)$$

with

$$D = \text{diag} (n_p) = \text{diag} (n_1, n_2, \dots, n_N) \quad (A7)$$

and

$$B = \text{diag} (b_p) = \text{diag} (b_1, b_2, \dots, b_N) \quad (A8)$$

Note that in (A6)

$$H = D^{-1} [G+BD^{-1}]^{-1} D^{-1} - B^{-1} D^{-1} \quad (A9)$$

π -Regularity and Full π -Stability on Commutative Rings

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الخلاصة

قدمت الحلقات المنتظمة من النمط π - من قبل ماكوي كأعمام للحلقات المنتظمة. في هذا البحث تم اعطاء وصف جديد لهذه الحلقات. بعين هذا الالهام تم عرض حلقات تامة الاستقرارية من النمط π - كأعمام لحلقات تامة الاستقرارية. العديد من الصفات والتمييزات لهذا الصنف من الحلقات درست. تم وصف الحلقات المنتظمة من النمط π - بدلالة حلقات تامة الاستقرارية من النمط π -، كما درست الانتظامية من النمط π - للحلقات التقليدية للقواسم. وأخيراً تم تحديد شكل المثاليات الجزئية العظمى لحلقات تامة الاستقرارية من النمط π -.

ABSTRACT

π - regular rings was introduced by N.H.Mc Coy as a generalization of Von Neumann regular rings. In this paper a new characterization of π -regular rings is established. Inspired of McCoy's definition we introduce fully π -stable rings as a generalization of fully stable rings. Various properties and characterization of such rings are obtained. Also, we characterize π -regular rings in terms of fully π -regular rings, and we study π -regularity of the classical rings of quotients. Finally, we determine the form of the maximal ideals in the class of fully π -stable rings.

INTRODUCTION

Nicholson in⁽¹⁾, introduced the concept of semi-regular modules as a generalization of regular modules. A submodule N of an R -module M is said to lie over a direct summand, if there exists a direct decomposition $M = P \oplus Q$ with $P \subseteq N$ and $Q \cap N$ is a small sub module of M . He termed an R -module M semi-regular if, each cyclic submodule of M lies over a projective direct summand. A ring R is called semi-regular, if it is semi-regular R -module. Regular rings are generalized by N.H. McCoy, namely π -regular rings. A ring R is called π -regular, if for each x in R , there exist y in R and a positive integer n such that $x^n = x^n yx^{n(2)}$. A submodule N of an R -module M is called stable if, $\alpha(N) \subseteq N$ for $\alpha \in$

$\text{Hom}_g(N, M)$. In case each submodule of M is stable, the R -module M is called fully stable. A ring R is fully stable, if it is fully stable R -module⁽³⁾.

Section one of this work is concerned with a new characterization of π -regular rings analogous to that of semi-regular. Analogous results on regular rings are proved for π -regular rings. Self-injective π -regular rings are characterized. Inspired by the definition of McCoy we investigate a generalization of fully stable rings, namely fully π -stable rings. Various characterizations of fully π -stable rings are established. We study π -regularity of the classical rings of quotients. Finally π -regular rings are characterized in terms of fully π -stable rings.

Section two is devoted to study the form of the maximal ideals of the fully π -stable (and hence π -regular) rings.

Throughout, R represents a commutative ring with identity, and all modules are left unitary.

π - Regular Rings and Fully π -Stable Rings

Recall that, a ring R is π -regular, if for each α in R , there exist b in R and a positive integer n such that $\alpha^n = \alpha^n b \alpha^n$ ⁽²⁾. This is equivalent to saying that, for each a in R , there exists b in R such that $a - aba \in L(R)$, where $L(R)$ is the prime radical of R ⁽⁴⁾. Thus regular rings are precisely π -regular rings with zero prime radical. It is well-known that, a ring R is regular if, and only if, each principal ideal is a direct summand of R ⁽⁵⁾. P.p. rings are defined as those rings in which each principal ideal is projective⁽³⁾. Then every regular ring is a p.p. ring.

We introduce the following: A ring R is said to be π -p.p. ring if, each x in R , there exists a positive integer m such that Rx^m is a projective ideal. Thus we have the following analogous result for π -regular rings.

Proposition

R is a π -regular ring if, and only if, for each x in R , there exists a positive integer n such that Rx^n is a direct summand of R (hence, every π -regular ring is π -p.p.).

Proof

For each x in R , there exist y in R and a positive integer n such that $x^n = x^n y x^n$. Put $e = x^n y$, then $e^2 = e$, which implies that $Rx^n = Re$. Hence $R = Rx^n \oplus R(1-e)$. Conversely, for each x in R , there exists a positive integer n such that Rx^n is a direct summand of R . Thus $Rx^n = Rg$ for some idempotent g in R . This equality implies that, there exist r, s in R such that $x^n = rg$ and $g = sx^n$, then $x^n = rg = rgsx^n = x^n sx^n$.

Let M be an R -module, the dual of M will be denoted by $M^* = \text{Hom}_R(M, R)$. A dual basis for M is a pair of subsets $\{x_i \mid I \in I\} \subseteq M^*$ (indexed by the

same set) such that for each $x \in M$, $\varphi_i(x) = 0$ for all but finitely many $i \in I$ and $x = \sum_i \varphi_i(x)x_i$. It is well-known that, M is (finitely generated) projective if, and only if, it has a (finite) dual basis⁽¹⁾. The R -modula M is called regular if, for each $m \in M$, $m = \alpha(m)m$ for some α in $M^{*(6)}$.

We need the following lemma⁽¹⁾:

Lemma

Let M be an R -modula and $x \in M$ be a regular element. If $\alpha \in M^*$ satisfies $\alpha(x)x = x$ and if we write $e = \alpha(x)$, then :

- (1) $e^2 = e$ and $x = ex$.
- (2) $Rx \xrightarrow{\alpha} Re$ is an isomorphism, so Rx is projective.
- (3) $M = Rx \oplus W$, where $W = \{w \in M \mid \alpha(w)x = 0\}$.

We introduce the following :

Definition

Let R be a ring. An ideal I of R is said to lie over a direct L -summand, if there exists a direct decomposition $R = P \oplus Q$ with $P \subseteq I$ and $Q \cap I \subseteq L(R)$.

Proposition

Let R be a ring, then the following statements are equivalent for an element x in R .

- (1) Rx lies over a projective L -summand of R .
- (2) There exists $\alpha \in R^*$ such that $(\alpha(x))^2 = \alpha(x)$ and $x - \alpha(x)x \in L(R)$.
- (3) There exists a regular element $y \in Rx$ such that $x - y \in L(R)$ and $Rx = Ry \oplus R(x-y)$.
- (4) There exists a regular element $y \in R$ such that $x - y \in L(R)$.
- (5) There exists $\wp: R \rightarrow Rx$ such that $\wp^2 = \wp$ (R) is projective and $x - \wp \in L(R)$.

Proof

(1) implies (2). Assume that, there exists a direct decomposition $R = P \oplus Q$ with $P \subseteq Rx$ is projective and $Q \cap Rx \subseteq L(R)$. Then P is a finitely generated projective R -modula, so it has a finite dual basis x_1, \dots, x_n and $\varphi_1, \dots, \varphi_n$. Put $x_i = r_i x$, and define $\alpha: P \rightarrow R$ by $\alpha(p) = \sum_i r_i \varphi_n(p)$, for each $p \in P$. Then α can be extended to all R by putting $\alpha(Q) = 0$. If $x = p + q$, where $p \in P$ and $q \in Q$, then $\alpha(x)x = p$ and $(\alpha(x))^2 = \alpha(x)$, so $x - \alpha(x)x = x - p = q \in Q \cap Rx \subseteq L(R)$.

(2) implies (3), Let $\alpha \in R^*$ such that $(\alpha(x))^2 = \alpha(x)$ and $x - \alpha(x)x \in L(R)$. Putting $y = \alpha(x)x$, then $\alpha(y)y = y$, so y is a regular element and $x - y \in L(R)$. Lemma (1.2) implies that, $R = Ry \oplus W$ where $W = \{w \in R \mid \alpha(w)y = 0\}$, thus $Rx \cap W = R(x-y)$. Now $Rx = Rx \cap (Ry \oplus W) = Ry \oplus R(x-y)$.

(3) implies (4). Obvious.

(4) implies (5). Suppose that there is a regular element in R such in R such that $x - y \in L(R)$. Then there exists $\alpha \in R^*$ such that $y = \alpha(y)y$. If $e = \alpha(y)$, then $x - ex = (1-e)(x-y) \in L(R)$. We claim that ex is regular. Indeed $e - \alpha(x) = \alpha(y-x) \in L(R)$. So, if $(1-e+\alpha(x))b = 1$ for some $b \in R$, then $\alpha(b) \in R^*$ and $[\alpha(b)(ex)](ex) = \alpha(bex)(ex) = e(ex)ex$. Hence, we can assume that $y \in Rx$. By lemma (1.2), $R = Ry \oplus E$, where $W = \{w \in R \mid \alpha(w)y = 0\}$. If $\wp : R \rightarrow Ry$ is the natural projection, then $\wp^2 = \wp$ and $\wp(R) = Ry$ is projective. It remains to show that $x - \wp(x) \in L(R)$. If $x = ry + w$ for some $r \in R$ and $w \in W$, then $0 = \alpha(x-ry)y = \alpha(x)y - ry$. So, $\wp(x) = ry = \alpha(x)y$. Hence $x - \wp(x) = x - \alpha(x)y = (x-y) - y\alpha(x-y) \in L(R)$.

(6) implies (1) obvious.

Definition

Let R be a ring, an element x in R is called L-regular if it satisfies the conditions of proposition (1.4). A ring R is called L-regular if, each element of R is L-regular.

The following corollary is immediate :

Corollary

For each two elements x and y in R , if y is L-regular and $x - y \in L(R)$, then x is L-regular.

Remarks

- (1) The regular rings are precisely the L-regular rings with zero prime radical.
- (2) Every L-regular ring with small Jacobson radical is semi-regular.
- (3) It follows from proposition (1.4)⁽²⁾ that the concept of L-regularity and π -regularity coincide.

It is well-known that a ring R is regular if, and only if, each principal ideal of R is direct summand if, and only if, each finitely generated ideal of R is direct summand. The following theorem is an analogous result for L-regular rings.

Theorem

The following statements are equivalent:

(1) R is L -regular.

(2) If I is a finitely generated ideal of R , then there exists an R -homomorphism $\wp : R \rightarrow I$ such that $\wp^2 = \wp$, $\wp(R)$ is projective and $(1 - \wp)(I) \subseteq L(R)$.

(3) Every finitely generated ideal of R lies over a projective L -summand.

Proof

(3) implies (1). It follows from proposition (1.4).

implies (3). Let I be any finitely generated ideal of R , then there exists $\wp : R \rightarrow I$ such that $\wp^2 = \wp$, $\wp(R)$ is projective and $(1 - \wp)(I) \subseteq L(R)$. Thus $R = \wp(R) \oplus (1 - \wp)(R)$ and $(1 - \wp)(R) \cap I = (1 - \wp)(I) \subseteq L(R)$.

implies (2). Notice that proposition (1.4) starts an induction on the number of generators of I . Suppose that $I = Rx_1 + \dots + Rx_n$ be a finitely generated ideal of R .

Then there exists $\beta : R \rightarrow Rx_n$ such that $\beta^2 = \beta$, $\beta(R)$ is projective and $(1 - \beta)(Rx_n) \subseteq L(R)$. Put $K = (1 - \beta) \left(\sum_{i=1}^{n-1} Rx_i \right)$, K is an ideal of R generated by $(n-1)$

elements. By induction, there exists $\delta : R \rightarrow K$ such that $\delta^2 = \delta$, $\delta(R)$ is projective and $(1 - \delta)(K) \subseteq L(R)$. Define $\wp = \beta + \delta - \delta\beta$. Then $\wp^2 = \wp$ and $\wp(R) \subseteq I$ and $(1 - \wp)(I) \subseteq L(R)$.

According to remark (1.7) (3), an ideal I is called L -regular if, for each x in I , there exists y in R and a positive integer n such that $x^n = x^n y x^n$. This is equivalent to saying that, there exists a positive integer n such that Rx^n is a direct summand of R (see proposition (1.1)).

It is well-known that, if R is a self-injective ring, then $R/J \oplus$ is regular and hence L -regular ring⁽¹⁾. On the other hand, in a self-injective ring we have $J(R) = Z(R)$ ⁽¹⁾. Thus every non-singular self-injective ring is L -regular. Self-injective L -regular rings are characterized as follows:

Theorem

Let R be a ring in which the singular submodule of each maximal ideal is reduced. Then the following statements are equivalent:

(1) R is a self-injective L -regular ring.

(2) R is a self-injective ring containing a maximal ideal which is L -regular in R .

(3) There exists a maximal ideal M of R such that, for each x in M , there exists a positive integer n such that $\text{ann}_R(x^n)$ is an injective R -module.

(4) R is a self-injective ring containing a non-singular maximal ideal.

Proof

(1) implies, (2) obvious

(2) implies (3). Let I be a maximal ideal which is L -regular in R . For any element $y \in I$, there exists a positive integer n such that Ry^n is direct summand. Hence $\text{ann}_R(y^n)$ is a direct summand of R and so an injective R -module.

(3) implies (4). Obviously, $R = \text{ann}_R(0)$ is an injective R -module. If $z \in Z(M)$, then $\text{ann}_R(z^m)$ is an essential ideal of R for each positive integer m . But $\text{ann}_R(z^n)$ is an injective R -module for some positive integer n . Then $R = \text{ann}_R(z^n)$. This implies that $z^n = 0$ and hence $z = 0$.

(4) implies (1). Let M be a non-singular maximal ideal of R . If M is essential, but $Z(M) = Z(R) \cap M$, then $Z(R) = 0$ and hence R is L -regular. Otherwise, if M is non-essential, then there exists a non-zero ideal N of R such that $M \cap N = (0)$. Maximality of M implies that $M \oplus N = R$, where $M = Re$ and $N = R(1-e)$, $e = e^2 \in R$. If $z (\neq 0) \in Z(R)$, then $z = ze - z(1-e)$, hence $ze \in Z(R) \cap M = Z(M) = 0$, which yields $z = z(1-2e)$, where $N = Rz$. Since $\text{ann}_R(z)$ is a direct summand of R and essential in R , then $R = \text{ann}_R(z)$ and hence $z = 0$, a contradiction. This shows that, in either case R is non-singular and hence L -regular.

Next, we introduce the concept of fully π -stable rings as generalization of fully stable rings, which is analogous to the definition of N.H.McCoy for π -regular rings.

Definition: An element x in R is said to be π -stable, if there exists a positive integer n such that $\alpha(Rx^n) \subseteq Rx^n$, for each R -homomorphism α of Rx^n into R . The ring R is called fully π -stable if all its elements are π -stable.

It is known that a ring R is regular if, and only if, R is a fully stable p.p. ring⁽³⁾.

Properties: If R is a fully π -stable ring and π .p.p. Then for each x in R , there exists a positive integer n . Define $\alpha: Rx^n \rightarrow Rx^n$ by $\alpha(r) = rx^n$ for each r in R . Then there exists an R -homomorphism $\beta: Rx^n \rightarrow R$ such that $\alpha \circ \beta = I_{Rx^n}$. Full π -stability of R implies that there exist an element $t \in R$ and a positive integer m such that $\beta(x^{nm}) = t(x^{nm})$. Now $x^{nm} = \alpha(\beta(x^{nm})) = \alpha(tx^{nm}) = tx^{nm}x^n = sx^{nm+1}$ where $s = tx^{n-1}$. Thus x^{nm} is π -regular element⁽⁴⁾.

It is clear that, every π -regular ring is fully π -stable, for the converse we have the following:

Corollary: Let R be a p.p. ring. Then R is a fully π -stable ring if, and only if, R is π -regular.

This theorem gives various characterizations of fully π -stable rings.

Theorem

The following statements are equivalent:

- (1) R is fully π -stable ring.
- (2) For any two elements x, y in R , there exists a positive integer n such that, if $y \notin Rx^n$, then $\text{ann}_R(x^n) \subsetneq \text{ann}_R(y)$.
- (3) For each x in R , there is a positive integer n such that $\text{ann}_R(\text{ann}_R(x^n)) = (x^n)$.
- (4) For any two elements x, y in R , there exists a positive integer n such that $\text{ann}_R[\text{ann}_R(x^n) + Ry] = Rx^n \cap \text{ann}_R(y)$.
- (5) For any two elements x, y in R , there exists a positive integer n such that $\text{ann}_R[\text{ann}_R(x^n) \cap Ry^n] = Rx^n + \text{ann}_R(y^n)$.

Proof: (1) implies (2). Assume that there are two elements x, y in R such that $y \notin Rx^n$ and $\text{ann}_R(x^n) \subseteq \text{ann}_R(y)$ for each positive integer n . Define $f: Rx^n \rightarrow R$ by $f(rx^n) = ry$ for each r in R . Then there is $t \in R$ such that $f(w) = tw$ for each w in Rx^n . In particular, $y = f(x^n) = tx^n \in Rx^n$, a contradiction

(2) implies (3). Let $y \in \text{ann}_R(\text{ann}_R(\text{ann}_R(x^n)))$ for each positive integer n . Then $\text{ann}_R(x^n) \subseteq \text{ann}_R(y)$. By (2), there exists a positive integer n such that $y \in Rx^n$. In particular $\text{ann}_R(\text{ann}_R(x^n)) \subseteq Rx^n$.

(3) Implies (4). Obvious.

(4) Implies (1). For each $x \in \text{ann}_R(x^n)$, then $sf(x^n) = f(sx^n) = 0$, hence $f(x^n) \in \text{ann}_R(\text{ann}_R(x^n)) = (x^n)$. Thus $f(Rx^n) \subseteq Rx^n$.

(5) implies (1). By taking $x = 1$ and by (3) we get (1),

implies (5). Let x, y be two elements of R . If $a \in \text{ann}_R[\text{ann}_R(x^m) \cap Ry^m]$ for each positive integer m , then $\text{ann}_R(x^m y^m) \subseteq \text{ann}_R(ay^m)$. By (2), there exists a positive integer n such that $ay^n \in Rx^n y^n$. Thus $ay^n = rx^n y^n$ for some $r \in R$, so $a - rx^n \in \text{ann}_R(y^n)$ and hence $a \in \text{ann}_R(y^n) + Rx^n$.

It is clear that every ideal of a fully stable ring is a fully stable R -module. For fully π -stable rings we have:

Corollary

Every reduced ideal of a fully π -stable ring is fully R -module.

Proof

Let I be a reduced ideal of R . For each x in I , there exists a positive integer n such that $\text{ann}_R(\text{ann}_R(x^n)) = (x^n)$. Since $\text{ann}_R(x^n) = \text{ann}_R(x)$, then $Rx \subseteq \text{ann}_R$

$(\text{ann}_R(x)) = \text{ann}_R(\text{ann}_R(x^n)) = (x^n) \subseteq (x)$. Therefore each principle ideal of I is annihilator. So I is a fully stable R -module⁽³⁾.

Corollary

Any reduced ideal of a fully π -stable ring is regular (In particular, every fully π -stable ring with zero prime radical is regular).

Proof

Let I be reduced ideal of a fully π -stable ring R . By cocollary (1.14), R is fully stable. Thus each principal ideal of R is annihilator. So, for each $x \in I$, $Rx = \text{ann}_R(\text{ann}_R(x)) = \text{ann}_R(\text{ann}_R(x^2)) = Rx^2$, which implies that $x = xyx$ for some $y \in R$.

Proposition

If R is a fully π -stable ring, then every non zero – divisor in R is invertible every fully π -stable ring is classical).

Proof

If x is any non zero-divisor in R , then there exists a positive integer n such that $\text{ann}_R(\text{ann}_R(x^n)) = (x^n)$, but $\text{ann}_R(x^n) = 0$, then $Rx^n = R$.

Corollary

Let R be a fully π -stable ring, then $Z(R) \subseteq J(R)$.

Proof

Let y be any element of $Z(R)$, then $\text{ann}_R(1-ry) = 0$ for each r in R , since $\text{ann}_R(y)$ is essential in R . Hence $1-ry$ is a non zero-divisor, then $1-ry$ is inevitable (proposition (1.16)). Thus $y \in J(R)$.

For the converse of proposition (1.16), recall that a ring R is chained of the ideals of R are linearly ordered by inclusion⁽⁸⁾.

Proposition

Let R be a chained ring. Then R is full π -stable ring if, and only if, every element of R is either a zero – divisor or invertible.

Proof

For each $x \in R$, suppose that there exists $y \in \text{ann}_R(\text{ann}_R(x^k))$ such that $y \notin (x^k)$ for each positive integer k . Then $Rx^k \subseteq Ry$. Let M be the unique maximal ideal of R ⁽⁸⁾. So $x^k = my$ for some $m \in M$. Thus $Ry \subseteq \text{ann}_R(\text{ann}_R(x^k)) = \text{ann}_R(\text{ann}_R(my))$. By taking the annihilator, we have $\text{ann}_R(my) \subseteq \text{ann}_R(y)$. Therefore

$Ry \cap \text{ann}_R \cap \text{ann}_R(m) = (0)$ and as the ideals of R are totally ordered, this implies that $\text{ann}_R(m) = (0)$ a contradiction, since m is a zero-divisor. Thus $\text{ann}_R(\text{ann}_R(x^n)) = (x^n)$ for some positive integer n .

R. Yue Chi in⁽⁹⁾ introduced the concept of p -injective modules as a generalization of injective modules to study regular rings. An R -module M is p -injective if, for each principal ideal P of R , each R -homomorphism of P into M extends to all R . A ring R is regular if, and only if, every R -module is p -injective⁽⁹⁾. Recall that an R -module M is π - p -injective if, for each x in R , there exists a positive integer n such that each R -homomorphism $\alpha : Rx^n \rightarrow M$ extends to all R . This is equivalent to saying that, for each x in R , there exists a positive integer n such that for each R -homomorphism α of Rx^n into M , there exists $m \in M$ such that $\alpha(t) = tm$ for each t in Rx^n . R is called π - p -injective ring if R is π - p -injective R -module. Observe that π - p -injectivity and full π -stability on rings coincide.

In this theorem we characterize π -regular rings in terms of π - p -injective R -modules:

Theorem

R is π -regular if, and only if, every R -module is π - p -injective.

Proof

Let M be an R -module and $x \in R$, then there is a positive integer n and $r \in R$ such that $x^n = x^n rx^n$. For each R -homomorphism $\alpha : Rx^n \rightarrow M$, $\alpha(x^n) = x^n \alpha(rx^n) = x^n y$ where $y = \alpha(rx^n) \in M$, then M is π - p -injective.

Conversely, for each $x \in R$, there exists a positive integer n such that Rx^n is π - p -injective. Consider the identity mapping of Rx^n . There exists $y \in Rx^n$ such that $x^n = I(x^n) = yx^n = x^n sx^n$ for some $s \in R$.

In this part, we study π -regularity of the classical rings of quotients:

Theorem

Let R be a ring with its classical ring of quotients Q . Then Q is π -regular if, and only if, every divisible torsionfree R -module is π - p -injective.

Proof

Assume first that Q is π -regular and M be a divisible torsionfree R -module. For every $x \in R$, there exists a positive integer n such that x^n is a regular element in Q . By the proof of [9 Theorem 3.3], M is canonically a Q -module. For each R -homomorphism $\alpha : Rx^n \rightarrow M$, define a Q -homomorphism $\beta : Qx^n \rightarrow M$ by

$\beta\left(\frac{q_1}{1_2}x^n\right) = \frac{\alpha(q_1x^n)}{q_2}$ for all $q_1, q_2 \in R$ such that q_2 is non zero-divisor. Thus α is

the restriction of β to Rx^n , by taking $q_2 = 1$. Since Q is π -regular, theorem (1.18) implies that, there exists an element $y \in M$ such that $\beta(u) = yu$ for each u in Qx^n . In particular $\alpha(x^n) = \beta(x^n) = yx^n$. hence M is π -p-injective R -module.

Conversely, assume that every divisible torsionfree R -module is π -p-injective. For each $y \in Q$, let $y = db^{-1}$ where $d, b \in R$ and b is non zero - divisor, then $y^m \in Q$ for each positive integer m such that $y^m = d^m b^{-m}$. The R -module $C = Qy^m$ is divisible torsionfree, hence π -p-injective. Then there exists a positive integer n such that $\alpha : Rd^n \rightarrow C$ is the inclusion mapping, where $Rd^n \subseteq C$. π -p-injectivity of C implies that, there exists $c \in C$ such that $\alpha(rd^n) = rd^n c$ for all $r \in R$. In particular, $d^n = d^n c = d^n qy^n$ for some $q \in Q$. Multiplying both sides by b^n we have $y^n = y^n qy^n$ which yields Q is π -regular.

Now, we give a new characteristic property for π -regular rings in terms of fully π -stable rings:

Theorem

The following statements are equivalents:

- (1) R is π -regular
- (2) R is a fully π -stable ring whose divisible torisonfree modules are π -p-injective.
- (3) For any non-trivial element x in R , there exists a positive integer n , non-trivial idempotent element e and a non zero - divisor element d such that $Rx^n = Red$.

Proof

(1) implies (2) and (3). Obvious, by using the fact that every π -regular ring is a classical.

(2) implies (1). Follows from proposition (1.16) and theorem (1.20).

(3) implies (1). For any non-trivial element x in R , there exist a positive integer n , a non trivial idempotent e and a non zero-divisor d such that $Rx^n = Red$. We claim that, every non zero-divisor in R is invertible. Let $c (\neq 0)$ be a non zero - divisor element in R with $Re \neq R$. By (3), there exists a positive integer k such that $Re^k = Rub$ where u is non - trivial idempotent and non zero-divisor b in R . Then $0 = \text{ann}_R(e^k) = \text{ann}_R(u) = R(1-u)$ which contradicts $u \neq 1$. Thus $Rx^n = Re$, hence R is π -regular.

Maximal Ideals of Fully π -stable Rings

We start this section with several results on fully π -stable rings.

Proposition

Let R be a fully π -stable ring and $Rx_1^{k_1} \oplus \dots \oplus Rx_n^{k_n}$ be a direct sum, where $x_i \in R$ and k_i is a positive integer ($i = 1, \dots, n$). Then any R -homomorphism of $Rx_1^{k_1} \oplus \dots \oplus Rx_n^{k_n}$ into R can be extended to an R -endomorphism of R .

Proof

Let $\alpha : Rx_1^{k_1} \oplus \dots \oplus Rx_n^{k_n} \rightarrow R$ be an R -homomorphism. Full π -stability of R implies that, there exist an element b in R and a positive integer t such that $\alpha[(x_1^{k_1} + \dots + x_n^{k_n})^t] = b(x_1^{tk_1} + \dots + x_n^{tk_n})$. Or $\alpha(x_1^{tk_1} + \dots + x_n^{tk_n}) = b(x_1^{tk_1} + \dots + x_n^{tk_n})$, since the sum is direct. For each $i = 1, \dots, n$, put $\alpha_i = \alpha|_{Rx_i^{k_i}} : Rx_i^{k_i} \rightarrow R$. Again by full π -stability, there exist a positive integer t_i and an element b_i such that $\alpha_i(x_i^{t_i k_i})^{t_i} = b_i(x_i^{t_i k_i})^{t_i}$, ($i = 1, \dots, n$). Thus in particular, $b(x_1^{t_1 k_1} + \dots + x_n^{t_n k_n}) = \alpha(x_1^{t_1 k_1} + \dots + x_n^{t_n k_n}) = \alpha_1(x_1^{t_1 k_1}) + \dots + \alpha_n(x_n^{t_n k_n}) = b_1 x_1^{t_1 k_1} + \dots + b_n x_1^{t_1 k_1} + \dots + b_n x_n^{t_1 k_n}$. Hence $(b-b_i)x_i^{t_i k_i} = \sum_{j=1}^n (b_j-b)x_j^{t_i k_j}$

$x_j^{t_i k_j} \in Rx_j^{t_i k_j} \cap (\sum_{\substack{j=1 \\ j \neq i}}^n Rx_j^{t_i k_j}) = (0)$ for each i . Therefore $b = b_i$ for each i . Define $\beta : R$

$\rightarrow R$ by $\beta(r) = br$ for each $r \in R$. then β is an extension of α .

Corollary

Let R be a fully π -stable ring and $Rx_1 \oplus \dots \oplus Rx_n$ be a direct sum, where $x_i \in R$ ($i = 1, \dots, n$). Then any R -homomorphism of $Rx_1 \oplus \dots \oplus Rx_n$ into R can be extended to an R -endomorphism of R .

Let R be a ring, if I and J are two ideals of R , then it is well-known that $\text{ann}_R(I \cap J) \supseteq \text{ann}_R(I) + \text{ann}_R(J)$, while, the other direction is not true. However, if R is a self-injective ring, then the equality holds⁽¹¹⁾. For fully π -stable rings we have the following:

Proposition

Let R be a fully π -stable ring and $Rx_1^{k_1} \oplus \dots \oplus Rx_n^{k_n}$ be a direct sum, where $x_i \in R$ and k_i is positive integer ($i = 1, \dots, n$). For each positive w with $1 \leq w \leq n$, put $S = Rx_1^{k_1} \oplus \dots \oplus Rx_w^{k_w}$ and $T = Rx_{w+1}^{k_{w+1}} \oplus \dots \oplus Rx_n^{k_n}$. Then $\text{ann}_R(S \cap T) = \text{ann}_R(S) + \text{ann}_R(T)$.

Proof

If $b \in \text{ann}_R(S \cap T)$, define $f: S + T \rightarrow R$ by $f(s+t) = bs$ for each $s \in S$ and $t \in T$. Proposition (2.1) implies that, f can be extended to an R -endo,orphism of R . Hence there exists an element $c \in R$ such that $f(x) = cx$ for every x in $S+T$ (see the proof of proposition (2.1)). In particular, $ct = f(t) = 0$, hence $c \in \text{ann}_R(T)$ and $cs = f(c) = bs$, which implies that $b - c \in \text{ann}_R(S)$. Now, $b = (b-c) + c \in \text{ann}_R(S) + \text{ann}_R(T)$.

Corollary

Let R be a fully π -stable ring and $Rx_1 \oplus \dots \oplus Rx_n$ be a direct sum, where $x_i \in R$ ($i=1, \dots, n$). For each positive integer w with $1 \leq w \leq n$, put $S = Rx_1 \oplus \dots \oplus Rx_w$ and $T = Rx_{w+1} \oplus \dots \oplus Rx_n$. Then $\text{ann}_R(S \cap T) = \text{ann}_R(S) + \text{ann}_R(T)$.

Theorem

Let R be a fully π -stable ring and $\bigoplus_{i \in I} H_i$ (I is index set) be a direct sum of ideals of R . Then $K \cap (\bigoplus_{i \in I} H_i) = \bigoplus_{i \in I} (K \cap H_i)$ for all ideals K of R .

Proof

Let $k \in K \cap (\bigoplus_{i \in I} H_i)$, then $k \in K$ and $k \in \sum_{i=1}^n h_i \in H_i$ ($i = 1, \dots, n$), $\bigoplus_{i=1}^n Rh_i$, is also a direct sum. Let $\pi: \bigoplus_{i=1}^n Rh_i \rightarrow Rh_i$ be the natural projection. By corollary (2,4) we have, for each i , $h_i = \pi_i(\sum_{i=1}^n h_i) = p_i(\sum_{i=1}^n h_i) = p_i k \in K \cap H_i$ where $p_i \in R$. Then $k = \sum_{i=1}^n h_i \in \bigoplus_{i \in I} (K \cap H_i)$. The pther direction is always hold.

Recall that an element u in R is uniform if, $u \neq 0$ and Ru is uniform R -module⁽¹²⁾. We introduce the following:

Definition

Let R be a ring. An element u in R is called π -uniform if, there esixts a positive integer n such that $u^n \neq 0$ and Ru^n is a uniform R -module.

It is clear that, each uniform element is π -uniform, while 2 is π -uniform element in the ring Z_{12} of integers modulo 12 but not uniform.

We use the concept of π -uniform elements in a fully π -stable rings to described maximal ideals. Thus we have the following:

Theorem

Let R be a fully π -stable ring. If u is π -uniform in R , then there exists a positive integer n such that $M_u^n = \{x \in R \mid \text{ann}_R(x) \cap Ru^n \neq 0\}$ is the unique maximal ideal of R containing $\text{ann}_R(u^n)$.

Proof: Let u be a π -uniform element in R , then there exists a positive integer n such that Ru^n is uniform R -module. For each $x, y \in M_u^n$, since $\text{ann}_R(Rx - Ry) \subseteq \text{ann}_R(R(x-y))$, then $\text{ann}_R(Rx - Ry) \cap Ru^n = [\text{ann}_R(x) \cap \text{ann}_R(y)] \cap Ru^n = [\text{ann}_R(x) \cap Ru^n] \cap [\text{ann}_R(y) \cap Ru^n] \neq 0$ so $x - y \in M_u^n$. Next $\text{ann}_R(x) \subseteq \text{ann}_R(rx)$ for each $r \in R$, then $0 \neq \text{ann}_R(x) \cap Ru^n \subseteq \text{ann}_R(rx) \cap Ru^n$. Thus M_u^n is an ideal of R . If $r \in \text{ann}_R(u^n)$, then $Ru^n \subseteq \text{ann}_R(\text{ann}_R(u^n)) \subseteq \text{ann}_R(r)$, therefore $\text{ann}_R(r) \cap Ru^n \neq 0$. Thus $\text{ann}_R(u^n) \subseteq M_u^n$. It is clear that $1 \notin M$. For each $x \notin M_u^n$, then $\text{ann}_R(x) \cap Ru^n = 0$, hence $R = \text{ann}_R[\text{ann}_R(x) \cap Ru^n]$. First, we claim that $\text{ann}_R(u^n x^n) \subseteq \text{ann}_R(u^n x)$. For each $b \in \text{ann}_R(u^n x^n)$, then $bu^n x^{n-1} \in \text{ann}_R(x) \cap Ru^n = 0$, which implies that $b \in \text{ann}_R(u^n x^{n-1})$. Continue in this way we get finally, $\text{ann}_R(u^n x^n) \subseteq \text{ann}_R(u^n x)$. Now, for each $a \in \text{ann}_R[\text{ann}_R(x) \cap Ru^n]$ we claim that $\text{ann}_R(u^n x) \subseteq \text{ann}_R(u^n a)$. For, if $w \in \text{ann}_R(u^n x)$, then $wu^n x = 0$, hence $wu^n \in \text{ann}_R(x) \cap Ru^n$, thus $wu^n a = 0$ which yields that $w \in \text{ann}_R(u^n x)$. Therefore $\text{ann}_R(u^n x^n) \subseteq \text{ann}_R(u^n a)$. Define $\alpha: Rx^n u^n \rightarrow R$ by $\alpha(rx^n u^n) = rau^n$ for each element $r \in R$. α is well-defined R -homomorphism. Full π -stability of R , implies that there exists a positive integer t such that $\alpha(R(x^n u^n)^t) \subseteq R(x^n u^n)^t \subseteq Ru^n x^n$, thus there exists an element $d \in R$ such that $au^n = dx^n u^n$, then $a - dx^n \in \text{ann}_R(u^n)$ and hence $a \in Rx + \text{ann}_R(u^n)$. Thus $R = \text{ann}_R[\text{ann}_R(x) \cap Ru^n] \subseteq Rx + \text{ann}_R(u^n) \subseteq Rx + M_u^n$, therefore, $R = Rx + M_u^n$, then M_u^n is a maximal ideal of R . Finally, suppose that L is a maximal ideal of R containing $\text{ann}_R(u^n)$ with $L \neq M_u^n$, then there exists an element $r \in L$ and $r \notin M_u^n$, hence $\text{ann}_R(r) \cap Ru^n = (0)$, as before we have that $Rr + \text{ann}_R(u^n) = R$, hence $Rr + L = R$ which implies that $L = R$ which contradicts the maximality of L .

It is natural to ask, if R is fully π -stable ring and M an arbitrary maximal ideal of R , does M have the form M_u^n for some π -uniform element u in R ?. Recall that, a ring R is Kasch if $\text{ann}_R(I) \neq 0$ for each maximal ideal I of R ⁽¹³⁾. We call an ideal I of R is π -uniform if there exists a positive integer n such that I^n is a uniform R -module.

Thus, we have the following:

Theorem:

Let R be a fully stable Kasch ring and assume that every non-zero ideal of R contains a π -uniform ideal. Then every maximal ideal of R is of the form M_u^n , for some π -uniform element u in R .

Proof: Let M be a maximal ideal of R , then $\text{ann}_R(M) \neq 0$. By the hypothesis, there exists a π -uniform ideal Ru such that $Ru \subseteq \text{ann}_R(M)$, then there exists a positive integer n with $Ru^n \subseteq Ru \subseteq \text{ann}_R(M)$. If $x \in M_u^n$, then $V = \text{ann}_R(x) \cap Ru^n \neq 0$. Hence as in the proof of the previous theorem, $\text{ann}_R(V) = Rx + \text{ann}_R(u^n)$. So $x \in \text{ann}_R(V)$, but $V \subseteq Ru^n \subseteq \text{ann}_R(M)$ which implies that $M \subseteq \text{ann}_R(\text{ann}_R(M)) \subseteq \text{ann}_R(V)$. Maximality of M gives $\text{ann}_R(V) = M$, so $x \in M$, thus $M_u^n \subseteq M$ and M contains $\text{ann}_R(u^n)$. The uniqueness of M_u^n , (previous theorem) implies that $M = M_u^n$.

The following lemma will be used later.

Lemma: Let R be a fully π -stable ring and $W = Ru_1^{n_1} \oplus \dots \oplus Ru_t^{n_t}$ be a direct sum, where each u_i is π -uniform element of R . If M is a maximla ideal of R which is not of the form M_u^n for any π -uniform element u of R , then there exists an element $m \in M$ such that $\text{ann}_R(1-m) \cap W$ is an essential ideal of W .

Proof

By the hypothesis $M \neq M_{u_i^{n_i}}$, there exists an element $m \in M$ and $m \notin M_{u_i^{n_i}}$, hence $\text{ann}_R(m) \cap Ru_i^{n_i} = 0$. Let $x \in \text{ann}_R(mu_i^{n_i})$, then $xu_i^{n_i} \in \text{ann}_R(m) \cap Ru_i^{n_i}$, so $x \in \text{ann}_R(u_i^{n_i})$, but $\text{ann}_R(m^{n_i}u_i^{n_i}) \subseteq \text{ann}_R(mu_i^{n_i})$, then $\text{ann}_R((mu_i)^{n_i}) \subseteq \text{ann}_R(u_i^{n_i})$. Full π -stability of R implies that $u_i^{n_i} = sm^{n_i}u_i^{n_i}$ for $s \in R$ (see the proof of theorem (2.7)). Therefore $(1-tm)u_i^{n_i} = 0$ for some $t \in R$ or $(1-m_1)u_i^{n_i} = 0$ where $m_1 = tm$. Thus $u_i^{n_i} \in \text{ann}_R(1-m_1) \cap Ru_i^{n_i}$, so $\text{ann}_R(1-m_1) \cap Ru_i^{n_i} \neq 0$ for every $i > 1$, then proposition (2.5) completes the proof. If $\text{ann}_R(1-m_1) \cap Ru_2^{n_2} = 0$. Define $\alpha : Ru_2^{n_2} \rightarrow Ru_2^{n_2}(1-m_1)$ by $\alpha(x) = x(1-m_1)$ for all $x \in Ru_2^{n_2}$. Then α begin an isomorphism and hence $Ru_2^{n_2}(1-m_1)$ is uniform ideal of R . As above, there exists $m' \in M$ such that $(1-m')(1-m_1)^{n_2}(u_2)^{n_2} = 0$, hence $(1-m')(1-m_1)^{n_2}u_2^{n_2} = 0$. Then $(1-m_2)u_2^{n_2} = 0$ where $m_2 = m_1' + m' - m'm_1'$, hence $\text{ann}_R(1-m_2) \cap Ru_1^{n_1} \neq 0$ for $i = 1, 2$. Continue in this way to obtain an element $m \in M$ such that $\text{ann}_R(1-m) \cap Ru_i^{n_i} \neq 0$ for $i = 1, 2, \dots, t$. Again proposition (2.5) implies that $\text{ann}_R(1-m) \cap W$ is an essential ideal of W .

It is known that, an R -module M is a finite dimensional if, and only if, M has an essential submodule which is a direct sum of finitely many uniform submodules⁽⁷⁾. This result motivates the following concept:

Definition

A ring R is said to be a finite π -dimensional if R has an essential ideal W such that $W = Ru_1^{n_1} \oplus \dots \oplus Ru_k^{n_k}$ where each u_i is π -uniform element of R .

In the following, we study another condition on fully π -stable rings to describe maximal ideals.

Theorem

Let R be a fully π -stable ring and finite π -dimensional, then every maximal ideal of R is of the form M_u for some π -uniform element u in R .

Proof

Let M be any maximal ideal of R , assume that M is not of the form M_u for every π -uniform element in R . Since R is finite π -dimensional, then $W = Ru_1^{n_1} \oplus Ru_2^{n_2} \oplus \dots \oplus Ru_k^{n_k}$ is essential in R , where each u_i is π -uniform element of R . Lemma (2.9) implies that there exists an element m in M such that $\text{ann}_R(1-m) \cap W$ is an essential in W and hence in R . Thus $\text{ann}_R(1-m)$ is an essential ideal of R . Then $1-m \in J(R)$ (corollary (1.17)), and hence $m = 1-(1-m)$ is invertible in R , then $M = R$ a contradiction, therefore there is a π -uniform element u in R such that $M = M_u$.

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Multistep Methods For Solving Non-Linear Integral Equations

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الخلاصة

في هذا البحث تم توسيع استخدام طرق متعددة الخطوات والمتضمنة قاعدة سمسون المتكورة، والدمج بين قاعدتي شبه المنحرف وسمسون وكذلك طريقة نيوتن - كريكوري، وذلك لحل المعادلات التكاملية اللاخطية من نوع فولتيرا من النوع الثاني. في هذه الطرق كان وقت تنفيذ البرامج العددية سريع نسبياً كما وتم مقارنة هذه الطرق من خلال بعض الامثلة الاختيارية.

ABSTRACT

The present paper extends the use of Multistep methods including: repeated Simpson's rule, the combination between trapezoidal and repeated Simpson's rule as well as newton Gregory to solve non-linear Volterra integral equations of the second Kind. These meethods use less memory and the computation time is shorter. Numerical calculations are persented establishing the validity of the methods.

INTRODUCTION

The basic theory for many classes of integral equations was well known⁽¹⁻³⁾. Due to a variety of technical and mathematical developments, this subject should experience rapid growth and take its place as a major branch of numerical analysis.

In this work we shall deal with the numerical solution of a large calass of integral equation which is non-linear Volterra integral equations of the second kind of the form:

$$U(x) = g(x) + \int_0^x k[x,t,u(t)]dt \quad 0 \leq x \leq b \quad \dots(1)$$

Where $g(x)$ is given function and k is a function of x,t and the unknown function $u(t)$.

Approximate Solution by Multistep Methods

Let:

$$\int_0^{x_i} k(x,t,u(t))dt = h \sum_{j=0}^i w_{ij}.k(x_i,t_j,u(t_j)) + Er \dots (2)$$

where the weights $\{w_{ij}\}$ being supposed given and Er is the remainder which can be negligible.

We can rewrite (1) with the and of (2) as:

$$u_i = g_i + h \sum_{j=0}^i w_{ij} k(x_i,t_j,u_j) \dots (3)$$

Eq. (3) represents a set of equations for determining an approximation to f at the points $x=x_i=ih, i=1,2,\dots,n. h= b/n$ with the adopted notation $u(x_i) = u_i; u(t_j)=u_j$ and $g(x_i) = g_i$.

Repeated Simpson's Rule

In the case when (2) is Simpson's rule. (3) have the form:

When i is even

$$U_i = g_i + h/3 \sum_{j=0}^{j-1} w_{ij} k(x_i,t_j, u_j) + h/3 k(x_i,t_i,u_{i1}), I = 2,4 \dots (4)$$

When i is odd

$$u_i = g_i + h/3 \sum_{j=0}^{j-1} w_{ij} - 3j [x_i, t_j, u_j] + 3h/8 (k[x_i, t_i - 3, u_i - 3] + 3k [x_i, t_i - 2, u_i - 2] + 3k [x_i, t_i - 1, u_i - 1] + k[x_i,t_i,u_{ij}], i = 3,5 \dots (5)$$

where $u_{i1} = g_i + hk [x_i, t_0, u_0]$

$$u_{i1} = g_i + (i/i-1)h \sum_{j=1}^{i-1} k(x_i, t_j, u_j); i=2,3,\dots,n \quad \dots(6)$$

the weights are $w_{i0} = w_{ij} = 1$ and $w_{ij} = 3-(-1)^j$; $1 \leq j \leq i-1$
 since $u_0 = g_0$ we can find u_1 as follows :

$$u_1 = g_1 + h/6 \{k(x_1, t_0, u_0) + 4k[x_1, t_1/2, u_{13}] + k[x_1, t_1, u_{12}]\}$$

where

$$\left. \begin{aligned} u_{11} &= g_1 + hk[x_1, t_0, u_0] \\ u_{12} &= g_1 + (1/2)h \{k[x_1, t_0, u_0] + [x_1, t_1, u_n]\} \\ u_{13} &= g_1/2 + (1/4)h\{[x_1/2, t_0, u_0] + k[x_1/2, t_1/2, u_0/2 + u_{12}/2]\} \end{aligned} \right\} \dots(7)$$

Combination Between Trapezoidal Rule and Simpson’s 1/3 Rule

For more accurate computation, we shall use the combination between the quadrature rule in the following manner:

Let $u_0 = g_0$

Define $u_{11} = g_1 + hk [x_1, t_0, u_0]$

Then $u_1 = g_1 + h/2 \{k(x_1, t_0, u_0) + k[x_1, t_1, u_{11}]\}$

Next

$$\text{Let } u_{ii} = g_i + (i/i-1)h \sum_{j=1}^{i-1} k[x_i, t_j, u_j]; i = 2,3$$

Then

$$u_j = g_i + h/3 \sum_{j=0}^{i-1} w_{ij} k[x_i, t_j, u_j] + h/3k [x_i, t_i, u_{i1}], i = 2,4,6 \quad \dots(8)$$

and

$$u_i = h_i + h/2 (k[x_i, t_0, u_0] + [x_i, t_1, u_1] + h/3 \sum_{j=1}^{i-1} k[x_i, t_j, u_j] + h/3k[x_i, t_i, u_{i1}]) \quad I=3,5,7,\dots (9)$$

The weights are given by $w_{i0} = w_{ii} = 1$, $w_{ij} = 3 - (-1)^j$; $1 \leq j \leq i-1$

Newton Gregory Method

A another type of quadrature formula, Newton Gregory, could also be sued to approximate the solution of (1) as follows:

Let $T(k,h)$ represent the trapezoidal rule approximation to $I(k)$ at interval

h.i.e. $I(k) = \int_0^{nh} k(x,t,u(t))dt$

There fore $T(k,h) = \frac{1}{2} h \{k[nh,o,u(o)] + 2 \sum_{j=1}^{j-1} k[nh,ih,u(ih)] + k[nh,nh,u(nh)]\}$

$$I(k) = T(k,n) - \frac{1}{12} h^2 \{k[nh,ih,u'(ih)] - k[nh,o,u'(o)] + \frac{1}{720} h k[nh,ih,u''(ih)] - k[nh,o,u''(o)] + \frac{B_{2n}}{(2m)!} h^{2m} [k(nh,ih,u^{(2m-1)}(ih)) - k(nh,o,u^{(2m-1)}(o))] + R \} \dots(10)$$

where the remainder R is given by

$$R = \frac{-B_{2m+2}}{(2m+2)!} h^{2m+2} (nh-o) k(nh, \epsilon, u^{2+2}(\epsilon))$$

And the coefficients B_i are the Bernoulli numbers.

Newton Gregory's formula obtained by replacing the derivatives in (10) by forward and back ward differences. We write $k_o = k(nh,o,u(o))$,

$$K_n = k(nh, nh, u(nh)), \Delta k_o = k(nh,h,u(h)) - k(nh,o,u(o))$$

$$\text{And } \nabla k_n = k(nh,nh,u(nh)) - k(nh,(n-1)h,u(n-1)h))$$

Then we have

$$I(k) = T(k,h) - \frac{1}{2} h \{ \nabla K(n,nh,u(nh)) - \Delta k(uh,o,u(o)) \} - \frac{1}{24} h \{ (\nabla^2 K(nh,nh,u(nh))) - \Delta^2 K(nh,o,u(o)) \} - \frac{19}{720} h \{ \nabla^3 K(nh,nh,u(nh)) - \Delta^2 K(uh,o,u(o)) \} + \dots$$

after simplifying the above formula, we get

$$u_1 = g_1 + h [5/12 k [x_1, t_0, u_0] + 13/12 k [x_1, t_1, u_1]] +$$

3- The Flowcharts

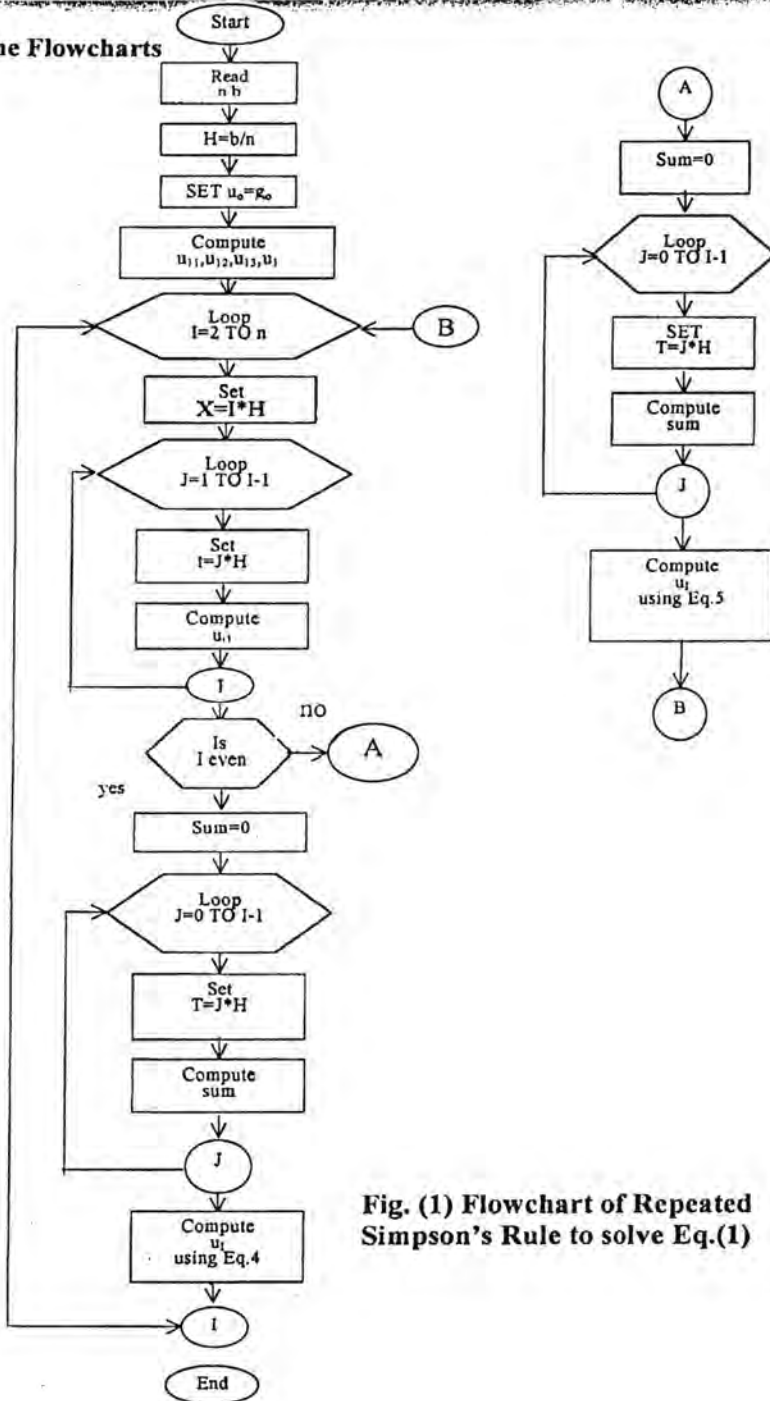


Fig. (1) Flowchart of Repeated Simpson's Rule to solve Eq.(1)

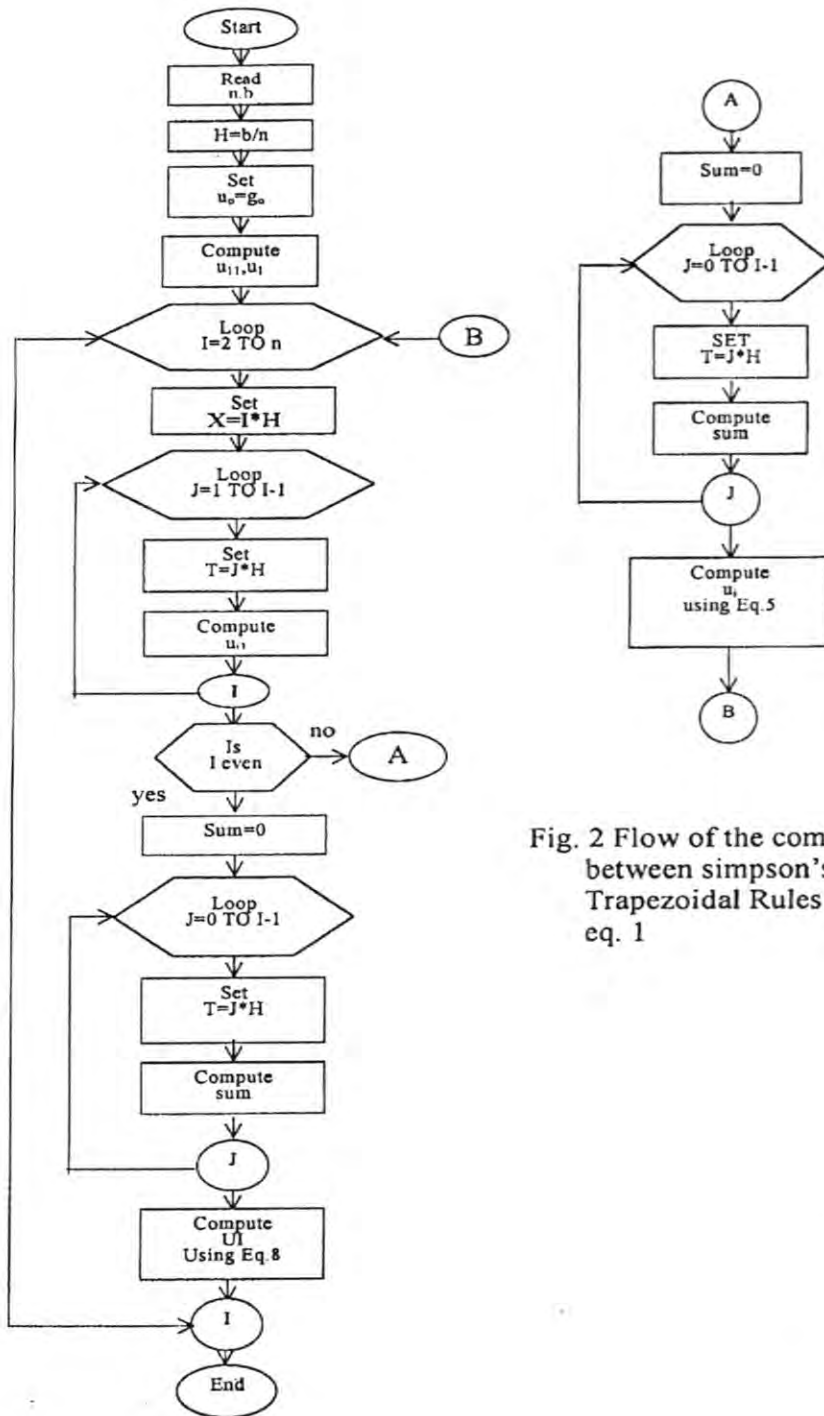
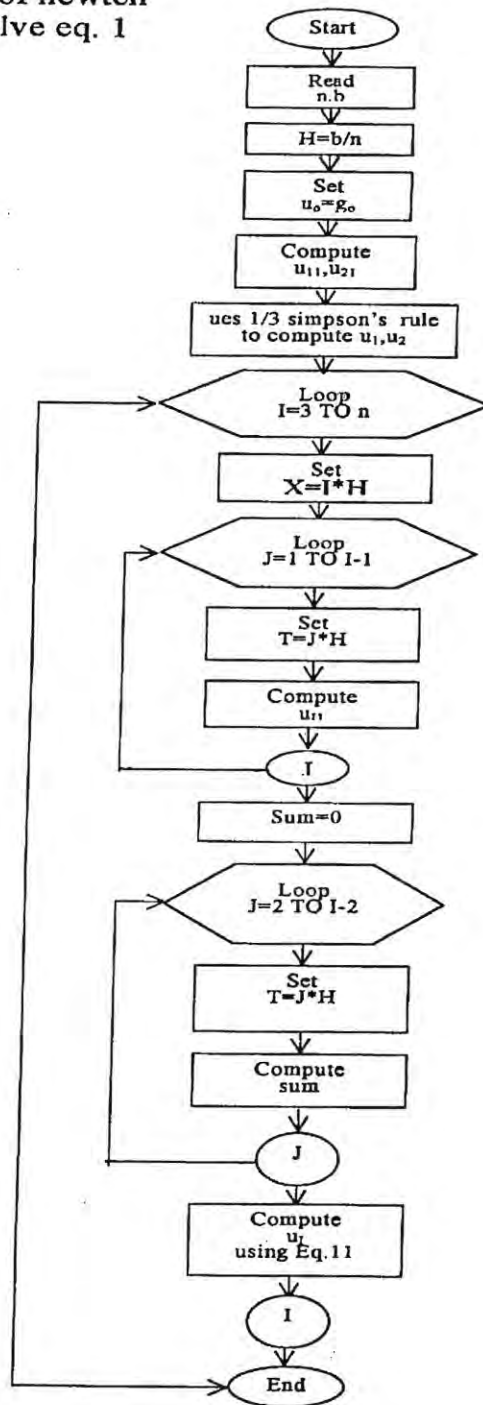


Fig. 2 Flow of the combination between simpson's 1/3 and Trapezoidal Rules to solve eq. 1

Fig. 3 Flow chart of newton Gregory to solve eq. 1



$$\sum_{j=2}^{i-2} k[x_i, t_j, u_j])] + \frac{13}{12} k [x_i, t_{i-1}, u_{i-1})] + 5/12k[x_i, k_i, u_{i1}] \dots (11)$$

$i = 3, \dots, n$

where

u_1 and u_2 are evaluated using simpson's rule

Numerical Examples

This section presents the results of three methods described in section (2) to solve the following integral equations.

Example (1)

Our first example is

$$U(x) = e^{-x} + \int_0^x e^{-(x+1)} (u(t) + e^{-u(t)}) dt \quad 0 \leq x \leq 1$$

This example was solved using repeated Simpson's rule, the combination between trapezoidal and Simpson's 1/3 rule as well as Newton Gregory method. Table (1) summarizes our results for $n = 10$ so that $h = 0.1$.

Table (1) Summary of Numerical solution of example (1) with the exact solution and the least square errors (L.S.F).

X	Repeated Simpson's	Trapezoidal + Simpson's 1/3	Newton Gregory	Exact $U(x) = \log(x+e)$
0	1	1	1	1
0.1	1.036127	1.036127	1.036127	1.036127
0.2	1.070983	1.070983	1.070983	1.070995
0.3	1.104658	1.104778	1.104786	1.104688
0.4	1.137234	1.137244	1.137358	1.137282
0.5	1.168765	1.188875	1.168894	1.168848
0.6	1.199340	1.199358	1.199457	1.199447
0.7	1.228979	1.229083	1.229106	1.229138
0.8	1.257785	1.257810	1.257891	1.257973
0.9	1.285741	1.285645	1.285863	1.285999
1	1.312973	1.313003	1.313065	1.313262
L.S.E	0.00000023	0.00000014	0.00000008	

Example (2)

Consider the following integral equation

$$U(x) = \frac{1}{2} - \frac{1}{2} e^{x^2} - x^2 + \int_0^x (x-t)e^{2xt} \cdot e^{u(t)} \quad 0 \leq t \leq x$$

With the exact solution

$$U(x) = -x^2 \quad 0 \leq x \leq 1$$

Table (2) lists the results obtained using methods which were described in section 2, to find approximations to the solution of the above equation. Included in the input was $n = 10$ and $h = 0.1$, the computations were performed using seven digits of precision.

Table (2) Summery of numerical solution of example (2) with the exact solution and the L.S.E.

X	Repeated Simpson's	Trapezoidal + Simpson's 1/3	Newton Gregory	Exact
0	0	0	0	0
0.1	-0.010000	-0.010000	-0.010000	-0.01
0.2	-0.039999	-0.039999	-0.039999	-0.04
0.3	-0.089997	-0.090122	-0.090074	-0.09
0.4	-0.159997	-0.159999	-0.160097	-0.16
0.5	-0.249994	-0.250203	-0.250118	-0.25
0.6	-0.359994	-0.360004	-0.360136	-0.36
0.7	-0.639989	-0.490252	-0.490152	-0.49
0.8	-0.809981	-0.640023	-0.640167	-0.64
0.9	-0.999981	-0.810255	-0.810180	-0.81
1	0.00000020	-1.00068	-0.000193	-1.0
L.S.E	0.00000020	0.00000019	0.00000017	

CONCLUDING REMARKS

We have shown how Newton-Gregory method can be used to substantially improve the accuracy for the solution of non-linear Volterra integral equation of the second kind compared with repeated Simpson's rule and the method of combination between trapezoidal and Simpson's 1/3 rule. Since Simpson's 1/3 rule cannot be used to approximate the solution of eq. (1) because not enough

points are available for the rule to be applicable when n is odd, so we used either the formula in (2-1) or (2-2).

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The Variation Of Wind Speed And Temperature With Height Within The Surface Boundary Layer

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الخلاصة

تم تقدير سرعة الرياح ودرجة حرارة الهواء على ارتفاع ٥٠م فوق سطح الأرض باستعمال العلاقة الخطية اللوغاريتمية. كذلك تمت مقارنة سرعة الرياح المحسوبة مع سرعة الرياح الحقيقية ودرجة الحرارة المحسوبة مع درجة الحرارة الحقيقية في الساعة 00 00 و 12 00 بالتوقيت العالمي. وجد ان التوافق كان جيداً بين سرعة الرياح المقدرة والحقيقية في الظروف غير المستقرة وأحسن مما هي عليه في الظروف المستقرة، ونفس الحالة تنطبق أيضاً على درجة الحرارة. كما ان تقدير درجة الحرارة للظروف المستقرة لايمكن الاعتماد عليها.

ABSTRACT

The log linear profile relationship is used to estimate wind speed and air temperature at 50m height. A comparison is made between the estimated and the observed wind speed then between the estimated and the observed temperature for 00 and 12 GMT. It is found that the agreement between the estimated and the observed wind speed for unstable conditions is better than that of stable conditions. The same trend is found for temperature. The estimation of temperature for stable conditions is not reliable.

INTRODUCTION

Knowledge of the variation of wind speed and temperature with height within the lowest 100m layer is of importance for aircraft operations, designing of tall ships and buildings and for air pollution studies.

In practice however, only surface weather observations such as 10m wind speed and 1.5m temperature are available most of the time. In such cases, there is a need for the description of mean wind speed and temperature profiles as some function of the available data.

Monin Obukhov Similarity Theory

The vertical variation in wind speed and temperature in the lowest layers of the atmosphere can be described within the framework of the Monin Obukhov similarity theory, which was proposed in 1953. This theory is based on the hypothesis that the non-dimensional wind speed and temperature gradients, ϕ_m and ϕ_h , defined by Paulson⁽¹⁾.

$$\frac{kz}{u_*} \left(\frac{\partial u}{\partial z} \right) = \phi_m \left(\frac{z}{L} \right) \quad \dots (1)$$

$$\frac{kz}{T_*} \left(\frac{\partial T}{\partial z} \right) = \phi_h \left(\frac{z}{L} \right) \quad \dots (2)$$

where ϕ_m is a function of z/L only, u is the wind speed at the height of z , u_* is the friction velocity and equal $\sqrt{\tau/\rho}$, τ shearing stress and ρ air density, k is the Von Karaman constant, T is the air temperature, T_* is the virtual temperature which can be formulated as:

$$T_* = -\left(\frac{1}{ku_*} \right) (H / \rho c_p) \quad \dots (3)$$

where H is the sensible heat flux, c_p is the specific heat of air at constant pressure. L is the Monin-Obukhov length defined as⁽²⁾:

$$L = -\frac{c_p \rho T u_*^3}{kgH} \quad \dots (4)$$

where g is the gravity of earth.

Several forms of ϕ_m is proposed for stable and unstable conditions (Yaglom, 1976). The most used forms of ϕ_m for unstable conditions ($L < 0$) are^(1,4,5,6).

$$\phi_m = 1 - (1 - 16z/L)^{-1/4} \quad \dots (5)$$

For stable conditions ($L > 0$), ϕ_m usually written as (Holtslag, 1984);

$$\phi_m = 1 + 5z/L \quad \dots (6)$$

Where as ϕ_h can be written as⁽⁶⁾

$$\phi_h = \left(1 - \frac{z}{L} \right)^{0.5} \quad \dots (7)$$

Profile Representations

Equations 1 and 2 can be interpreted and the resulting equations are⁽⁸⁾:

$$\Delta u = \frac{u_*}{k} \left[\ln \left(\frac{z_2}{z_1} \right) - \psi_m \left(\frac{z_2}{L} \right) + \psi_m \left(\frac{z_1}{L} \right) \right] \quad \dots (8)$$

$$\Delta T = \frac{T_*}{k} \left[\ln\left(\frac{z_2}{z_1}\right) - \psi_h\left(\frac{z_2}{L}\right) + \psi_h\left(\frac{z_1}{L}\right) \right] \quad \dots (9)$$

where Δu and ΔT are the wind speed and temperature differences between two height z_1 and z_2 in the atmospheric surface layer respectively. ψ_m and ψ_h are stability corrections of wind speed and temperature, respectively which are given by ⁽⁹⁾:

$$\psi_m = \ln\left(\frac{1+x}{2}\right)^2 + \ln\left(\frac{1+x^2}{2}\right) - 2 \tan^{-1}(x) + \frac{\pi}{2} \quad \dots (10)$$

where for unstable conditions :

$$\psi_m = -\alpha \frac{z}{L} \quad \dots (11)$$

and for stable conditions :

$$\psi_m = 2 \ln\left(\frac{1+x^2}{2}\right) \quad \dots (12)$$

where $x = \phi_m^{-1}$ and $\alpha = 5$ is an empirical parameter.

Determination of the roughness length z_0

The friction velocity may be written as ⁽¹⁰⁾:

$$u_* = uk \left(\ln \frac{z}{z_0} - \psi_m \right)^{-1} \quad \dots (13)$$

where z_0 is the roughness length which represents a parameter that characterize the aerodynamic roughness of the underlying surface. When equation (13) is written for two levels in the surface boundary layer (subscripts 2 and 1) for neutral stability conditions i.e., $\psi_m = 0$ the results will be :

$$\frac{u_2}{u_1} = \frac{\ln \frac{z_2}{z_0}}{\ln \frac{z_1}{z_0}} \dots (14)$$

Equation (14) is used to estimate z_0 for different wind directions as shown in table (1).

Table (1) Distribution of roughness length with directions

Directions	Z_0 , Roughness length in cm
186-15	2
16-60	6
61-90	4
91-170	3

Estimation of sensible heat flux H

The sensible heat flux is the heat transformed from the ground to the atmosphere. Typically, this rate is 1/3 to 1/2 the heat absorbed by the surface, the remainder is divided between heat stored in the ground and the heat used to evaporate water from soil and plants.

The sensible heat flux may be expressed as ⁽¹¹⁾:

$$H = c_p \overline{\rho w T'} \dots (15)$$

Where w is the vertical velocity, T is the temperature and the overbar indicates a time average and the prime denotes deviations from the averaged quantity. Since there are no available instruments to measure the turbulent quantities, such as the quantity $(w'T')$ in equation (15) for Baghdad station, sensible heat flux H can not be measured directly.

The model of De Bruin and Holtslag ⁽¹²⁾ is used to calculate the quantity $w'T'$ because of the lack of measurement facilities. In this model, sensible heat flux can be written as :

$$H = \frac{(1 - \alpha) + \left(\frac{\gamma}{s}\right)}{1 + \left(\frac{\gamma}{s}\right)} (R_n - G) - \beta \dots (16)$$

Where, α and β are empirical parameters and for grass-covered surface $\alpha = 1$ and $\beta = 20 \text{ Wm}^{-2}$ ⁽¹²⁾. No values for the parameters for Baghdad are available. So in this work the above mentioned are used.

$\frac{\gamma}{s}$ is a function of temperature, $s = \partial q_s / \partial T$, q_s is saturation specific heat of air at constant pressure.

The equation which governs the relation between $\frac{\gamma}{s}$ and the temperature is ⁽⁸⁾:

$$\frac{\gamma}{s} = 1.43 \text{ EXP} (-0.056 T) \quad \dots(17)$$

The soil heat flux G is given by the following equation ^(12,13):

$$G = c_G R_n \quad \dots(18)$$

Where c_G is a constant equal to 0.1 and R_n is the net radiation received by the earth which can be expressed by the equation ⁽¹²⁾:

$$R_n = \frac{(1-r)K + c_1 T^6 - \sigma T^4 + c_2 N}{1 + c_3} \quad \dots(19)$$

Where, r is the albedo of the surface. A value of 0.15 is taken as a representative to the area around the station. σ is the Stefan-Boltzmann constant. N total cloud cover where $N=1$ is for cloudy sky condition, $c_1 = 5.31 \times 10^{-13} \text{ Wm}^{-2}\text{K}^{-6}$ is a constant of proportionality between the incoming long wave radiation and the air temperature in the absence of clouds $c_2 = 60 \text{ Wm}^{-2}$ is a constant appropriate for mid-latitudes. $c_3 = 0.12 \text{ Wm}^{-2}$ is a constant can be regarded as heat coefficient for the surface. K is the incoming solar radiation, which can be expressed by ⁽⁸⁾:

$$K = (a_1 \sin SA + a_2) (1 - b_1 N^{b_2}) \quad \dots (20)$$

Where SA is the solar elevation, a_1 and a_2 are turbidity coefficients. For Baghdad, the values for a_1 is 990 and a_2 is -30 which gives a fair average ⁽⁸⁾.

b_1 and b_2 are empirical coefficients have typical values of 0.75 and 3.4 respectively ⁽⁹⁾.

Estimation of Monin – Obukhov length L

To evaluate K, applying equation (13) uses iteration method. The first step is to estimate u_* by putting $z/L = 0$ so that $\phi_m = 1$ and $\psi_m = 0$. With sensible heat flux obtained from equation (16), an estimation of L is made. With this estimate, equation (13) is used again to improve the estimated value of u_* and son on. It appears that usually not more than three interactions are needed to achieve an accuracy of 5% in successive values of L.

Estimation of wind speed and temperature at 50 m by log- linear relationships

The computations starts with an estimate of the friction velocity u_* by iteration method. Throughout the iteration, stability corrections of wind speed and temperature ψ_m and ψ_h will be computed by using the equations (10), (11) and (12) respectively. Finally these corrections will be introduced to the equations (8) and (9) to give estimates of wind speed and temperature for the desired height. The equation (8) is not appropriate for the very stable conditions i.e., for $z/L > 0.5$. Insread, the following equation will be used to estimate the wind speed u_2 at any height z_2 from the surface measurements of wind speed at height z_1 ⁽⁶⁾:

$$U_2 = u_1 (LN/LZ) \quad \dots(21)$$

Where,

$$LN = \ln(z_2/z_0) + 7 \ln (z_2/L) + 4.25 (z_2/L) - 0.5 / (z_2/L) + 0.852 \quad \dots(22)$$

And,

$$LZ = \ln(z_1/z_0) + 5(z_1/L) \quad \dots (23)$$

Figure (1) shows comparison between the estimated wind speed and the observed wind speed at 50m height for stable and unstable conditions. Similarly figure (2) shows comparison between the estimated and the observed temperature. The results presented in table (2) and (3) for wind speed and temperature, respectively.

Table 2: The root mean square errors (rms) of the observed and estimated wind speed at 50m height in the stable and conditions.

Month	1	2	3	4	5	6	7	8	9	10	11	12
Rms for stable conditions	0.97	0.72	0.72	0.82	2.4	0.79	0.99	1.38	1.08	1.16	0.89	1.26
Rms, for unstable conditions	0.74	0.71	1.29	0.75	Missing data	0.64	0.67	0.77	0.71	0.55	0.88	0.49

Table 3: The root mean square errors (rms) of the observed and estimated temperature at 50m height in the stable and unstable conditions

Month	1	2	3	4	5	6	7	8	9	10	11	12
Rms for stable conditions	11.3 6	5.19	5.06	2.9	2.32	1.65	1.87	1.75	5.3	5.97	12.9 3	19.6 7
Rms, for unstable conditions	0.74	0.71	1.29	0.75	Missing data	0.64	0.67	0.77	0.71	0.55	0.88	0.49

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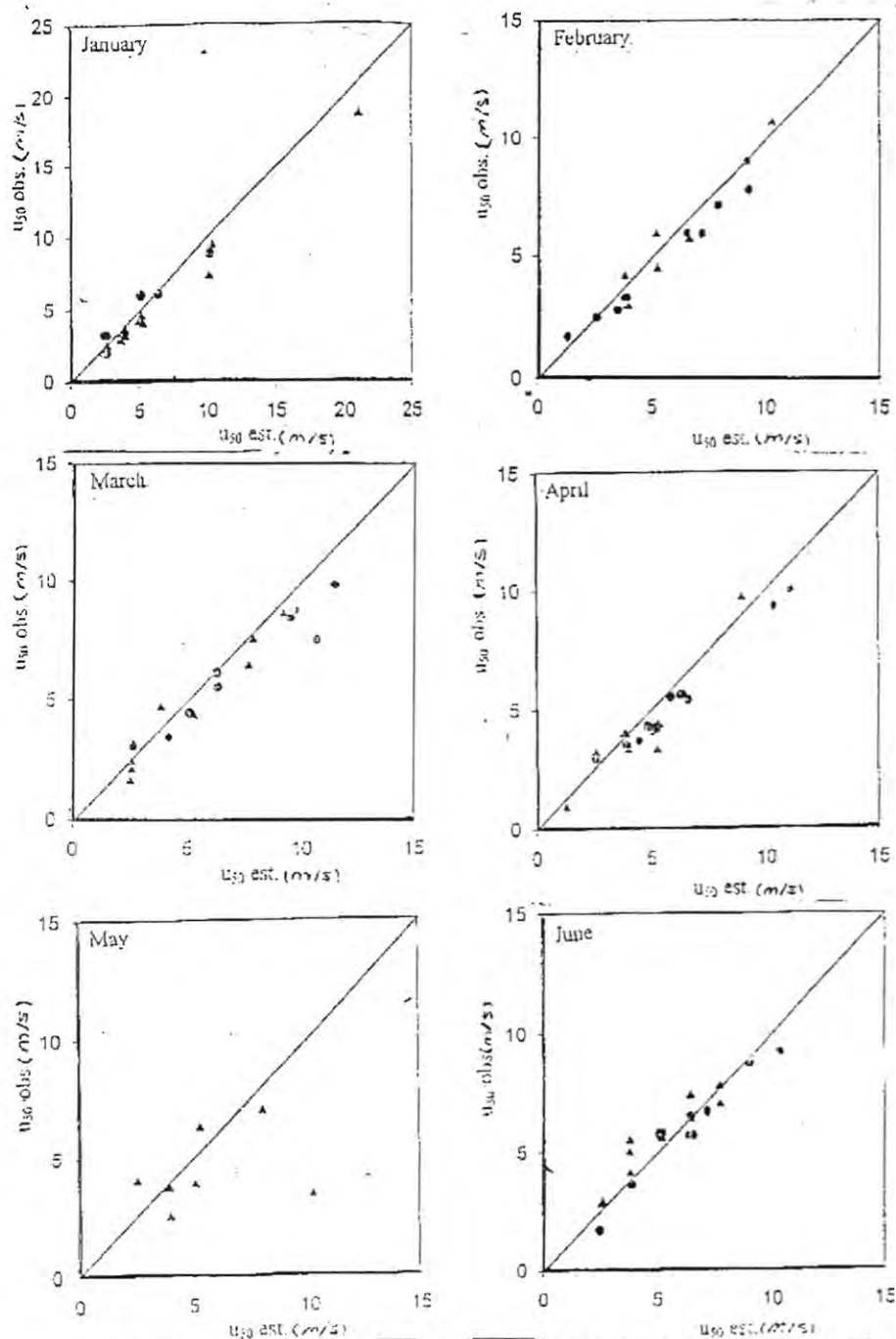
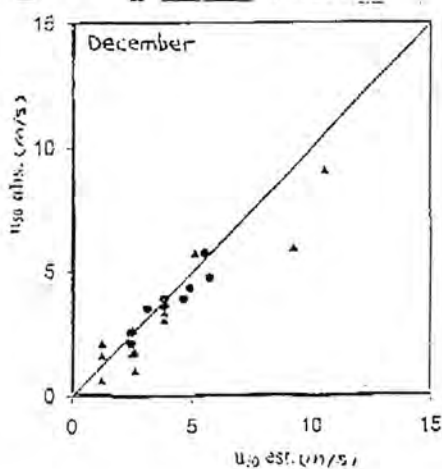
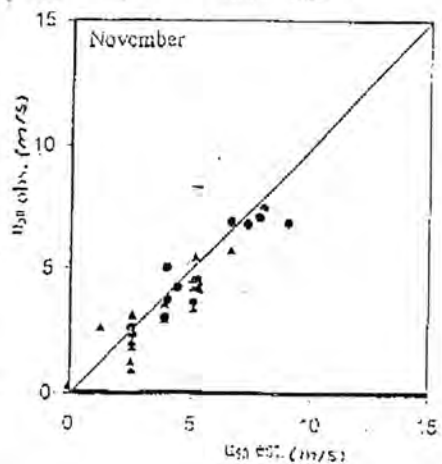
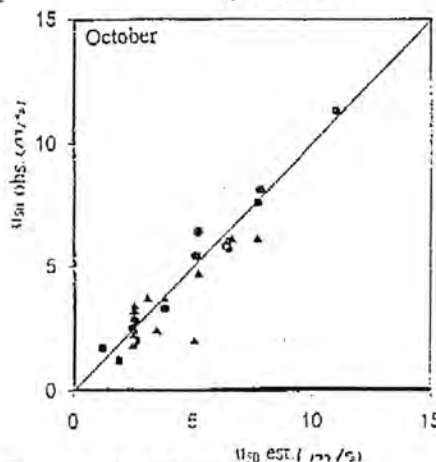
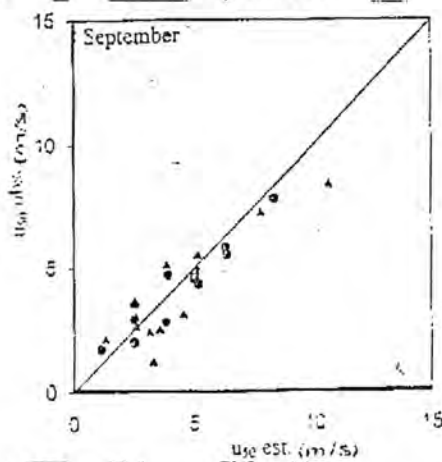
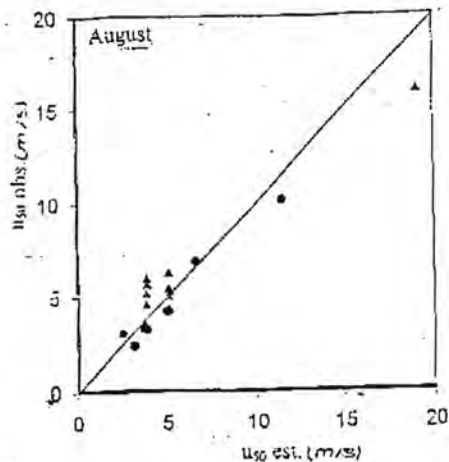
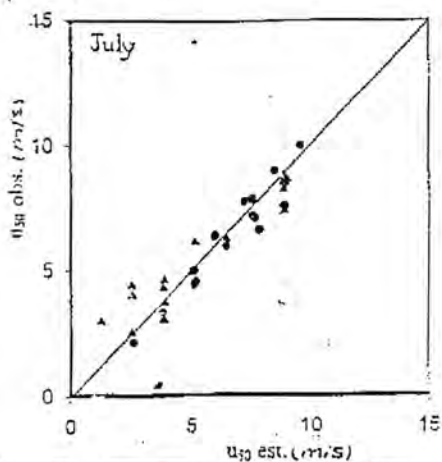


Fig. (1) Comparison between the estimated and the observed wind speed for 1986. \blacktriangle refers to stable conditions and \bullet refers to unstable conditions.



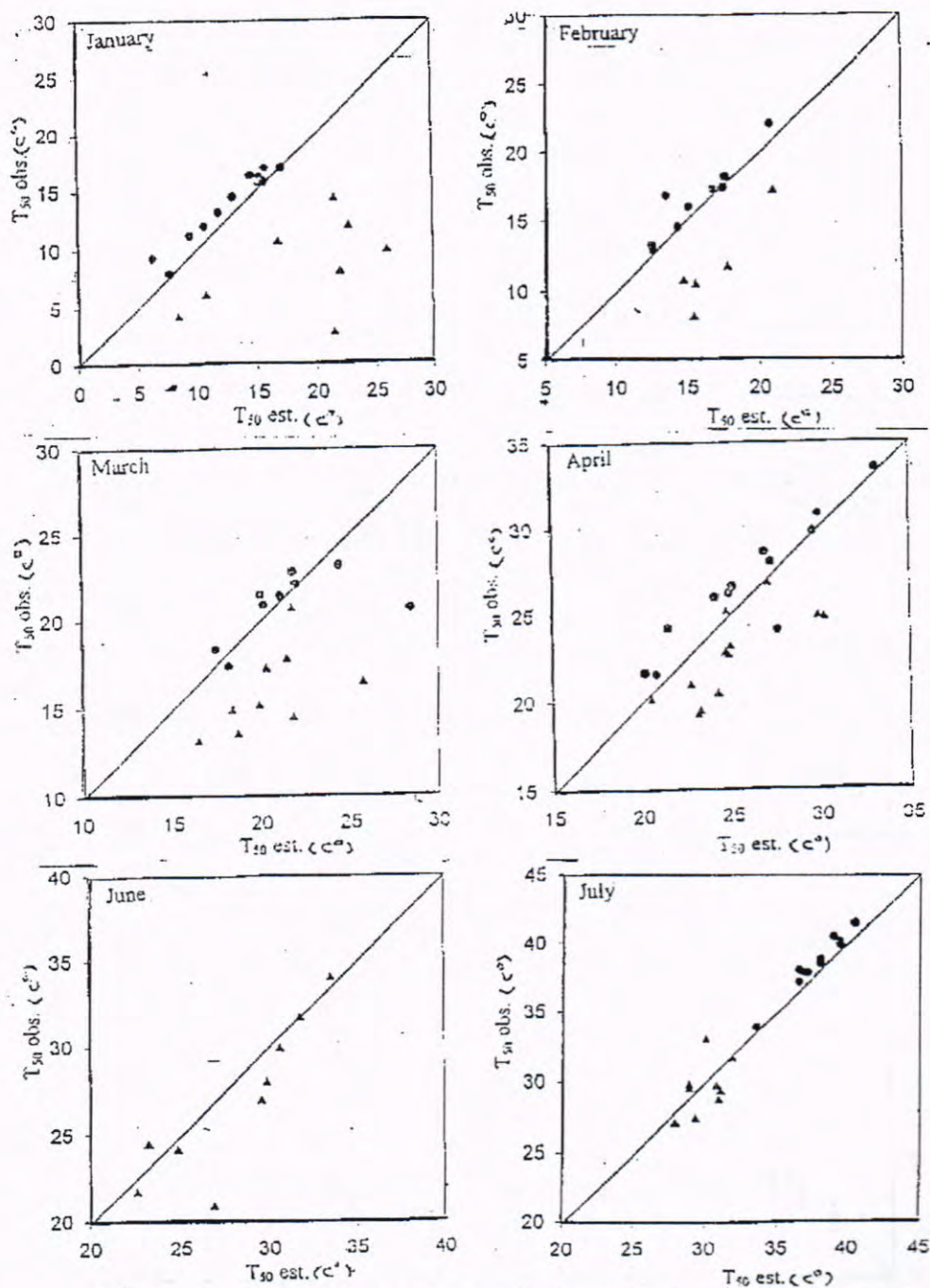


Fig. (2) Comparison between the estimated and the observed temperature for 1986. \blacktriangle refers to stable conditions and \bullet refers to unstable conditions.

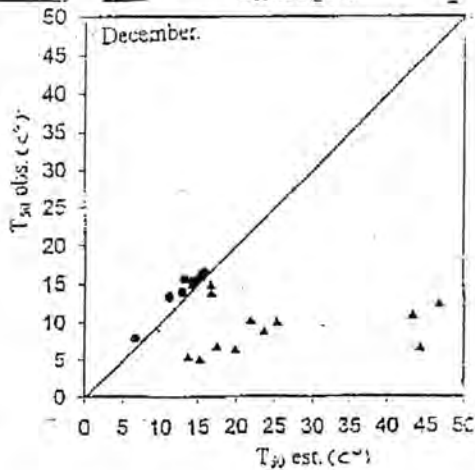
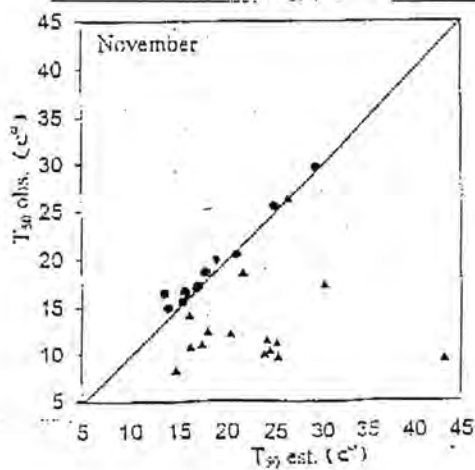
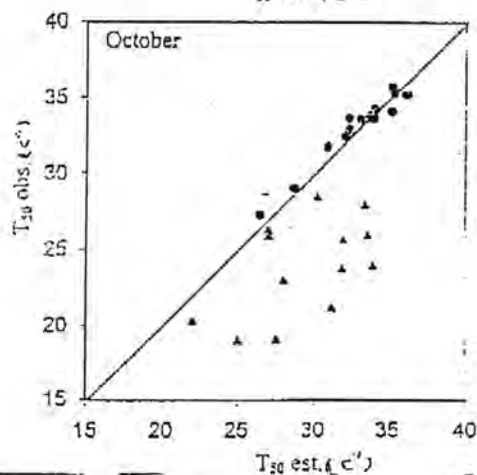
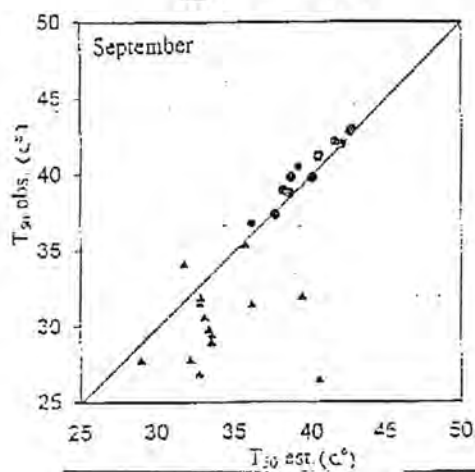
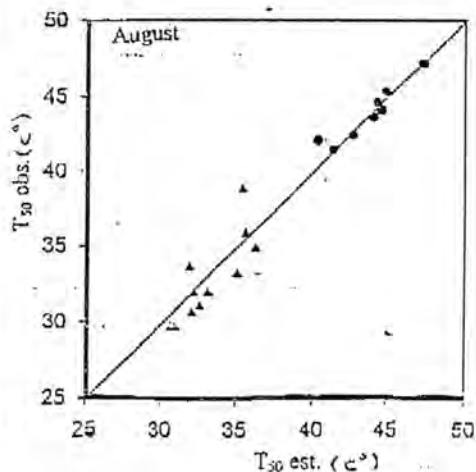
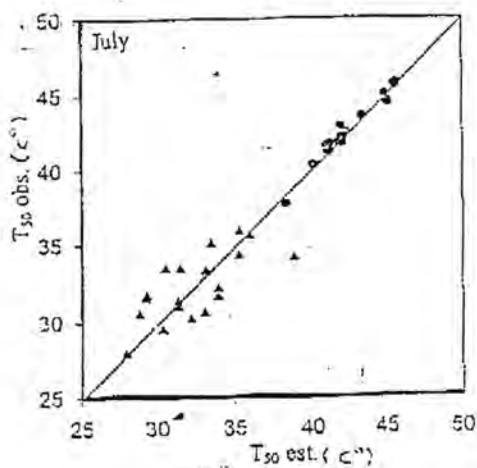


Table (2) shows that the relation between the estimated and the observed wind speeds varies due to stability variations. For stable conditions, the root mean square error is about 0.72 to 2.42. The use of equation (21) solves the problem in estimating wind speed in the very stable conditions. As such the average value of (rms) in the stable condition indicates that accuracy of the logarithmic law is acceptable. The agreement between the estimated and the observed wind speed is better for the unstable conditions. It is found that the root mean square error (rms) for the unstable conditions is between 0.49 and 1.29, which is less than root mean square error for the stable conditions. This confirms the superiority of the Monin – Obukhov theory in the case of unstable conditions.

Since Monin – Obukhov similarity is based on assumptions of stationary and homogeneous conditions; the deviation from such conditions may introduce some error in the calculations.

The estimation of temperature as it is clear in the figures is less reliable in the stable conditions. Table (3) indicates that the root mean square error (rms) is in the range 1.65 to 19.67. This is due to the imperfection of Monin – Obukhov similarity theory in the stable conditions.

CONCLUSIONS

1. The comparison, which is made between the estimated and the observed wind speed by using the similarity theory scheme at 50m height, indicates that the estimation in the unstable conditions is better than that in the stable conditions. This belongs to the imperfection of this theory in the stable conditions.
2. The estimated temperatures at 50m height are in good agreement with the observed temperature in the unstable conditions but they are erroneous in the stable conditions especially in winter months, this can be attributed to the imperfection of the similarity scheme in the stable conditions.

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Depolarization Effects Due to Some Atmospheric Constituents

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ABSTRACT

The non-spherical shape of rain, snow, sand and dust particles contribute to depolarization of the microwave signal on both earth-earth and earth-space paths. The aim of this research is to model and investigate the depolarization effects due to these atmospheric constituents. The theoretical concepts of depolarization problem and the microphysical properties of the propagation media are reviewed. Computer programs were developed to simulate the depolarization effects. Results suggest that rain, snow, dust, and sand particles play an important role in producing differential phase shift ($\Delta\phi$) and differential attenuation (ΔA). For rainy and snowy media $\Delta\phi$ and ΔA increases with increasing frequency and precipitation. The effects of temperature is that, in rainy medium the increase in temperature causes in ΔA and an increase in $\Delta\phi$ while in snowy medium, the increase in temperature causes increase in ΔA while $\Delta\phi$ remains constant. For dust and sand storms $\Delta\phi$ and ΔA tend to increase with increasing frequency and moisture contents. The results are presented in a way that helps communication engineers to deduce information about the depolarization effects in any weather condition.

INTRODUCTION

In view of great development using depolarization techniques in communication system using microwave frequency, it is necessary to investigate the effects of atmospheric constituents on the depolarization properties of the signal. Depolarization refers to the change in the state of polarization of the electromagnetic wave due to variety of atmospheric particles. The problem of depolarization due to rain, snow, sand, and dust particles was investigated by many authors^(1-8,12). This paper presents a review of the polarization parameters computations and examines effects due to some atmospheric particles. The specific effects examined are differential phase shift and differential attenuation which are defined as the differences between the phase shifts and between the attenuation of horizontal and vertical waves. Rain, snow, dust and sand particles are assumed to contain identically shaped ellipsoidal and equaligned particles with the same orientation. The first assumption implies that there is no strong dependence of shape and size and the assumption is justifiable since the vast

majority of particles are aligned with longest axes horizontal and their shortest axes vertical^(9,10,11).

Theoretical Aspects

The horizontal and vertical complex propagation constants of the medium $k_{H,V}$ (m^{-1}) can be expressed by⁽⁴⁾ :

$$K_{H,V} = k_0 + \frac{\pi k_0}{12} N \int_{D_{min}}^{D_{max}} Q_{H,V}(e,m) D^3 N(D) dD \quad \dots(1)$$

Where D is the diameter of a sphere with volume equal to that of spheroid, $N(D)$ is the particle size distribution (m^{-4}), D_{max} and D_{min} are the maximum and minimum diameter of particle, and k_0 is the free propagation constant (m^{-1}) [$k_0 = 2\pi/\lambda, \lambda$ is the wavelength (m)]. $Q_{H,V}$ are functions of the eccentricity e and refractivity m of the particle as follows⁽⁴⁾ :

$$Q_{H,V} = \frac{m^2 - 1}{1 + P_{H,V}(m^2 - 1)} \quad \dots(2)$$

Where

$$P_v = \frac{1}{e^2} \left[1 - \left(\frac{1 - e^2}{e^2} \right)^{1/2} \sin^{-1} e \right]$$

$$P_H (1 - P_v)^{1/2}$$

The eccentricity e is a function of the semi-minor and semi-major axes (a and b) $e^2 = 1 - a^2/b^2$. The refractivity m is related to the complex dielectric constant ϵ by $m^2 = \epsilon$.

For dust and sand storms, it is more convenient to express $K_{H,V}$ in terms of the probability density function $P(D)$ ⁽⁸⁾ :

$$K_{H,V} = k_0 + \left(\frac{5.67 \times 10^{-4}}{V_0} \int_{D_{min}}^{D_{max}} Q_{H,V}(e,m) D^3 P(D) dD \right) \left(\int_{D_{min}}^{D_{max}} D^2 P(D) dD \right)^{-1} \quad \dots(3)$$

The one-way differential phase shift $\Delta\phi_L$ (deg/km) and one way differential attenuation ΔA_L (dB/km) for horizontally polarized waves are defined by⁽⁸⁾ :

$$\Delta\phi_L = \frac{180}{\pi} \times 10^3 \operatorname{Re}(K_{IH}-K_V) \quad \dots(4)$$

$$\Delta A_L = 0.4343 \times 10^4 \operatorname{Im}(K_{IH}-K_V) \quad \dots(5)$$

where $\operatorname{Re}(K_{IH}-K_V)$ and $\operatorname{Im}(K_{IH}-K_V)$ signifies the real and imaginary parts of the complex term $(K_{IH}-K_V)$.

RESULTS AND DISCUSSION

Differential attenuation due to rain and snow were computed versus frequency for different atmospheric conditions. Fig (1) shows differential phase shift and differential attenuation versus frequency for different rainfall rates. It is clear that for frequencies less than 10 GHz there is a sharp increase in $\Delta\phi$ as frequent increases. While for frequencies greater than 10 GHz there is a gradual increase in $\Delta\phi$. The results also suggest that as rainfall increases $\Delta\phi$ increases spontaneously for any given frequency, this mainly due to the fact that $\Delta\phi$ depends on the scattering term and the latter increases with increasing drop size and frequency. It is also evident that $\Delta\phi$ is significant for any given frequency and temperature. The behavior of ΔA is almost similar to that of $\Delta\phi$. Fig (2) illustrates the effect of temperature on both $\Delta\phi$ and ΔA . It is seen that below 30 GHz the temperature has no effect on $\Delta\phi$ and above 30 GHz $\Delta\phi$ tend to increase with increasing temperature. The result shows that ΔA is sensitive for temperature at all frequencies and it tends to increase with decreasing temperature.

Figures (3) and (4) shows the results of computations for snow. It is clear that at low frequencies $\Delta\phi$ and ΔA increases with increasing frequency and snowfall and $\Delta\phi$ is independent of temperature while ΔA increases with increasing temperature.

The results for dust and sand particles are shown in figures (5) and (6). It is clear that very dense storms, i.e. storms with visibility less than few meters, cause a depolarization in the signal through the induced $\Delta\phi$ and ΔA of noticeable importance. It is noticed that at 37 GHz and 10% moisture content (H_2O) the corresponding $\Delta\phi$ is less than that at 24 GHz and 10% H_2O . This is attributed to the fact that real part of dielectric constants at 37 GHz and much less than at 24 GHz. At 37 GHz and 5% H_2O the corresponding ΔA is less than that at 24 GHz and 5% H_2O . This is attributed to the fact that the imaginary part of dielectric constant at 37 GHz and 5% H_2O is much less than that at 24 GHz and 5% H_2O . The results also show that at frequency range (108 GHz) there is a sharp increase in both $\Delta\phi$ and ΔA while at the frequency range (14-37 GHz) there is a small increase in both $\Delta\phi$ and ΔA with decreasing visibility. The effect of moisture

content of storm on both is evident $\Delta\phi$ and ΔA is very evident since they increase sharply with increasing moisture.

CONCLUSION

Investigations of depolarization effects due to some atmospheric constituents have been carried out using computer simulation. It has been found that rain, snow, dust, and sand particles are the most important atmospheric elements affecting the polarization of the signal. The results showed that differential phase shift and differential attenuation are dependent on the frequency of the signal, the precipitation rates of rain and snow, the density and moisture content of dust and sand storms. Results are presented in a way that helps communication engineers in extracting information on the depolarization effects of the signal for any weather condition.

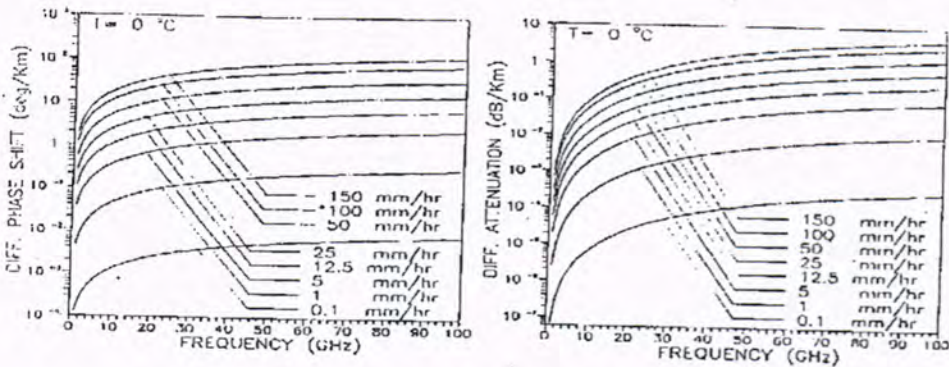


Fig (1): Differential phase shift and differential attenuation as a function of frequency for different rainfall rates.

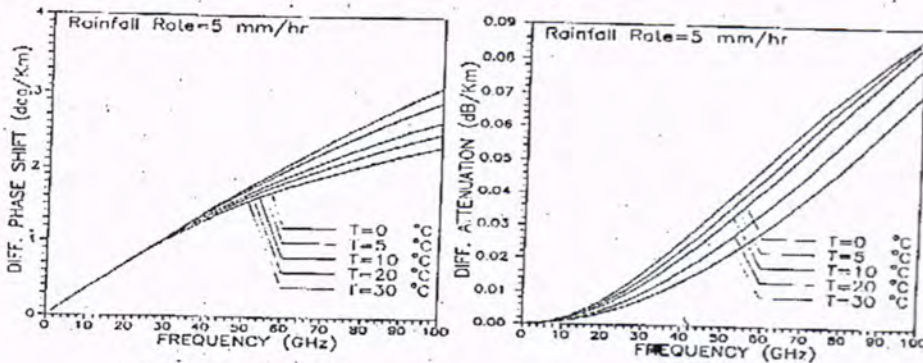


Fig (2): Differential phase shift and differential attenuation as a function of frequency for different temperatures.

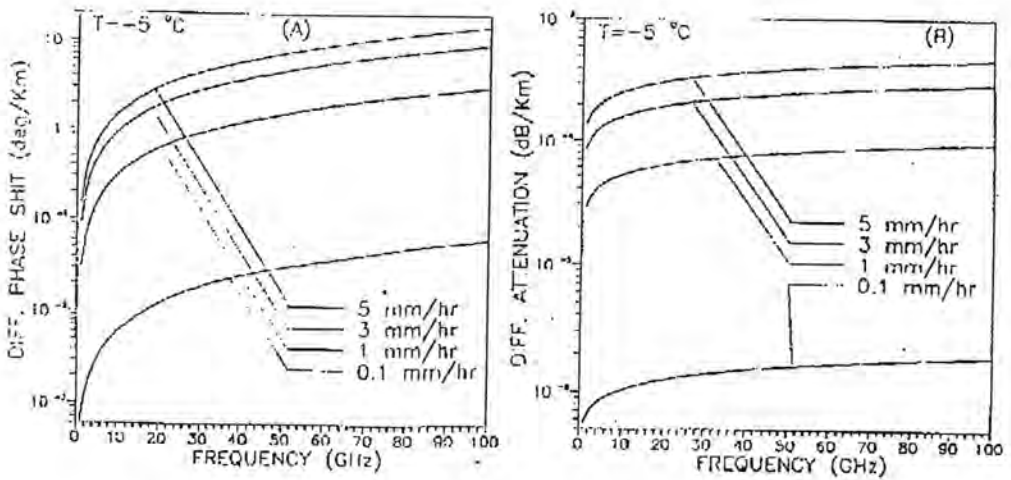


Fig (3): Differential phase shift and differential attenuation as a function of frequency for different snowfall rates.

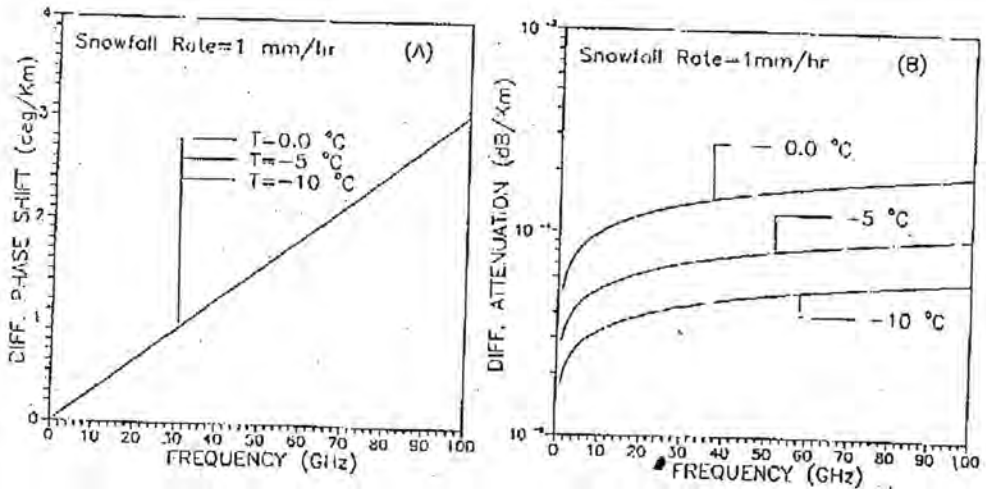


Fig (4): Differential phase shift and differential attenuation as a function of frequency for different temperatures.

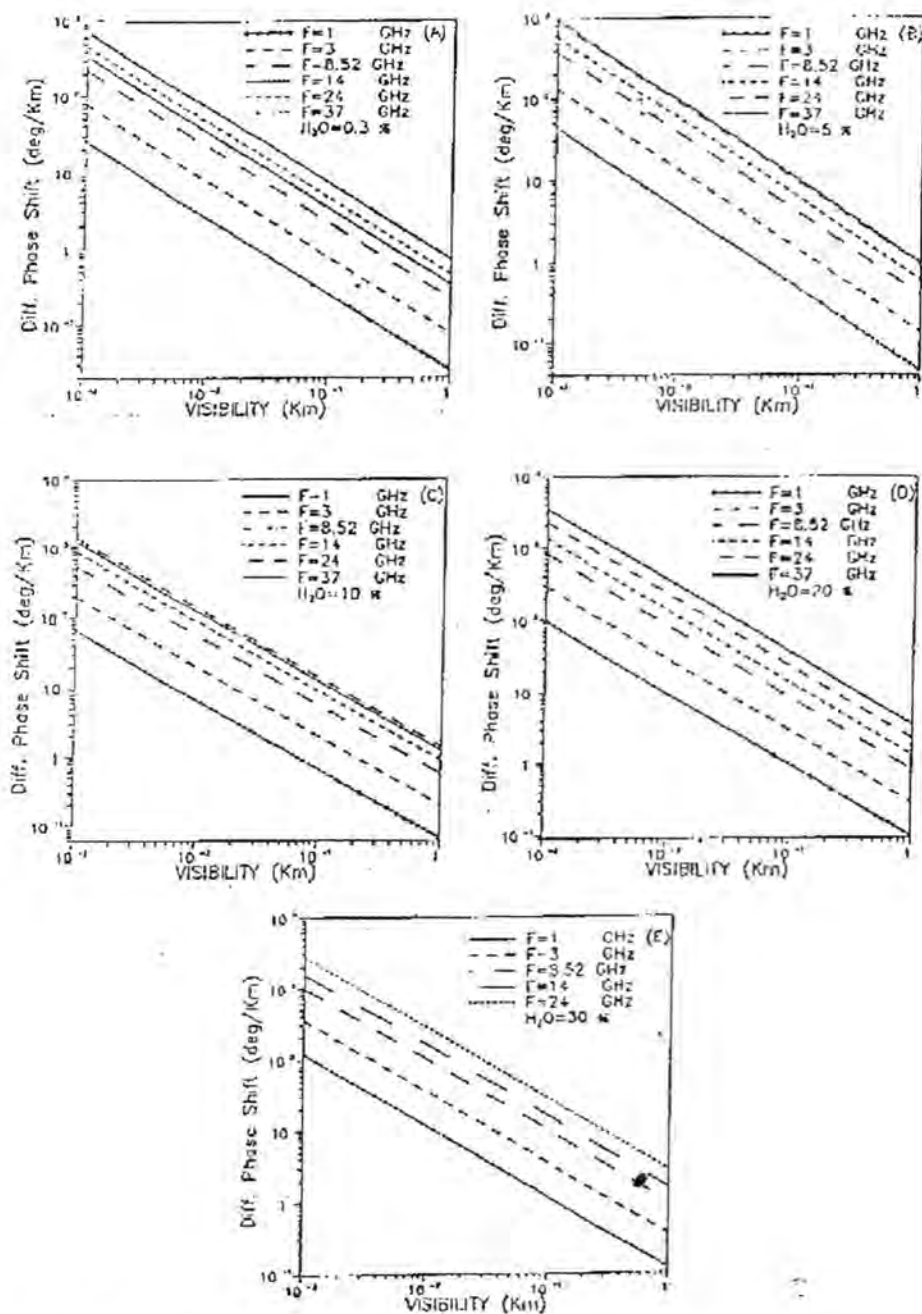


Fig (5): Differential phase shift versus visibility for various frequencies and moisture content.

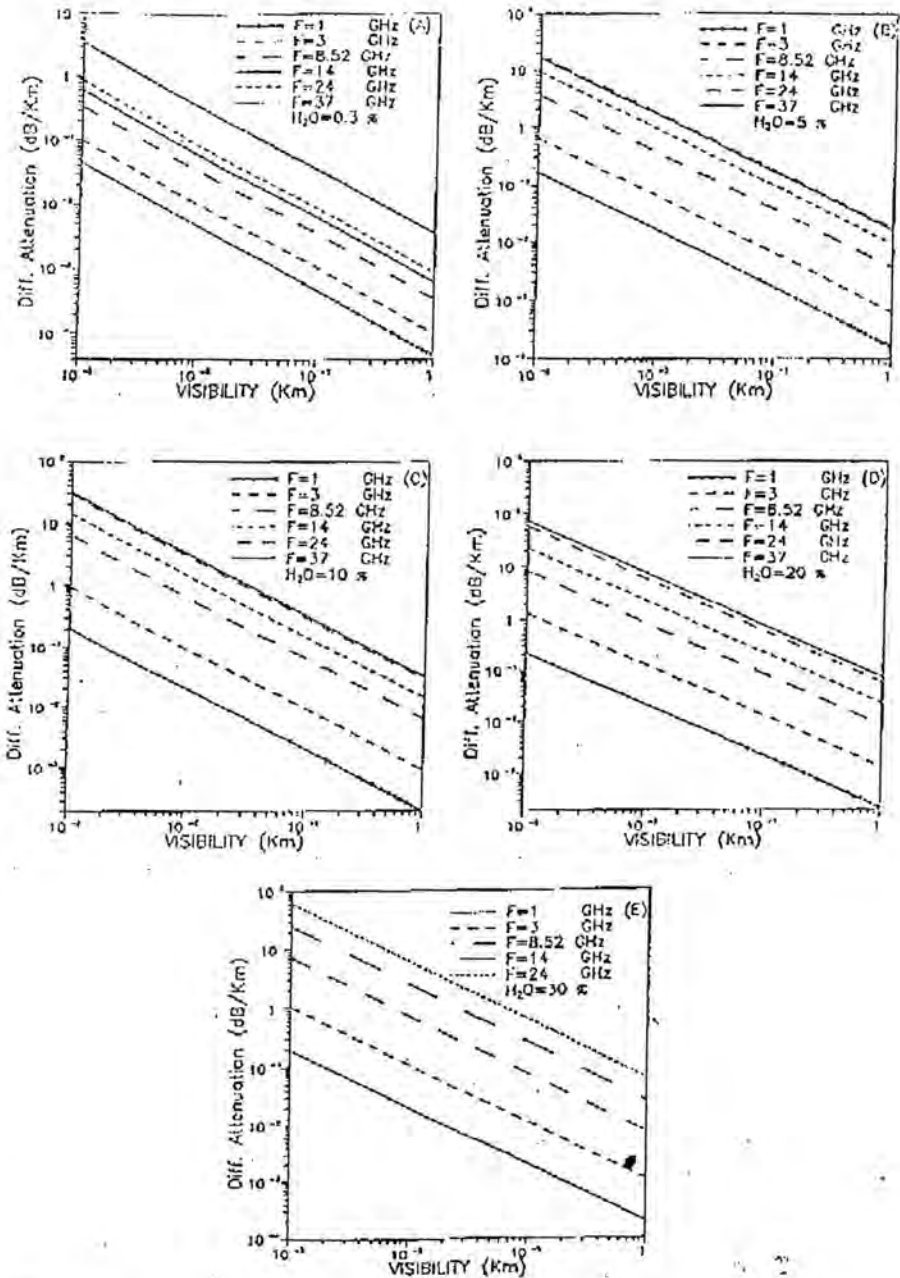


Fig (6): Differential phase shift versus visibility for various frequencies and moisture content.

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An Operational One – Dimensional Cloud Model

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ABSTRACT

The aim of this research is to develop a computer based one-dimensional cloud model using the formulation of Curic and Janc. The model includes the effects of forced lifting and entrainment, One the basis of radiosonde data, the model provides information about themicrophysical properties of cloud as a function of height. These include vertical velocity, mixing ratio for cloud, rain and snow as well as radar reflectivity. Results showed that the model, which was implemented on a personal computer, could be useful too for investigating the microphysics properties of connective clouds especially when instrumentation for such investigation are not available.

INTRODUCTION

One-dimensional steady-state cloud were investigated by many authors⁽¹⁻⁶⁾. These models were not very useful for forecasting the effects of precipitation loading on the cloud dynamics. One-dimensional time-dependent models were published by Weinstein (1970)⁽⁷⁾, Wisner et al. (1972)⁽⁸⁾ and Curic and Janc (1987, 1988, 1989)⁽⁹⁻¹¹⁾. Curic and Janc (1993)⁽¹²⁾ have showed that their entrainment formulation with combined effects of turbulent and dynamic processes together with forced lifting produce more acceptable results in forecasting the cloud-top heights. Cloud modeling is a very useful tool for investigating the cloud microphysical properties of clouds. The objective of this work is to develop an operational computer based model using the formulations of Curic and Janc (1993)⁽¹²⁾. Such model will be very practical for many application since instrumentation for measuring cloud microphysical are not available.

Model Equations and Numerical Techniques

The model equations used in this work are fully discussed by Curic and Janc (1993)⁽¹²⁾. The following is a brief description of these equations :

a) Equation of vertical velocity

The vertical velocity equation includes two components, the vertical velocity at the cloud base, which is derived form the third equation of motion, and the forced component :

$$w = w_0 + \int_0^{\Delta t} \left[-w \frac{\partial w}{\partial z} + \frac{g}{a} \frac{T_v P' - T_v'}{p T_v'} - \mu w - g(Q_l + Q_i) \right] dt \quad \dots (1)$$

where t is time, g the acceleration due to gravity, a the buoyancy reduction coefficient taken to be 1.05, T_v the vertical temperature, p the pressure, μ the entrainment rate, and Q_l and Q_i the respective liquid and solid water substance mixing ratios. The prime denotes an environment quantity; the zero subscript, a value at the beginning of the time interval Δt .

On the vertical velocity calculated from eq. (1) at the cloud base h is added to the forced component given by $w_{hr} = W_0 \sin \pi \frac{t - t_0}{\tau_0}$ where W_0 is the amplitude of the forced lifting, τ_0 its initiation time. Here we assume that the forced lifting amplitude is constant during the integration time for the sake of simplicity. W_0 and τ_0 are set to 6 ms^{-1} and 45 min, respectively.

b) Equation of entrainment rate

This equation describes entrainment processes of turbulent character and the organized entrainment processes through the vertical velocity advection :

$$\begin{aligned} \mu &= c_0 + c_1 |w| ds^{0.5} + c_2 \left[\frac{w(\partial w / \partial z)}{ds} \right]^{0.5} \quad \text{for } w \frac{\partial w}{\partial z} \geq 0 \\ \mu &= c_0 + c_1 |w| ds^{0.5} - c_2 \left[\frac{w(\partial w / \partial z)}{ds} \right]^{0.5} \quad \text{for } w \frac{\partial w}{\partial z} < 0 \end{aligned} \quad \dots (2)$$

where

$$ds = \mu(2r + dr) \left[(dr)^2 + (dz)^2 \right]^{0.5} \quad \dots (3)$$

and dr and dz are differential changes of the core radius r and the height, respectively, while c_0 , c_1 , and c_2 are constants.

c) Thermodynamic equation

The cloud air temperature is calculated from the first law of thermodynamics :

$$\frac{dT}{dt} = \gamma_a w \frac{T}{T} + \mu \left(\delta T + \beta_2 \frac{L}{c_p} \delta q \right) - \beta_2 \frac{L}{c_p} \frac{dq}{dt} + \frac{L_s}{c_p} P_{SUB} + (1 - \beta_2) \frac{L_v}{c_p} P_{REVP} + \frac{L_f}{c_p} P_F + \frac{c_w}{c_p} T_c P_{MELT} - \left[\sum_k DQ_k (w - U_k) + (c_w Q_{cw} + c_i Q_{ci} \beta_i) w \right] \gamma \dots(4)$$

where

$$D = \begin{cases} c_w k = r \\ \beta_i c_i k = s, h \end{cases}$$

$$\beta_i = \begin{cases} 1 & T < 273 \text{ K} \\ 0 & \text{otherwise} \end{cases}$$

$$\beta_2 = \begin{cases} 1 & Q_{ci} + Q_{cw} > 0 \\ 0 & Q_{ci} + Q_{cw} < 0 \end{cases}$$

and *r*, *s* and *h* are related to rain, snow, and hail fractions. *L_v*, *L_f*, *L_s* are latent heats of evaporation, freezing and sublimation, respectively; *L* represents either latent heat of evaporation (*L_v*) or sublimation (*L_s*); *c_w* and *c_i* are specific heats with respect to water and ice; *Q_r*, *Q_h* and *Q_s* are mixing ratios for water vapor, cloud ice, cloud water, rain, hail, and snow, respectively; δT is the difference between ambient air and cloud air temperatures, δq the difference between ambient air water vapor mixing ratio and saturated water vapor mixing ratio with respect to water or ice at cloud air temperature; *T_c* is the cloud air temperature (°C); γ_a and γ are dry adiabatic and cloud air temperature lapse rates with *P_{SUB}*, *P_{REVP}*, *P_{MELT}* and *P_F* are production terms due to sublimation, evaporation, melting, and freezing processes by Lin et al. (1983).

d) Microphysical equation

These includes the continuity equation for water vapor and non-precipitating elements (water clouds and ice clouds) and the continuity equation of the presipitating water (rain, snow, hail). These equations are given by :

$$\frac{dQ}{dt} = - \mu Q - P_r - P_s - P_h \dots(5)$$

$$\frac{dQ_k}{dt} = - \mu Q_k + P_k + \frac{1}{\rho} (U Q_k \rho) \dots (6)$$

where Q is the sum of the mixing ratios of water vapor, cloud water and cloud ice, $k = r, s, h$ (rain, snow, hail) respectively, and U_k is the terminal velocity.

The above equations were solved using numerical techniques, namely, the finite differences. A Fortran language was used to develop the computer code which was implemented on a Pentium II personal computer using Prospero Fortran Compiler. The input required for the program are the entrainment coefficients and the radiosond date, i.e. pressure, temperature, and dew point as a function of height. The output consists data as a function of height, these are vertical velocity, mixing ratios for cloud, rain and snow (or ice), and radar reflectivity. The output is produced for each 15 sec interval for the duration of the cloud development.

RESULTS AND DISCUSSION

The model was test using standard US atmosphere, mid-latitude summer and winter soundings. The results were compared with the work of Curic and Janc (1993) and it was found that the program gives an acceptabel results and slight differences found were attributed to the differences in the sounding data. Several experiments were carried out using sounding data measured in Iraq. Figures (1) to (3) show samples for the results of these experiments. The microphysical properties of cloud are displayed as a function of height starting from the cloud base. These are the vertical velocity (w), mixing ration for cloud (QC), mixing for rain (QR), and mixing ratio for ice (QI), as well as the radar reflectivity (Z). Fig (1) show the effect of the entrainment coefficients (c_0, c_1, c_2) assuming zero forced lifting. Various combinations of these coefficients were used. The effects of the entrinment on the microphysical are very evident. Fig (2) shows the effect of the forced lifting amplitude (W). Three values were selected, 6, 12, 18m/s, it is seen that the vertical velocity decreased when W was changed from 6 m/s to 12 m/s and increased above the 6 m/s values when W was changed to 18 m/s, this may be due to the nature of the sinusoidal nature of the forced lifting component. The effects on $QC, QR, QI,$ and Z was that they decreased with increasing W and became independent of W after the value of 12 m/s. Fig (3) illustrate the effects of duration of forced lifting (TAU), two values were chosen 30 and 45 min, it is seen that the increase in the duration of the forced lifting tend to increase the microphysical properties of the cloud. These small experiments show that the model may used operationally to satisfy several needs. We plan to use the model in cloud seeding project carried on in Iraq.

CONCLUSIONS

A computer based one-dimensional model was developed on the basis of the formulation of Curic and Janc (1993). The model includes the effects of forced lifting and entrainment. Several tests and experiments were carried out, the results show that the model give and acceptable values of the microphysical properties of cloud. It can be used to determine these properties for any radiosondig data and it can be operated to satisfy several needs in investigating clod properties specially when instrumentation are not available to carry out such measurements.

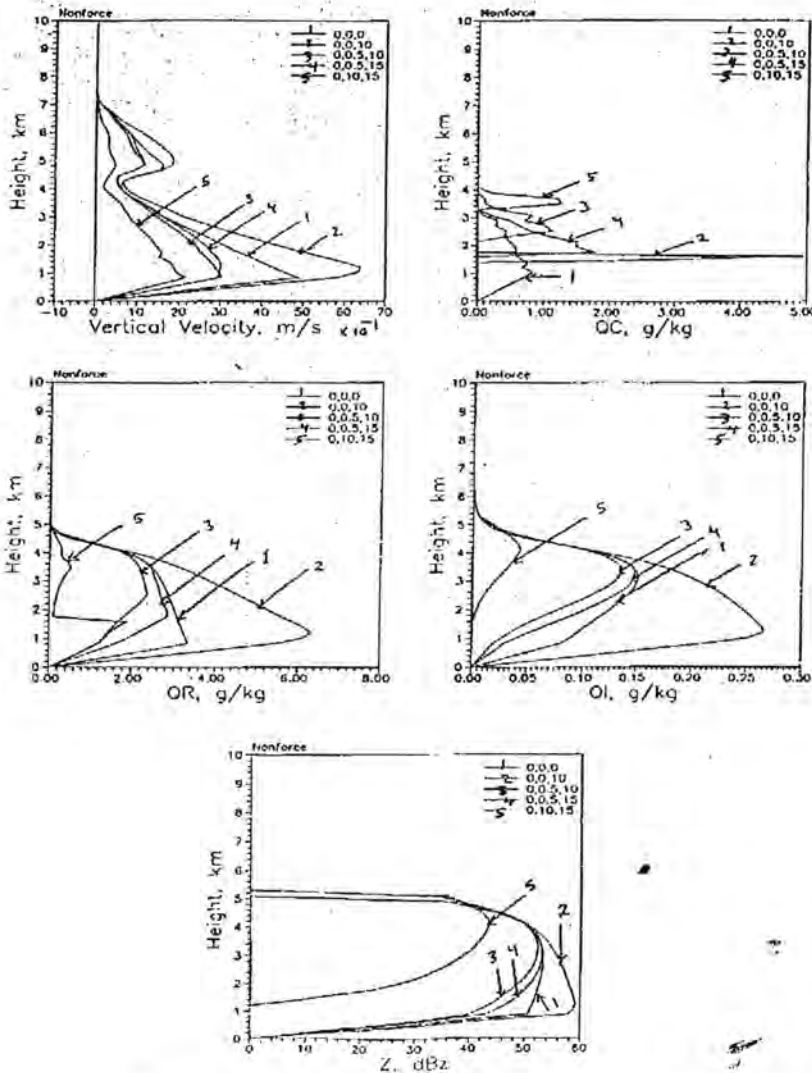


Fig 1. Microphysical properties of cloud for various combinations of entrainment coefficient (c_0, c_1, c_2) for $t=5min$.

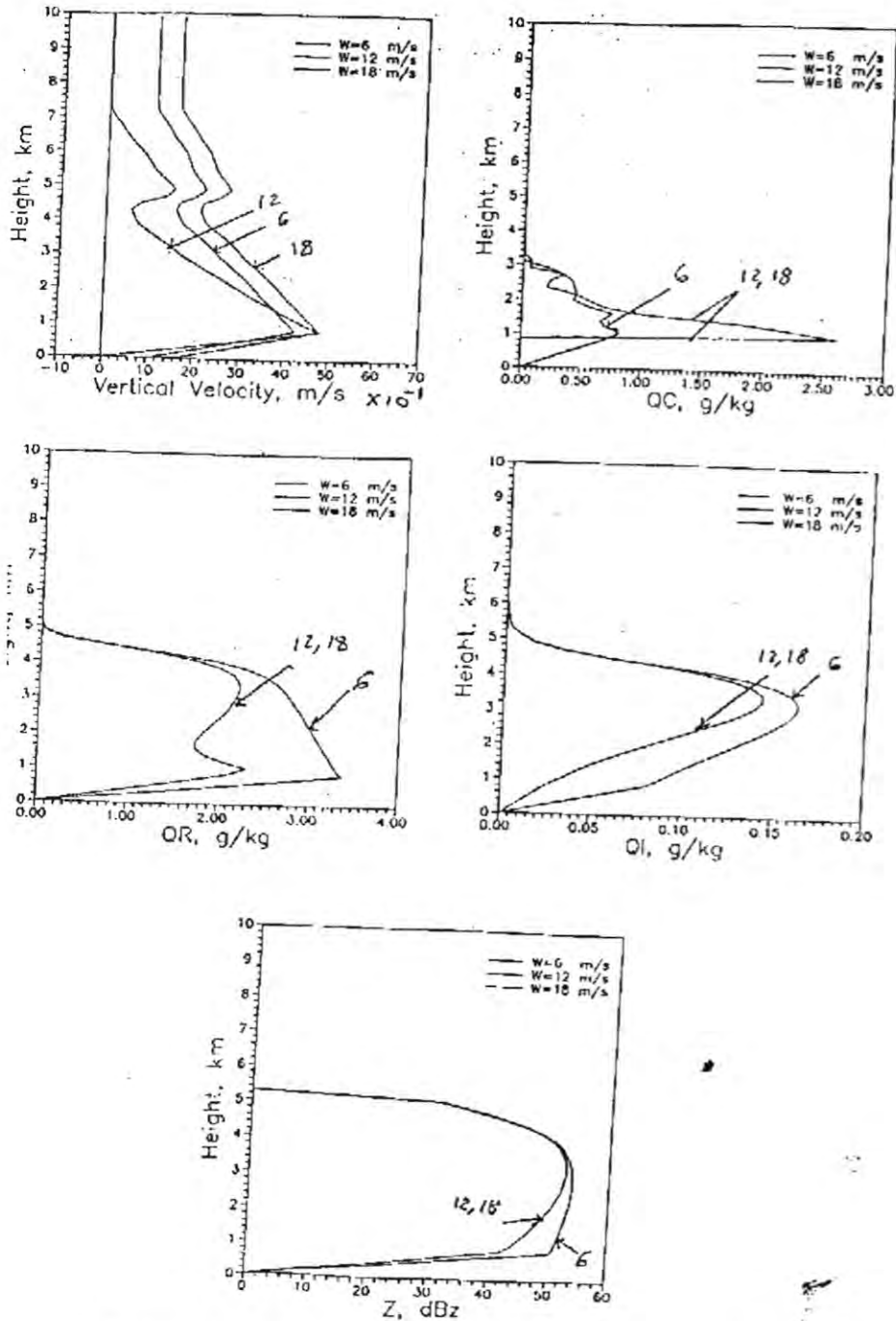


Fig 2. The effects of the forced lifting amplitude (w) on microphysical properties of cloud for $t=5$ min.

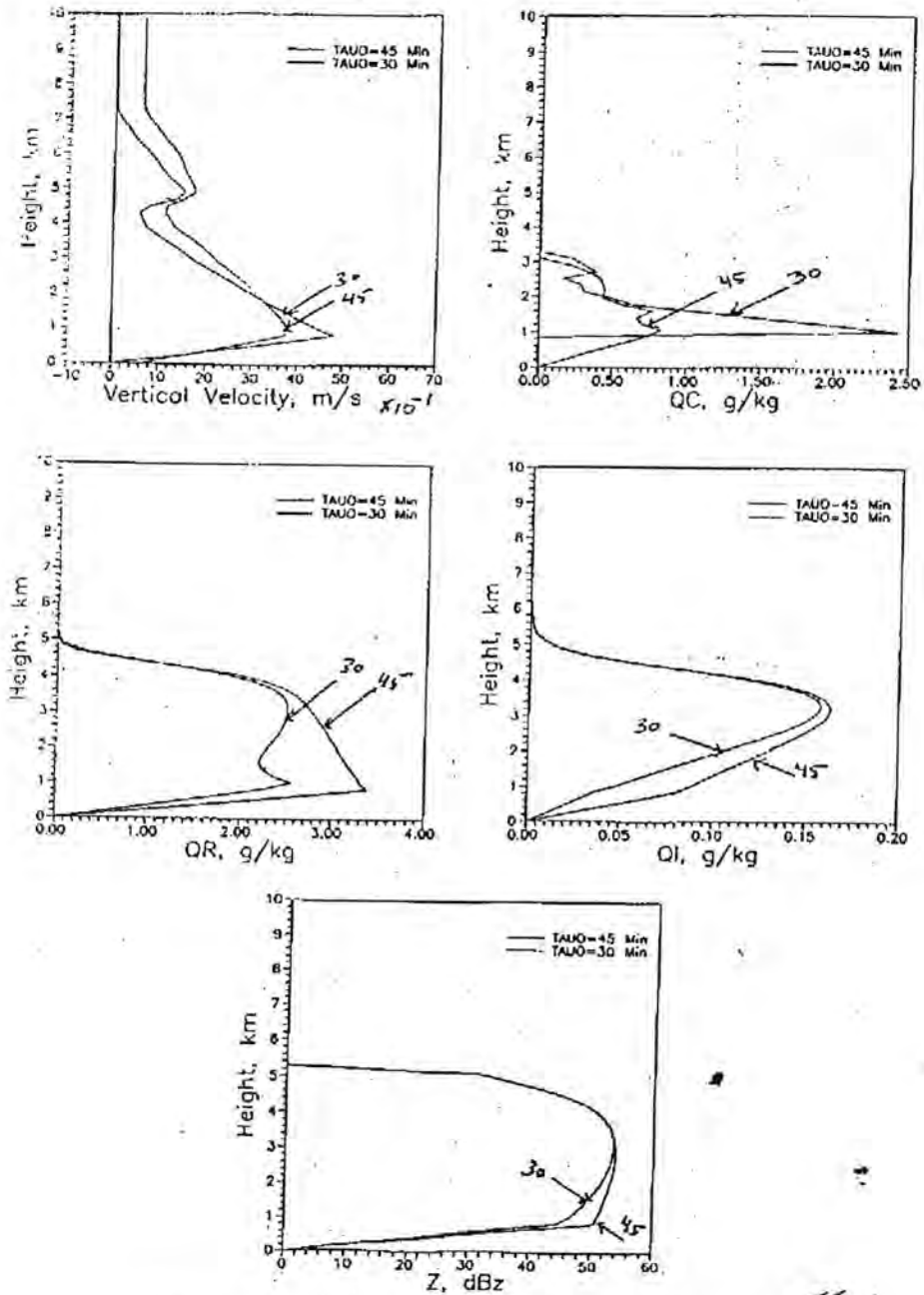


Fig 3. The effects of the duration of forced lifting (TAU) on microphysical properties of cloud for $t=5$ min.

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An Intelligent Theorem Prover

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الخلاصة

يعد موضوع برهنة النظريات أحد مواضيع الذكاء الاصطناعي والذي يهتم ببناء أنظمة استدلال ذكية. تعتمد معظم مبرهنات النظريات في عملها على تطبيق أحد تحسينات مبدأ القرار على مجموعة من الفقرات المتناقضة. يمثل النمو الآسي لفضاء الفقرات اضافة لشرط توقف عملية البرهنة المشكلتان الرئيسيتان لموضوع برهنة النظريات. ولتجاوز هاتين المشكلتين تم استحداث ثلاثة تطويرات : أولاً، عملية التقليل والتي تحدد وتحذف كل الفقرات المعتلة التي لا تشارك في تكوين البرهان. ثانياً، طريقة المجموعة اللامتزنة والتي تمثل شرطاً ضرورياً لاستنتاج التناقض. ثالثاً، قانون القرار الموجه والذي يختار ألياً القرار المناسب اعتماداً على الفقرات والتي بدورها يتم اختيارها بعناية. حسنت تلك التطويرات السلوكية العامة لمبرهنات النظريات ورفعت كفاءتها، فقد تقلص فضاء الفقرات بشكل ملحوظ. وتعزز كذلك شرط توقف البرهنة. أما عملية البرهنة ذاتها فقد أصبحت مقادة باتجاه التناقض. وبتنفيذ تلك التطويرات اظهر النظام تمييزاً ذكياً للمعلومات غير الضرورية للبرهان. كذلك اصبح التشخيص المبكر لغياب التناقض ممكناً. اضافة لذلك ، فان النظام اظهر استقلالية الاختيار لقوانين الاستنتاج المناسبة لكل حالة. ونظراً للسلوكيات الذكية الأضافية التي اظهرها النظام، فقد سميناه مبرهن النظريات الذكي.

ABSTRACT

Theorem Proving is the field of Artificial Intelligence that is interested in building reasoning systems. Most of current theorem provers are based on the application of resolution principle extensions on a refutation set of clauses. The main problems of theorem proving are the exponential growth of the clause space, and the termination condition of proving process.

In order to overcome these problems, three improvements are introduced. First, the reduction process, that detects and deletes singular clauses that are not involved in a proof. Second, the unbalanced set method, which characterizes a necessary condition for contradiction. Third, the directed resolution, that selects an appropriate resolution extension with respect to a carefully selected clause.

These improvements have improved the proving behavior and increased its efficiency. The clause space is significantly reduced. Also, the termination condition is more enhanced. Moreover, the proving process is directed towards the contradiction. By implementing these improvements, the resulting system has showed an intelligent recognition of unnecessary information, an early diagnosing of satisfiability, and an independent selection of inference rules. Because of these additional intelligent activities, the resulting system is called an Intelligent Theorem Prover.

INTRODUCTION

1.1 Historical background

Artificial Intelligence is the study of ideas that enable computers to behave intelligently as human⁽¹⁷⁾. Some researchers consider the ability to reason as the major ability that characterizes the human being intelligence. Reasoning and human ability of thinking were major questions for centuries. Aristotle (384-322 BC) was the first to try to describe the laws of thinking. He was successful in formulating 19 correct laws (called syllogisms). They were a significant part of Aristotle huge and great achievement in the history of philosophy and science, which was the construction of logic⁽⁴⁾. Since the foundation of logic and until the middle of the 19th century, logic was expressed in a natural language with a little use of symbols. G. Boole (1850) invented a calculus of logic⁽⁹⁾ and clarified the subject of symbolic logic. Then the convergence between logic and mathematics had started. G. Freg (1855) introduced predicates (or logical functions) to represent the logical relationships between objects and gave some rules for making deductions. His language of predicates with his rules of deductions, were called the predicate calculus⁽⁴⁾⁽⁵⁾. In 1931, Godel succeeded in proving the completeness of the predicate calculus. From that time, predicate calculus was considered the powerful language of describing and proving⁽⁹⁾.

1.2 Mechanical Theorem Proving

The correctness of inferences is related to the logical structure of the inference rule and not the domain of the problem. This fact has encouraged many researchers to design mechanical methods for deriving conclusions from a given axioms expressed as predicate calculus expressions regardless of the domain of the problem under consideration⁽⁴⁾.

1.3 Pre-resolution methods

Mechanical theorem proving was performed with investigating the given axioms and repeatedly applying complete inference rules on them until the required theorem is deduced. This operation is not easy since there might be a

relatively tremendous number of unnecessary deductions from the axioms. Also, there is a problem in guiding the deduction process towards the required theorem. Moreover, the completeness of the inference rule may not be guaranteed. In addition, the termination of the process of applying the inference rule may not be guaranteed^[19].

The alternative method of mechanical theorem proving is performed by refutation, since searching for a contradiction is simpler than searching for a specific theorem. Contradiction can be generally detected if exist^[19]. This sort of proving doesn't require a complete inference rule. In fact there is a requirement of a new characteristic, which is the refutation-completeness that is the inference rule must deduce the contradiction if existed^[19]. The set of axioms and the negated theorem is called a refutation system^[4].

1.4 Resolution principle

In 1965, J. A. Robinson developed the resolution principle. This principle was based on searching for contradictory predicates among statement^[4]. Resolution is an inference rule that takes two statements as input, and produces another statement as output. The output statement, the resolvent, represents a true statement consistent with the input statements as the result of resolving them^[13]. The resolvent is one conclusion we can draw, but not necessarily the only one^[5]. The application of resolution requires that every thing must be in clause form. By clause form we mean that the information is expressed as an ANDing of ORed literals. By literal we mean a negated or un-negated predicate. So any problem expressed in predicate calculus must be transformed into a logically equivalent clause form. The procedure of transformation, must take on account the quantification of variables, implications and nesting of (&, |, ~) connectives^[13].

Resolution requires pairs of opposites within the two input clauses. That is, one input clause must contain a literal, call it E1 for which $\sim E2$ occurs in the other input clause, and where E1 matches E2 using a suitable substitution of variables, if necessary. The resolvent of the two input clauses is the ORing of them after canceling E1 and $\sim E2$, and eliminating any duplicate or redundant expressions in the resolvent. If the resolvent consists of only one literal, unit clause, then we have proved a fact. If the resolvent consists of no literals, empty (or null) clause, then we have proved a contradiction^{[4][19]}. Resolution is a sound rule of inference^{[4][5][17][19]}, any resolvent logically follows from the input clauses. If duplicate literals, factors, are deleted, resolution will become refutation complete inference rule^{[17][19]}. Resolution deduction becomes more useful when we do several resolutions in succession, every resolvent can participate many times as an input clause to a next resolution step^[13]. Resolution has a structural form that is not related to the subject or even the domain of the theorem under consideration. One of the magnificent advantages of resolution is the fact that

many inference rules can be proved as being a resolution step. Modus ponens, modus tolens, backward chaining, forward chaining, rule collapsing and many other inference rules are special cases of resolution^[13]. When predicates have variable arguments, a unification process is needed to match the two contradicted literals. Unification process searches for a suitable substitution of variables that makes two contradicted literals identical except for sign^[5].

1.5 Extensions (refinements) of resolution

Since the development of resolution principle, many extensions have been made. hyperresolution, negative hyperresolution, ur-resolution and many other kinds are examples of such extensions^[19]. Since the original principle uses two clauses, it is called binary resolution^[4]. These extensions to resolution were developed to provide additional efficiency to the process. Strategies (both ordering and restriction) also designed for guiding the search of contradiction and controlling the clause space size^[19].

1.6 Basic elements of theorem proving

The requirements of mechanical theorem proving is divided into five basic elements:

1. Representation of information: Predicate calculus is the most suitable representation^{[9] [15]}. If we use resolution, we must convert the representation into a logically equivalent clause form, and the information must be provided as a refutation system^[4].
2. Inference rules: We must choose a suitable inference rule to be applied on the chosen representation. Any chosen inference rule must be sound, and when used with a refutation system, it must be refutation complete^[19].
3. Strategies: We must use suitable strategies for guiding the search for contradiction and controlling the theorem prover reasoning. These strategies are classified as ordering strategy or restriction strategy^[19].
4. Redundancy control: Redundancy occurs when we infer the same conclusion repeatedly (no need for keeping more than one copy of a clause). Another kind of redundancy can be detected if we have clauses that are logically included by another clauses (subsumption process detects and deletes them). Tautologies are also redundant clauses (always evaluate to true). Redundant clauses must be detected and can be deleted without affecting the original clause state^{[5] [19]}.
5. Unification: There must be a procedure that finds, if one exists, a most general unifier for any two given expressions such that when applied to both expressions, they become identical except for sign. If the unification fails, the

resolution step cannot be performed. It is needed if the representation contains variable(s) ^[2].

1.7 Theorem provers examples

A number of existing theorem provers are available: L. T. a short hand of Logic Theorist, is a theorem prover designed by Simon and Newell which can discover some primitive proofs in symbolic logic using heuristic techniques similar to those used by human. LMT and AM are another examples of theorem provers ^[19]. The most recently developed theorem prover is Otter ^[18] which is rather easy to use, requiring one to prepare an input file that contains no more than a statement of the problem and an instruction concerning the type of resolution to employ ^[20].

1.8 Theorem proving problems

In spite of all of that, the two major problems, which are the exponential growth of the clause space that leads to a combinatorial explosion and the termination condition of the theorem prover, still exist. The previous improvements have really decreased the number of generated clauses, but the exponential character still exists. By the use of restriction strategies and the redundancy control process, the termination condition is now more likely to be reached but this is not guaranteed. Theorem proving still of a semi-decidable character. We can prove the set unsatisfiability, but proving the set satisfiability is not guaranteed ^[17]. In 1971, Stephen Cook announced in his paper "The complexity of Theorem Proving" that the satisfiability is a NP- Complete problem ^[7].

1.9 Goal of this work

The combinatorial explosion is a natural consequence of an evolutionary set of clauses. So, if we can select an appropriate inference rule to each selected clause, find more clever strategies for guiding the search to the contradiction, employ more new redundancy control process and diagnose the satisfiability of the set earlier, then the effect of those two critical problems will be greatly reduced.

2 The Proposed Intelligent Theorem Prover (ITP)

To enhance the theorem proving process, we introduce three improvements: the reduction process, the diagnosing process, and the directed resolution process. These improvements overcome some of the problems that face the subject of Theorem Proving.

2.1 The Reduction Process

This process is capable of detecting and deleting a class of unnecessary singular clauses from a set of clauses.

Definition 1'. The predicate p is said to be singular in a set of clauses S if and only if either p appears only in a single clause in S or all occurrences of p in S are in either positive or negative literals but not in both. A literal is called a singular literal if its predicate is singular in S .

Suppose we have the following set of clauses:

$$\begin{aligned} & p(X) \mid \sim q(X) \mid \sim r(a) \mid \sim r(b) \mid r(X). \\ & p(a) \mid q(b) \mid s(m). \\ & \sim s(m) \mid p(c). \\ & s(Y) \mid q(Y). \end{aligned}$$

Then, according to definition 1, the predicate p is singular since all its occurrences are in positive literals. Also the predicate r is singular since it appears only in the first clause. If we consider the positive literals of a clause as conclusions and the negative literals as conditions, then positive singular predicates are unproductive conclusions and negative singular predicates are unreachable conditions. The case in which a singular predicate occurs only in one clause is a general case of tautology that is always a true and denotes a trivial and unrelated information.

Definition 2: Let S is a set of clauses. The clause A in S is called a singular clause if and only if there exists a singular literal in A .

In the previous set of clauses, the first three clauses are singular according to definition 2. Singular clauses resemble unnecessary information.

Informally, any clause with a singular literal will never lead to the empty clause (i.e., contradiction). We will not be able to resolve upon a singular literal since there is no contradictory literal in the whole set. If L is a singular literal then any singular clause contains it will be resolved into yet singular clauses. In the best cases, a singular clause will be resolved into a unit clause that contains only the singular literal. So it will not participate in any future resolution because it

cannot be resolved. The absence of the complementary literal in the set of clauses will keep the singular literal in the set and will never be deleted. As an important result, singular clauses will never lead to a contradiction. Thus no singular clause will participate in the proof if it exists. So we can delete them without affecting the refutation deduction. Any set of clauses can be reduced to a smaller set by deleting all singular clauses without affecting the satisfiability or unsatisfiability state of the original set.

Theorem 1: Let S is a set of clauses and let S_r is the set S after deleting all singular clauses. Then S_r is unsatisfiable if and only if S is unsatisfiable.

Proof: (if-part)

Let S is unsatisfiable. Assume that S_r is satisfiable, then there exists a model I of S_r . Let L is a singular literal in S . Since neither L nor $\sim L$ is in S_r , then neither L nor $\sim L$ is in I . So any interpretation of S that contains I and L (assigned to true) is a model of S . So S is satisfiable. But this contradicts our assumption that S is unsatisfiable. So S_r is unsatisfiable.

(only if-part)

Suppose S_r is unsatisfiable. Then S_r evaluates to false in every interpretation. So the conjunction of any clause to S_r still unsatisfiable since it is ANDed with false. So any set from which S_r is a subset is also unsatisfiable. But S_r is a subset of S , so S is unsatisfiable.

Definition 3: A set of clauses S is said to be stable if and only if it contains no singular clauses.

Sometimes deleting singular clauses leads to new singular clauses that were hidden by the original singular ones. Suppose we have a singular literal L , then singular clauses containing L may contain another literals that might be the only contradictory literals to other literals in other non-singular clauses. If we delete the singular clauses, then those non-singular clauses will become singular since their contradictory literals are no more exist. The resulting singular clauses can also be deleted without affecting the state of the set. If we have unsatisfiable set S , then deletion of the singular clauses in it with respect to some singular literals gives S_{r1} that is also unsatisfiable. Now consider S_{r1} as the original unsatisfiable set, if other singular clauses appear then deleting them will give S_{r2} which is also unsatisfiable according to theorem 1. By continuing in the process until no more singular clauses appear, the resulting set is unsatisfiable just as the original set S . If S is satisfiable then the resulting set is also satisfiable.

The reduction algorithm outline.

Initially, we build a predicate PredTable from a given set of clauses S. The number of entries in this table equals the number of distinct predicate symbols in S. Each entry contains the predicate name Pred, a list of pairs PosList to index each clause containing Pred in a positive literal, and another list of pairs NegList to index each clause containing Pred in a negative literal. Each pair contains the number j of the corresponding clause and the weight w of that clause. Each list is sorted in ascending order with respect to weights.

Algorithm reduction (PredTable)

algorithm theoremProver :

input information;

build the improved set of support queue SupQueue;

build the queue of ordered positive clauses PosQueue;

build the queue of ordered negative clauses NegQueue;

build the predicate table PredTable;

Contradiction \leftarrow false; {initial state}

Pcount \leftarrow 0; *Ncount* \leftarrow 0; {for positive and negative clauses}

reduction (PredTable); {activate the Reduction process}

if empty (SupQueue) or empty (PredTable) then

announce ("failure .. no contradiction");

else

This algorithm is activated at the beginning of proving process and reactivated whenever already existing clauses were (backward) subsumed.

Example: Suppose we have the following set of clauses:

1. $\text{divided}(X, 2) \mid \sim\text{even}(X)$. {even number are divisible by 2}
2. $\text{divided}(X, 3) \mid \sim\text{sumDigits}(X, S) \mid \sim\text{divided}(S, 3)$. {if sum of number digits is divisible by 3, it is divisible by 3}
3. $\text{divided}(X, 5) \mid \sim\text{start}(X, 5)$. {if a number starts with 5, it is divisible by 5}
4. $\text{divided}(X, 10) \mid \sim\text{start}(X, 0)$. {if number starts with 10, it is divisible by 10}
5. $\text{even}(X) \mid \sim\text{double}(X)$. {any duplicate number is even}
6. $\text{start}(X, 0) \mid \sim\text{multiple}(X, 10)$. {any multiple of 10, starts with 0}
7. $\text{mod}(X, Y, 0) \mid \sim\text{divided}(X, Y)$. {X divided by Y means $X \bmod Y = 0$ }
8. $\text{equal}(Y, X) \mid \text{equal}(Y, 1) \mid \sim\text{divided}(X, Y) \mid \sim\text{prime}(X)$. {if a divisor of a number isn't equal to the number or 1, the number is not prime}
9. $\sim\text{double}(X) \mid \sim\text{prime}(X)$. {any duplicate number is not a prime}

10. $\sim \text{even}(X) \mid \sim \text{greater}(X, 2) \mid \sim \text{prime}(X)$. {any even number greater than 2 is not a prime}.

Suppose we have the following theorem:

Forall Num(double (Num) \rightarrow divided (Num, 2)).

If we negate the theorem and transform it into clause form the result will be :

11. double (sko),

12. \sim divided (sko, 2).

Let us build the predicate table, PredTable:

Predicate	Positive List	Negative List
Divided	1,3,4,2	12,7,2,8
Even	5	1,10
SumDigits	--	2
Start	6	3,4
Multiple	--	6
Double	11	5,9
Mod	7	--
Prime	--	9,10,8
Equal	8	--
Greater	--	10

Table 1. Initial predicate table.

According to the definition of the singular predicate the predicates sumDigits, multiple, mod, prime, equal, and greater are singular since they have no contradictory predicate in any clause. So, clauses 2,6,7,8,9 and 10 are singular. We can reduce the table, PredTable, into:

Predicate	Positive List	NegativeList
Divided	1,3,4	12
Even	5	1
Start	--	3,4
Double	11	5

Table 2. Predicate table after deleting singular clauses (first cycle).

Notice the appearance of a hidden singular predicate start. So, clauses 3 and 4 are singular. We can reduce the table, PredTable, into:

Predicate	PositiveList	Negative List
Divided	1	12
Even	5	1
Double	11	5

Table 3. Predicate table after deleting singular clauses (second cycle).

Since the table has no more singular clauses, the set of clauses 1, 5, 11, and 12 is stable and logically equivalent to the original set of clauses. The reduction process has decreased the original 12 clauses into only 4 clauses while preserving the state of the set. Deleting unnecessary clauses can be considered as a sixth basic element of the theorem prover. The reduction process may enhance the termination conditions of the theorem prover because it may delete the clauses of the negated theorem or transform the state of the set into an unbalanced state (will be explained shortly). The combinatorial explosion is relatively decreased with the reduction process.

2.2 The Diagnosing Process

This process determines a case in which a set of clauses is satisfiable even if there is still more resolvable clauses (case of a set that does not contain at least one positive clause and another negative clause). Other cases of set satisfiability are determined by the assistance of the improved set of support strategy and the above reduction process.

Theorem 2: For any two clauses A and B, the resolvent of a binary resolution in which A and B are the parent clauses will be (if exist):

1. Mixed clause if both A and B were Mixed clauses.
2. Mixed or Positive clause if A was Mixed and B was Positive.
3. Mixed or Negative clause if A was Mixed and B was Negative.
4. No resolvent is possible if both A and B were either Positive or Negative clauses.

Proof: Considering each case respectively and each L_k denotes a positive literal:

1. Let $A = L_1 | \sim L_2 | R_a$ and $B = L_3 | \sim L_4 | R_b$, are two mixed clauses such that R_a and R_b , are disjunction of zero or more literals and L_1 unifies with L_4 under a substitution Φ then by the application of binary resolution, the resolvent is: $C = (\sim L_2 | L_3 | R_a | R_b) \Phi$ which is obviously mixed clause whatever the content of

- R_a and R_b , (notice that C contains at least one positive literal L_3 and at least another negative literal $\sim L_2$). Hence, the resolvent of A and B will always be mixed (if exist).
2. Let $A = L_1 \mid R_a$ and $B = \sim L_2 \mid L_3 \mid R_b$ are positive and mixed clauses respectively, where R_a is a disjunction of zero or more positive literals and R_b , is a disjunction of zero or more literals and L_1 unifies with L_2 under a substitution Φ . By the application of binary resolution, the resolvent is: $C = (L_3 \mid R_a \mid R_b) \Phi$ which is positive if R_b , is a disjunction of zero or positive literals and is mixed if R_b , contains at least one negative literal. Hence the resolvent of A and B will always be either positive or mixed.
 3. Let $A = \sim L_1 \mid R_a$ and $B = L_2 \mid \sim L_3 \mid R_b$, are negative and mixed clauses respectively, where R_a is a disjunction of zero or more negative literals and R_b is disjunction of zero or more literals and L_1 unifies with L_2 under a substitution Φ . By application of binary resolution, the resolvent is: $C = (\sim L_3 \mid R_a \mid R_b) \Phi$ which is negative if R_b , is a disjunction of zero or negative literals, and mixed if R_b , contains at least one positive literal. Hence, the resolvent of A and B will always be either negative or mixed.
 4. Let both A and B are positive clauses. It is obvious that for each (positive) literal in A , there is no a contradictory (negative) literal in B since they are all positive. So, no possible resolution can be applied on two positive clauses. Similarly, if both A and B are negative clauses, then for each (negative) literal in A there is no contradictory (positive) literal in B since they are all negative. So, no possible resolution can be applied on two negative clauses. We can conclude that no resolvent is possible between A and B if they both were either positive or negative.

Definition 4: A non-empty set S of clauses is said to be, balanced if it contains at least one positive clause and at least one negative clause. A set S is said to be unbalanced if it is not balanced.

Theorem 3 (the unbalanced set theory): If a set of clauses S is unbalanced then it is satisfiable.

Proof: We know that S is satisfiable if there is no deduction of the empty clause (i.e., deduction of contradiction). It is obvious that the empty clause is neither mixed nor positive nor negative because it contains no literals. Since S is unbalanced then it is in one of the following cases:

- 1.S is a set of either positive or negative clauses: According to conclusion 4 of theorem 2, there is no possible resolvent at all. So, it is impossible to deduce the empty clause from S.
- 2.S is a set of only mixed clauses: According to conclusion 1 of theorem 2, only mixed clauses can be resolved from S. But the empty clause is not mixed. So, it is impossible to deduce the empty clause from S.
- 3.S is a set of mixed and positive clauses: According to conclusions 1,2, and 4 of theorem 2, only positive and mixed clauses can be resolved from S (any two selected parents are either both positive or both mixed or one positive and one mixed). Since the empty clause is neither positive nor mixed, it is impossible to deduce the empty clause from S.
- 4.S is a set of mixed and negative clauses: According to conclusions 1, 3, 4 of theorem 2, only negative and mixed clauses can be resolved from S (any two selected parents are either both negative or both mixed or one negative and one mixed). Since the empty clause is neither negative nor mixed, it is impossible to deduce the empty clause from S.

From 1, 2, 3, and 4 we conclude that the empty clause cannot be deduced from the unbalanced set S. Since resolution is a refutation complete inference rule, then S must be satisfiable. So any unbalanced set of clauses is satisfiable.

Unbalanced set method as an algorithm outline

We can use theorem 3 to detect a case in which the satisfiability of a given set is assured. We can terminate the resolution process and announce the failure of the theorem whenever the set of clauses becomes unbalanced (that is either no positive or no negative clauses exist) even if there is still more resolvable clauses in the set. The balanced algorithm consists of three parts that are distributed among the theorem prover. Initially, two counters Pcount and Ncount are assigned to the number of positive and negative clauses respectively in the set. The three parts are:

1. Checking the set balance: This part is responsible for checking the balance state of the set. Whenever any sign is lost (the respective counter becomes zero), the prover terminates with failure. Otherwise, proceeds. Checking part:
 - if either Pcount = empty or Ncount = empty then terminate with failure.
2. Increment the sign counters: This part is activated whenever a new clause is resolved and inserted into the set. Incrementing part(Clause):
 - if positive (Clause) then increment (Pcount) else if negative (Clause) then increment (Ncount).
3. Decrement the sign counters: This part is activated whenever an already existing clause has been deleted either by backward subsumption or by the reduction process. Decrementpart(ClauseList):

- For each Clause C in ClauseList:
 - if backward subsumption or reduction process of ClauseList then if positive (C) then decrement (Pcount) else if negative (C) then decrement (Ncount).
- Activate Checking {check the set satisfiability}

Example : Suppose we have the following stable set of clauses:

1. $\text{divided}(X, 2) \mid \sim \text{even}(X)$. {even numbers are divisible on 2}
2. $\text{even}(X) \mid \sim \text{double}(X)$. {any duplicate number is even}
3. $\text{double}(X) \mid \sim \text{even}(X)$. {any even number is duplicate}

Suppose we have the following theorem:

Forall Num ($\sim \text{double}(\text{Num}) \rightarrow \text{divided}(\text{Num}, 2)$).

{undoubtedly, the theorem is incorrect and hence the set is satisfiable}

If we negate the theorem and transform it into clause form the result will be :

4. $\sim \text{double}(\text{sko})$.
5. $\sim \text{divided}(\text{sko}, 2)$.

The resulting predicate table, PredTable, will be :

Predicate	Positive List	Negative List
Divided	1	5
Even	2	1.3
Double	3	4.2

Table 4. Predicate table

Although the resulting set is stable, it is unbalanced. Notice that we do not have any positive clause in the set. So, according to the unbalanced method, the set is satisfiable and we can terminate the process of resolution and announce that the theorem is not proved. We can notice the following :

- The unbalanced method detects a case in which the set is satisfiable.
- The set balancing state is initially detected and redetected each time a clause is deleted due to a backward subsumption or reduced singular clause.
- The set balancing state is maintained by the use of two counters, Pcount and Ncount.
- Balanced set is not necessary unsatisfiable. But unbalanced set is for sure satisfiable. That is balancing condition is necessary for unstisfiability but not sufficient.

2.3 The Directed Resolution

In theorem proving, the available tools are the available resolution extensions such as binary resolution, factoring, both types of hyperresolution, ur-resolution. Each of them is efficient on some set of clauses and inefficient on others. Even for refutation complete inference rules, it may take many proof steps with a generation of numerous numbers of resolvents. The criterion of selecting a suitable inference rule is ambiguous, since there are many factors that affect the resolution process. On the other hand, if we apply a number of inference rules on a set we will obtain a huge number of clauses. Our experiments on various kinds of inference rules, sets of clauses, and strategies have suggested the following :

The proposed selection criterion is to select an inference rule according to the selected clause not to the whole set. There must be an effective strategy for selecting clauses. We have formulated a dynamic inference rule combined with an improved strategy.

The improved set support: Our experiments with the known set of support strategies have suggested the following improvements :

- It is better to build a queue of supported predicates within supported clauses instead of supported clauses only. Each supported clause is investigated, and each predicate in it is given a priority according to the number of its contradictory predicate occurrences in the set (using the PredTable of the reduction process).
- Employing the weighting ordering strategy, each clause will be assigned a weight. This weight corresponds to the number of literals it contains. The queues will be sorted in ascending order with respect to weights.
- In any resolution step, we employ a unit preference strategy to select the clauses from the unsupported clauses. The selected clause must have the contradictory predicate with respect to the supported predicate of the supported selected clause (PredTable is to be sorted with respect to the clause weights).

Our procedure can be summarized as follows:

- i. Select the special hypothesis and the negated theorem clauses and consider them the set of support.
- ii. Build the set of support queue. Each entry in the set of support queue is a triple containing the clause number, its respective weight (the number of literals it contains) and the supported predicate within the clause. The supported clause has a number of entries, one for each predicate. These entries are sorted in ascending order according to the number of contradictory predicate clauses by the assistance of the predicate table PredTable.
- iii. Build additional three queues, the positive clauses queue, the negative clauses queue, and the mixed clauses queue. Each entry of these queues is a pair of the clause number and its corresponding weight only. Sort each queue in ascending order with respect to the weights.

The Directed Resolution Inference Rule: In order to build an efficient inference rule, we have combined binary resolution, factoring, positive hyperresolution, negative hyperresolution, and ur-resolution in one inference rule, the directed resolution inference rule. The process of this rule is as follows

From the set of support queue SupQueue, with respect to its supported predicate (and using PredTable), determine the list of contradictory predicate clauses. Perform a binary resolution step upon the supported predicate. If the binary resolvent (if exist) is negative, then it is considered as a nucleus for a positive hyperresolution step. A successful hyper step may resolve either a contradiction or a positive resolvent. If the hyper step fails, the latest intermediate hyper clause is kept if it is a unit clause. The same steps hold if the binary resolvent is positive (interchanging the term positive with negative in the previous context). In the case of a mixed binary resolvent, both positive and negative hyperresolution are applied on it separately, thus resolving positive and negative clauses (if exist). Factoring is implicitly applied within each resolution step (if there are factors). The binary resolvent is kept in the set unless subsumed by one of its decedents. If the set of support becomes empty, the proving process is terminated and the set is considered a satisfiable set.

The Directed Resolution algorithm outline

algorithm directedResolution (C(n, p, w), FinalRes):

{n: clause number & p: supported predicate & w: assigned weight}

determine ($\sim p$, ContraList, PredTable);

{the clauses list of contradictory predicates to p using the PredTable}

if not empty (ContraList) then

determine (E=entry(n, w), ContraList);

perform (binary, C, E, Resolvent);

if not negative (Resolvent) then {positive binary resolvent}

determine (negative, NegSats);

perform (negativeHyper, Resolvent, NegSats, FinalRes);

end if

if not positive (Resolvent) then {negative binary resolvent}

determine (positive, PosSats);

perform (positiveHyper, Resolvent, PosSats, FinalRes);

end if end if

end directedResolution

Example: Consider the following set of clauses:

1. $p(X,Y,Z) \mid \sim q(X) \mid \sim q(Z)$.
2. $\sim p(X,Y,Z) \mid m(X) \mid m(Y) \mid m(Z)$.
3. $\sim m(1)$.
4. $\sim m(2)$.
5. $\sim m(3)$.
6. $q(1)$.
7. $q(2)$.
8. $q(3)$.

Suppose our negated theorem is clause 8. The resulting predicate table, PredTable, and set-support queue, SupQueue, will be:

Predicate	Positive List	Negative List
P	1	2
Q	6,7,8	1
M	2	3,4,5

Table 5, Predicate table

SupQueue = (e(8,q, positive, 1)).

If we pick the supported clauses 8 and resolve it upon the supported predicate p with contradictory predicate in clauses 1, we will obtain: $p(3, Y, Z) \mid \sim q(Y) \mid \sim q(Z)$ as a binary resolvent. Since it is mixed, we will apply positive and negative hyperresolution as follows :

9. $p(3, 1, 1)$.
10. $P(3, 1, 2)$.
11. $P(3, 1, 3)$.
- ...
16. $p(3, 3, 2)$.
17. $P(3, 3, 3)$.

Are the positive hyperresolvents. Notice that no negative hyperresolvent is possible since the only contradictory p is in mixed clauses that cannot be satellites. The next supported clauses will be 9 which will be binary resolved with clause 2 (upon the contradictory predicate p) yielding: $m(3) \mid m(1) \mid m(1)$ as a binary resolvent. Since it is a positive clause, a negative hyperresolution is

applied (this clause as a nucleus and clauses 3 and 5 as satellites). If we consider clause 3 as two distinct satellites, then the result will be the empty. The proof steps are as follows :

- 9. $p(3, 1, 1)$ from clauses 8, 1, 6, and 6.
- 10. Empty clause from clauses 9, 2, 3, 3, and 5.

From this result we find that our prover resolved 10 clauses until resolves the empty clause. But if we consider the same example with positive hyperresolution (even with the use of set-of-support), the resolvents will be:

- 9. $p(3, 1, 1)$.
- ...
- 27. $p(1, 3, 3)$.

Selecting clause 2 as a nucleus we will have additional 19 positive clauses for each positive satellite from 9 to 27 as follows :

- 28. $m(3) | m(1) | m(1)$.
- ...
- 46. $m(1) | m(3) | m(3)$.

Now we can pick clause 28 and resolve it with the nucleus clause 3 to obtain:

- 47. $m(3) | m(1)$.

By selecting the nucleus clause 3 negative we resolve:

- 48. $m(3)$.

Finally by selecting the nucleus clause 5 we resolve :

- 49. empty clause.

Notice that hyperresolution resolved 41 clauses until resolving the empty clause. In this case the proof is :

- | | |
|--------------------------|------------------------------|
| 9. $p(3, 1, 1)$ | from clauses 8, 6, 6, and 1. |
| 28. $m(3) m(1) m(1)$ | from clauses 9 and 2. |
| 47. $m(3) m(1)$ | from clauses 28 and 3. |
| 48. $m(3)$ | from clauses 47 and 3. |

49. empty clause from clauses 48 and 5.

This example shows clearly the efficiency of our directed resolution compared to positive hyperresolution. A similar huge size will be obtained if we use other extension of resolution such as negative hyperresolution. We notice the following:

- Our inference rule, directed resolution, tends to find the shortest resolvents, so the direction towards the empty clause is progressing. We keep the binary-step resolvent (unless subsumed by its descendants) to preserve the refutation completeness in some critical cases.
- In the worst case, the directed resolution will behave as efficient as an appropriately chosen hyperresolution type on the given set of clauses.
- The improved set of support strategy reduces the number of generated clauses in a resolution cycle because it resolves only on a specific predicate.
- The application of a positive-hyperresolution-step on a negative resolvent from the binary-step is more likely to resolve an empty clause. In some cases the resolvent will be positive. The same discussion holds for negative hyperresolution.

In the case of mixed clause binary resolvent, it is preferable to transform it into positive and negative clauses to increase the chance of contradiction.

- Factoring is implicitly used whenever possible. Subsumption in each direction is employed. Tautologies are also detected and deleted from the set of clauses. Moreover any backward subsumption activates the reduction process and diagnosing process.
- With the assistance of the predicate table, searching for resolvable clauses and contradictory predicates among them is indexed compared with the combinatorial searching for contradict literals among sequentially inspected clauses in the old fashion theorem provers. The directed resolution is more efficient.
- Because theorem proving is NP-Complete problem, we direct the process toward the most fruitful direction.

The Intelligent Theorem Prover, All Together

The introduced new characteristics of our theorem prover compared to its ancestors are as follows:

- The use of multiple inference rules with an intelligent selection of the appropriate inference rules in each step with respect to the clause under consideration. Thus directing the process toward a solution.
- The permanent monitoring of the clause set for an early and intelligent diagnosing the satisfiability of the set.

- The intelligent detecting of the clause set for the described class of unnecessary clauses and deleting them.
- The introduction of additional termination conditions applied to the theorem prover.

Our theorem prover can be programmed with any efficient programming language (we programmed it with Turbo PROLOG v.2 programming language). It will be stated here as an algorithm. It is divided into three parts as follows:

- Input part: This part is responsible for accepting information representing the problem under consideration. This information can be entered in a window either in a predicate calculus form (a transformation process will be applied) or in a clause form. During the transformation process, each existentially quantified variable is replaced with a scripted skolem symbol (sko k) and every universally quantified variable is replaced with a scripted symbol (VAR k). Whenever, a new replacement occurs the script is incremented. Each transformation step is displayed in a second window and the resulting logically equivalent clauses are displayed in a third window.
- Processing part: Initially, a predicate table is displayed for the original set of clauses. A brief set-support, counters for the positive and negative clauses, counters for subsumed, reduced, and tautology clauses are displayed on several windows. A window for each resolved clause is also displayed. The theorem prover terminates when either a contradiction or no contradiction is found.
- Output part: The user can select one or more of the following output modes: Textual proof steps, Graphical proof steps, Printed proof steps, and Statistics.

The Intelligent Prover Algorithm Outline:

```

algorithm theoremProver :
  input information;
  build the improved set of support queue SupQueue;
  build the queue of ordered positive clauses PosQueue;
  build the queue of ordered negative clauses NegQueue;
  build the predicate table PredTable;
  Contradiction  $\leftarrow$  false; {initial state}
  Pcount  $\leftarrow$  0; Ncount  $\leftarrow$  0; {for positive and negative clauses}
  reduction (PredTable); {activate the Reduction process}
  if empty (SupQueue) or empty (PredTable) then
    announce ("failure .. no contradiction");
  else

```



```
while not empty (SupQueue) do
  determine (SupClause, SupQueue);
  directedResolution (SupClause, Resovent);
  testResult (Resolvent);    {for subsumption,...etc}
end while
if found (Contradiction) then
  announce ("success .. contradiction is found");
  outputResult;
else announce ("failure .. no contradiction")
end if end if
end theoremProver
```

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مجلة

علوم الملائتتطرية

السنة ٢٠٠١

عدد ٢

مجلد ١٢

تصدرها كلية العلوم بالجامعة المستنصرية - بغداد - العراق

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عزل وتشخيص بكتريا التهابات المجاري البولية بنظام api وقابليتها على مقاومة المضادات الحيوية

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ABSTRACT

A total of (1600) urine samples, collected randomly from patients of 3 hospitals in Baghdad city, suffering from urinary tract infection (U.T.I), were examined for general urine analysis, urine culture and antibiotics sensitivity test. Only (126) urine samples revealed the presence of pus cells during the general urine analysis. Each urine sample was cultured on blood and MacConky agars. The isolated colonies were counted and estimated according to Kass scale. The results showed that (107) urine samples contained significant growth, (7) contained non significant growth, while (12) showed no growth. Staphylococcus bacteria and api E20 for Enterobacteriaceae and Pseudomonas. It was found that *Escherichia coli* was the predominant bacteria in the U.T.I. followed by each of the Followings: Staphylococcus, Klebsiella, Pseudomonas, Enterobacter, Proteus, Serratia, Acinetobacter and Providencia. The antibiotics sensitivity test showed that gram positive bacteria were completely sensitive to Nitrofurantoin, Chloramphenicol and Gentamicin, while gram negative bacteria were sensitive to Trimethoprim, Chloramphenicol and Cefotaxime.

الخلاصة

جمعت (1600) عينة ادرار من مرضى يعانون من التهابات المجاري البولية من ثلاث مستشفيات في بغداد، حيث اخضعت العينات لفحوصات الادرار العام والزرع البكتيري وفحص الحساسية للمضادات الحيوية. اظهرت النتائج احتواء (126) عينة على خلايا خراج. زرعت العينات على وسطي الدم ومكونكي و قدرت اعدادها على اساس مقياس كاس (Kass)، حيث احتوت (107) عينة منها على اعداد معنوية من المستعمرات و (7) على اعداد غير معنوية، فيما لم تعط (12) عينة منها نموا بكتيريا. اعتمد في التشخيص على نظام Staphylococcus api E لتشخيص بكتريا Staphylococcus و api E 20 لتشخيص البكتريا المعوية و Pseudomonas. اظهرت النتائج ان بكتريا *Escherichia coli* هي الاكثر انتشارا تليها كل من Staphylococcus، Klebsiella، Pseudomonas، Enterobacter، Acinetobacter و Serratia، Proteus، Providencia، اما فحص الحساسية للمضادات

رعد خليل الحسيني وجماعته

الحياة فقد اظهر ان البكتريا موجبة كرام كانت حساسة كلياً لثلاثة مضادات حيوية هي النايتروفورانتين و الكلورامفينيكول والجنتاميسين، فيما كانت السالبة لكرام حساسة للمضادات تراي مثيريم و كلورامفينيكول وسيفوناكسيم.

المقدمة

تعد التهابات المجاري البولية من المشاكل الطبية المزمنة، فهي تصيب الذكور والاناث ومن مختلف الفئات العمرية خلال موسم السنة، حيث تلي اصابات الجهاز التنفسي العلوي انتشاراً⁽¹⁾. وتكون البكتريا في مقدمة العوامل المسببة لهذا الالتهاب، وتعزل بكثرة من عينات الادرار، خلال الفحوصات المختبرية الروتينية.

اشارت العديد من الدراسات الى ضرورة اجراء الفحص العام للادرار قبل اجراء أي عملية جراحية للمجرى البولي⁽²⁾ للكشف عن وجود البكتريا المسببة وامكانية معالجتها بالمضادات الحيوية، ومن اهم انواع البكتريا تسبباً في مثل هذه الالتهابات ايشركيا القولون (*Escherichia coli*) والمكورات العنقودية (*Staphylococcus*)، غالباً ما يرافق هذه الالتهابات ظهور الخلايا متعددة النوى (بولي مورف)، الا انه يصعب في بعض الاحيان عزل الكائن المسبب للمرض بالطرق الزرعية الروتينية كحالات التدرن الكلوي والاصابة بالمايكوبلازما والسيلان⁽³⁾.

تسجل يومياً في مستشفيات العراق مختلف حالات التهابات المجاري البولية والبكتريا المسببة لها، وتستخدم العديد من المضادات الحيوية لعلاجها، الا ان الحاجة الى التشخيص الدقيق لمسببات تلك الالتهابات وانتخاب الطرق الكفوءة في التشخيص والعلاج اصبح من الامور التي تحتاج الى المزيد من الجهود والدراسات الميدانية، لذا فقد تضمنت الدراسة الحالية عزل وتشخيص البكتريا المسببة لالتهابات المجرى البولي من كلا الجنسين ولجميع الفئات العمرية بنظام *api* لتحديد الانواع الاكثر انتشاراً وتسبباً للمرض، والتعرف على نسب الاصابة بالبكتريا سالبة وموجبة صبغة كرام، كذلك اجراء فحص الحساسية للمضادات الحيوية للوقوف على افضل المضادات الملائمة لعلاج كل نوع من انواع البكتريا المعزولة من حالات الالتهاب.

طرائق العمل

جمع العينات

جمعت (1600) عينة ادرار تعود لمرضى في مستشفيين بمدينة بغداد (احدهما في جانب الكرخ والاخرى في جانب الرصافة) ممن يعانون التهابات المجاري البولية، اضافة الى احد مختبرات الصحة العامة في بغداد، جمعت العينات في قناني زجاجية معقمة، حيث اوصي كل مريض ان تكون عينة الادرار المطلوبة هي من منتصف الادرار بعد اهمال البداية (mid - stream urine).

الفحص العام للادرار

اجري التحليل العام للادرار مباشرة بعد جمع العينات، حيث شمل التعرف على الصفات الفيزيائية المتضمنة لون الادرار ودرجة العكرة اضافة الى التحري عن وجود الالبومين بطريقة التسخين واستعمال حامض الخليك (3%) لاختبار التضبب المتولد بعد التسخين⁽⁴⁾، يدل عدم ظهوره على سلبية الفحص، اما عند ظهوره فقد قدرت شدته كالاتي: تضبب قليل (ضعيف)، تضبب ملحوظ (+)، تضبب شديد الوضوح (++)، تضبب مع تحين ملحوظ (+++)، تضبب مع تجبن شديد (++++) . كما وتم التحري عن السكر، حيث استخدمت طريقة كاشف بندكت وملاحظة تغير لون الكاشف من الازرق الى الاحمر الفاتح كدلالة على ايجابية الفحص (+)، او الاخضر الغامق (++) او الاصفر (+++) واخيراً الاحمر النحاسي (++++) اعتماداً على كمية السكر في الادرار⁽⁴⁾.

الفحص المجهري للادرار

نبذت عينات الادرار في جهاز النبذ المركزي المنضدي (3500 دورة في الدقيقة) لمدة (10-15) دقيقة، حيث استخدم الادرار الطافي في فحص الالبومين. وبعد مجانسة الراسب بالمازج لمدة دقيقة واحدة وضعت قطرة منه على شريحة زجاجية وغطيت بغطاء الشريحة، ثم فحصت تحت المجهر لمشاهدة خلايا الخراج (pus)، والتي قدرت اعدادها في الحقل المجهري واعطيت لها العلامات (قليل، +، ++، +++، ++++) التي تعادل (1-6, 8-15, 16-25, 26-30, 36-100) خلية / حقل مجهري على التوالي، وعند تغطية الحقل المجهري باكملة اعطيت الرمز Full High Power Field (FHP). اما كريات الدم الحمراء فقد قدرت اعدادها باستخدام نفس الطريقة التي استخدمت في تقدير خلايا الخراج. فضلاً عن التحري عن المحتويات الاخرى التي شملت وجود الفطريات التي تعود الى جنس الكانديدا (Candida) والطفيلي *Trichomonas vaginalis* المرافق عادة للحالات المرضية التناسلية.

زرع الادرار

زرعت العينات باخذ نقلة بالناقل (Loop) من الادرار وزرعها على اطباق حاوية على وسطي اكار الدم (Blood agar) ومكوني (MacConkey agar). وبعد الحضان بحرارة (37)° م لمدة (18-24) ساعة، حسبت اعداد المستعمرات النامية وتم التعبير عن كثافة النمو كالاتي: نمو ضعيف (Scanty)، متوسط (Moderate)، كثيف (Heavy)⁽⁵⁾.

تشخيص العزلات

شخصت العزلات بصورة اولية اعتماداً على الصفات الزرعية (النمو) والمجهورية (للخلايا بعد تصبيغها بصبغة كرام). اعتمد التشخيص النهائي للمستعمرات النامية على استخدام كل من عدتي الفحص (*api Staph*) للمكورات العنقودية و (*api E20*) لافراد العائلة المعوية وبكتريا السيدوموناس، حيث تضمنت هاتين العدتين الاختبارات البايوكيميائية الاتية :

Indol, V.P., TDA, Oxidase, Nitrites, Alkaline Phosphatase

وذلك كما ورد في نشرة *api* لشركة BioMerieux الفرنسية سنة 1995.

فحص الحساسية للمضادات الحيائية

استخدم في هذا الفحص الوسط الزرعي Hinton-Mueller واقرص المضادات الحيائية قطر (6.3) ملم (شركة Oxoide) وكما ورد في⁽⁶⁾. تم قياس مناطق زوال النمو (بالملم) حول الاقراص والتي قورنت مع نتائج العزلات القياسية الاتية :

1. *Escherichia coli* (ATCC 25922).

حيث قورنت معها عزلات البكتريا المعوية :

2. *Staphylococcus aureus* (ATCC 25923)

وقورنت معها المكورات العنقودية.

3. *Pseudomonas aeruginosa*

والتي قورنت معها عزلات السيدوموناس.

اعتبر قطر زوال النمو المساوي او الذي يقل بلملم واحد عن قطر زوال النمو للعزلة القياسية مساوياً لها، وهذا يعني ان العزلة حساسة للمضاد، اما اذا كان الفرق مساوياً الى اثنين ملم او اكثر تعد العزلة مقاومة للمضاد.

النتائج والمناقشة

تحديد معنوية النمو البكتيري

تشير نتائج تحليل عينات الادرار الى ان نسب الاصابة عند النساء (80%) اعلى مما عليها في الرجال (20%)، وقد اكدت دراسات سابقة⁽⁵⁾ ارتباط الاصابة بالجنس، حيث كانت عند النساء مقارنة بالرجال كنسبة 1/10.

ويتضح من الجدول رقم (1) تركيز عينات الادرار ذات النمو البكتيري غير المعنوي (اقل من 30 مستعمرة للنقطة الواحدة) عند النساء وانعدامها عند الرجال، ويمكن تفسير ذلك بسبب الاختلاف

التشريحي بين الذكور والاناث حيث ان قصر الاحليل (urethra) عند النساء يجعل الادرار اكثر عرضة للتلوث من الذكور.

اعتمد تعريف Kass لعام 1957 في تحديد معنوية النمو البكتيري في الادرار (significant bacteriuria) بعدد 10^5 / مل من نوع واحد من البكتريا⁽⁷⁾. وبهذا الخصوص فقد اوضح Sleigh et al⁽⁵⁾ ان العدد 10^5 / مل ادرار يكون معادلاً لحوالي (300) مستعمرة او اكثر نامية على الوسط الزرعي، في حيث ان الاعداد البكتيرية بين 10^4 و 10^5 تعني ان الاصابة غير مؤكدة وتحتاج الى تكرار الفحص على عينة ادرار اخرى، كون هذه الاعداد تعادل (30) مستعمرة في الطبق الزرعي، اما اذا كانت اعداد البكتريا اقل من 10^4 / مل وهو ما يعادل اقل من (30) مستعمرة للطبق الزرعي الواحد، يعد اما غير معنوياً او تلوئاً.

جدول (1) : اعداد عينات الادرار المزروعة ونسب الاصابة عند الذكور والاناث

جنس المريض	عدد العينات	العينات ذات النمو الجرثومي المعنوي	العينات ذات النمو الجرثومي غير المعنوي	العينات غير الحاوية على نمو جرثومي
		(%) العدد	(%) العدد	(%) العدد
اناث	101	10187(86.1)	7(6.9)	7(6.9)
ذكور	25	20(80)	-	5(20)
المجموع الكلي	126	107(84.9)	7(5.6)	12(9.5)

يحتوي ادرار الاحليل عند النساء السليمات بين 10^3 و 10^4 وحدة تكوين مستعمرة (cfu) / ملي ادرار من مختلف انواع البكتريا⁽⁸⁾، لذا يوجه المرضى عند جمع العينات بالحصول على الادرار الوسطي تجنباً للتلوث، ويظهر من الجدول السابق ان نسب نماذج الادرار التي لم تظهر نمواً بكتيرياً كانت عند الذكور (20%) مقارنة بالاناث (6.9%)، وتبين ان غالبية الذكور ضمن هذه الفئة كانوا يعانون من امراض تناسلية زهرية اهمها مرض السيلان، لاسيما عند الشباب منهم، حيث امكن التأكد من هذه النتيجة باجراء صبغة كرام لخراج الاحليل ومشاهدة بكتريا السيلان (*Neisseria gonorrhoeae*) داخل وخارج خلايا الخراج، وظهرت الحالة ايضاً عند الاناث ولو بشكل اقل. ومن التفسيرات الاخرى لعدم وجود نمو بكتيري في عينات الادرار هو احتمال الاصابة بالتدرن الكلوي او المايكوبلازما، او وجود بكتريا نادرة مثل انواع *Leptospira spp.* اضافة الى رواشح Cytomegalo virus⁽³⁾، مما يقود الى الاستنتاج بعدم احتواء العينة على نمو بالرغم من وجود اعداد كبيرة من خلايا الخراج.

التشخيص البكتيريولوجي

أدى تشخيص البكتريا بعدة *api* الى الحصول على الاجناس والانواع المثبتة في الجدول رقم (2)، حيث يتضح من الجدول ان البكتيريا ايشركيا القولون هي الاكثر تردداً من الانواع الاخرى وذلك عندما بلغت نسبة عزلها (52.8%) من المجموع الكلي للعزلات البالغ (108) وكانت نسبة توажدها في الاناث تفوق باكثر من اربعة اضعاف تلك النسبة في الذكور. تلتها عزلات الجنس *Staphylococcus aureus* وعزلت بنسبة (17.6%) حيث كان النوع *S. aureus* اكثرها عزلاً وبنسبة (13%)، فيما احتلت بقية انواع الجنس نسب عزل ضئيلة كما هو موضح في الجدول. اما بكتريا *Klebsiella* فقد توأجدت بنسبة (11.1%) مقارنة ببكتريا *Pseudomonas* والتي بلغت نسبها (6.6%)، ثم *Enterobacter* بنسبة (5.6%)، تلتها *Proteus* بنسبة (3.7%)، اما بخصوص بكتريا *Serratia*، *Providencia*، *Acinetobacter* فقد عزلت كل منها بنسب ضئيلة جداً (0.9%) وعند النساء فقط. ومن مجموع العينات المزروعة احتوت واحدة فقط نمواً مزدوجاً (ايشركيا القولون + *Klebsiella*)، وقد وصف مثل هذا النوع من الالتهابات بالالتهاب المزمن⁽⁵⁾.

الجدول رقم (2) التشخيص النوعي لاجناس البكتريا المعزولة من ادرار الذكور والاناث

المجموع الكلي و (%)	عدد النماذج		النوع	جنس البكتريا
	اناث	ذكور		
57(52.8)	46	11	<i>coli</i>	<i>Escherichia</i>
14(13.0)	12	2	<i>aureus</i>	<i>Staphylococcus</i>
2(1.9)	2	-	<i>saprophyticus</i>	
1(0.9)	1	-	<i>epidermidis</i>	
1(0.9)	1	-	<i>capitis</i>	
1(0.9)	1	-	<i>haemolyticus</i>	<i>Klebsiella</i>
8(7.4)	6	2	<i>pneumoniae</i>	
4(3.7)	4	-	<i>ozaenae</i>	<i>Pseudomonas</i>
2(1.9)	1	1	<i>fluorescence</i>	
2(1.9)	1	1	<i>stutzeri</i>	
2(1.9)	2	-	<i>maltophilia</i>	
1(0.9)	-	1	<i>aeruginosa</i>	<i>Enterobacter</i>
4(3.7)	4	-	<i>agglomerans</i>	
2(1.9)	2	-	<i>cloacae</i>	
4(3.7)	2	2	<i>mirabilis</i>	
1(0.9)	1	-	<i>odorifera</i>	<i>Serratia</i>
1(0.9)	1	-	<i>calacoacetcus</i>	<i>Acinetobacter</i>
1(0.9)	1	-	<i>stuartii</i>	<i>Providencia</i>
108(100)	88	20		المجموع

والتأثيرات الناتجة عن تناول الأدوية المضادة للبكتيريا. في دراسة أجراها (4) في مستشفى جامعة القاهرة، وجد أن 74% من المرضى الذين يعانون من التهابات المسالك البولية لديهم مستويات عالية من البكتيريا في البول، و 15% منهم لديهم مستويات عالية من البكتيريا في الدم. وفي دراسة أجراها (5) في مستشفى جامعة القاهرة، وجد أن 85% من المرضى الذين يعانون من التهابات المسالك البولية لديهم مستويات عالية من البكتيريا في البول، و 20% منهم لديهم مستويات عالية من البكتيريا في الدم.

ومن الجدير بالذكر أن *Enterobacter* البكتيريا الأكثر شيوعاً في العينين، و 15% من العينين المصابة بالبكتيريا، و 30% من العينين المصابة بالعدوى الفطرية. وفي دراسة أجراها (6) في مستشفى جامعة القاهرة، وجد أن 45% من المرضى الذين يعانون من التهابات العينين لديهم مستويات عالية من البكتيريا في العين، و 30% منهم لديهم مستويات عالية من البكتيريا في الدم. وفي دراسة أجراها (7) في مستشفى جامعة القاهرة، وجد أن 60% من المرضى الذين يعانون من التهابات العينين لديهم مستويات عالية من البكتيريا في العين، و 40% منهم لديهم مستويات عالية من البكتيريا في الدم. وفي دراسة أجراها (8) في مستشفى جامعة القاهرة، وجد أن 70% من المرضى الذين يعانون من التهابات العينين لديهم مستويات عالية من البكتيريا في العين، و 50% منهم لديهم مستويات عالية من البكتيريا في الدم. وفي دراسة أجراها (9) في مستشفى جامعة القاهرة، وجد أن 80% من المرضى الذين يعانون من التهابات العينين لديهم مستويات عالية من البكتيريا في العين، و 60% منهم لديهم مستويات عالية من البكتيريا في الدم. وفي دراسة أجراها (10) في مستشفى جامعة القاهرة، وجد أن 90% من المرضى الذين يعانون من التهابات العينين لديهم مستويات عالية من البكتيريا في العين، و 70% منهم لديهم مستويات عالية من البكتيريا في الدم.

في دراسة أجراها (11) في مستشفى جامعة القاهرة، وجد أن 95% من المرضى الذين يعانون من التهابات العينين لديهم مستويات عالية من البكتيريا في العين، و 75% منهم لديهم مستويات عالية من البكتيريا في الدم. وفي دراسة أجراها (12) في مستشفى جامعة القاهرة، وجد أن 100% من المرضى الذين يعانون من التهابات العينين لديهم مستويات عالية من البكتيريا في العين، و 80% منهم لديهم مستويات عالية من البكتيريا في الدم. وفي دراسة أجراها (13) في مستشفى جامعة القاهرة، وجد أن 100% من المرضى الذين يعانون من التهابات العينين لديهم مستويات عالية من البكتيريا في العين، و 90% منهم لديهم مستويات عالية من البكتيريا في الدم.

عزل وتشخيص بكتريا التهابات المجاري البولية بنظام *api* وقابليتها على مقاومة المضادات الحيوية

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الخراج مع شدة الاصابة، اذ تراوحت من قليل الى تغطية الحقل المجهرى المفحوص (HPF). ان ملاحظة وجود خلايا الخراج في الادرار ترافق عادة وجود البكتريا باعداد معنوية⁽¹⁵⁾. تظهر كريات الدم الحمراء مرافقة للخراج المصاحب لحالات التهابات المجاري البولية، كذلك مصاحبة وجود الالبومين مع بعض حالات التهابات الكلوية، وان الترابط بين وجود خلايا الخراج وكريات الدم الحمراء والالبومين يولد فكرة عن شدة الاصابة. اشار احد الباحثين⁽¹⁶⁾ بأن وجود خلايا الخراج مع اعداد بكتريا اكثر من 10^2 وحدة تكوين مستعمرة / مل دليل على وجود التهاب في المثانة ولايعد كذلك عند وجود عدد بكتريا يتجاوز 10^5 ولكن دون وجود خلايا خراج.

جدول (3) عزلات بكتريا الادرار موزعة حسب الفئات العمرية وجنس المصاب

رقم الفئة	عمر الفئة (سنة)	عينات الادرار			الادرار ذو النمو المعنوي		الادرار غير الحاوي على		ادرار ذو نمو غير معنوي		<i>E.coli</i>		Staphylococcus		Klebsiella		Pseu-domonas	
		م	ا	ذ	ا	ذ	ا	ذ	ا	ذ	ا	ذ	ا	ذ	ا	ذ	ا	ذ
1	اقل من 20	-	13	13	12	-	-	-	1	-	9	-	2	-	1	-	-	-
2	20-30	4	48	52	3	41	6	2	2	18	1	10	1	6	-	1	-	
3	31-40	6	22	28	5	20	2	1	3	9*	1	2	1	3*	-	3	-	
4	41-50	5	6	11	3	3	2	3	1	2	1	-	1	-	-	-	-	
5	51-60	4	4	8	4	4	-	-	-	3	4	-	-	1	-	-	-	
6	اكثر من 60	6	8	14	5	7	2	-	-	2	4	-	2	-	1	-	2	
	المجموع	25	101	126	20	87	12	7	11	46	11	2	17	10	3	4	3	

• نموا مشتركا لكل من *E.coli* و *klebsiella* ذ + ذكور ا = اناث

م = المجموع

تابع جدول (3)

رقم الفئة	عمر الفئة (سنة)	Enterobacter		Proteus		Serratia		Acinetobacter		Providencia	
		ا	ذ	ا	ذ	ا	ذ	ا	ذ	ا	ذ
1	اقل من 20	-	-	-	-	-	-	-	-	-	-
2	20-30	-	5	-	-	1	-	-	-	-	-
3	31-40	-	1	-	2	-	-	-	-	-	-
4	41-50	-	-	-	2	-	-	-	-	-	-
5	51-60	-	-	-	-	-	-	-	-	-	-
6	اكثر من 60	-	-	-	-	-	-	1	-	-	-
	المجموع	-	6	2	2	1	-	1	-	-	1

جدول (4) تأثير الاصابة بأجناس البكتريا المختلفة على الادرار

الالبومين			كريات الدم الحمراء			خلايا الخراج		اجناس البكتريا
ب	أ	ج	ب	أ	ج	ب	أ	المعزولة
14	4	1	11	41	9	46	2	Escherichia
4	1	-	6	13	3	16	-	Staphylococcus
3	-	-	3	8	2	10	-	Klebsiella
1	-	-	-	6	1	6	-	Pseudomonas
-	-	1	-	4	-	6	-	Entrobacter
2	-	-	2	2	-	3	-	Proteus
-	-	-	-	1	1	-	-	Serratia
-	-	-	1	-	-	1	-	Acinetobacter
-	-	-	-	1	-	1	-	Providencia
2	-	1	2	3	2	10	-	لا يوجد نمو
1	-	-	2	2	-	6	1	نمو غير معنوي
27	5	3	27	81	18	105	3	المجموع

١ = الاعداد القليلة من (1-7) خلية / الحقل المجهرى

ب = من + (8-15) الى 4 + (36-100) خلية / الحقل المجهرى

ج = FHP = تغطية الحقل المجهرى باكماله

أ = عكرة خفيفة بعد تسخين الادرار

ب = تضبيب واضح الى حالة التضبيب الشديد

حساسية البكتريا للمضادات الحياتية

اتضح من الدراسة وجود مقاومة عالية للمضادات الحياتية في غالبية العزلات بغض النظر عن نوع تفاعلها في صبغة كرام الجدول رقم (5) حيث ابدت البكتريا مقاومة عالية لمضادى الريفامبين والسيفالكسين، حيث بلغ عدد العزلات المقاومة لهذين المضادين (103)، (102) من مجموع (108) عزلة حسب التعاقب، بينما كانت المقاومة على اقلها لكل من مضادى الكلورامفينكول والنايتروقيورانتين حيث كان عدد العزلات المقاومة لهذين المضادين (64)، (66) من مجموع (108) عزلة وعلى التوالي. اما بقية المضادات الموجودة في الجدول فقد ابدت البكتريا تجاهها نسب مقاومة مختلفة. ومن الجدير ذكره ان اختلاف تراكيز الاقراص يولد تأثيرا متغيرا على البكتريا والتأثير لا يتناسب طرديا مع تركيز المضادات، فمضاد التراي مثيرم بالرغم من قلة تركيزه القرصي الا ان تأثيره كان اعلى بكثير من مضادات اخرى ذات محتوى قرصي عالى كالكاربينسلين والسيفالكسين.

جدول (5) حساسية البكتريا المعزولة من الادرار للمضادات الحيوية المستخدمة (طريقة الاقراص)

Proteus. spp.		Pseudom . spp.		Enterob. spp.		Klebsiella. spp.		Staph. spp.		E.coli		انواع المضادات
م	ح	م	ح	م	ح	م	ح	م	ح	م	ح	
4	-	7	-	6	-	11	1	17	2	55	2	سيفالكسين (30)
1	3	7	-	4	2	12	-	14	5	47	10	امبسلين (25)
2	2	5	2	4	2	7	5	15	4	37	20	سيفوتاكسيم (30)
4	-	7	-	5	1	12	-	14	5	40	17	حامض النالدكس (30)
3	1	7	-	4	2	11	1	1	18	38	19	نايتروفورانتين (200)
4	-	7	-	6	-	12	-	15	4	56	1	ريفاميسين (2)
3	1	6	1	5	1	10	2	12	7	49	8	حنتاميسين (10)
3	1	6	1	5	1	8	4	11	8	29	28	كلورامفنكول (30)
3	1	7	-	3	3	6	6	18	1	31	26	تراي مثيريم (1.25)
3	1	7	-	4	2	12	-	18	1	50	7	كاربنسلين (100)
3	1	7	-	4	2	8	4	13	6	47	10	نتراسايكاين (10)

تابع جدول (5)

المجموع		Providencia. spp.		Acinetob. spp.		Serratia . sp.		انواع المضادات
م	ح	م	ح	م	ح	م	ح	
102	6	1	-	1	-	-	1	سيفالكسين (30)
87	21	-	1	1	-	1	-	امبسلين (25)
71	37	-	1	1	-	-	1	سيفوتاكسيم (30)
85	23	1	-	1	-	1	-	حامض النالدكس (30)
66	42	1	-	1	-	-	1	نايتروفورانتين (200)
103	50	1	-	1	-	1	-	ريفاميسين (2)
88	20	1	-	1	-	1	-	حنتاميسين (10)
64	44	1	-	1	-	-	1	كلورامفنكول (30)
70	38	-	1	1	-	1	-	تراي مثيريم (1.25)
96	12	-	1	1	-	1	-	كاربنسلين (100)
84	24	1	-	1	-	-	1	نتراسايكاين (10)

لوحظ من نتائج الدراسة وجود اختلاف في مديات المقاومة للمضادات المستخدمة، الجدول رقم (6) بين البكتريا موجبة وسالبة كرام، فقد امكن ترتيب انتشار المقاومة للمضادات في البكتريا سالبة كرام تنازليا على الشكل التالي ريفامبين، كاربنسلين، جنتاميسين، امبسلين، تيتراسايكلين، نايتروفيورانين، سيفوتاكسيم، كلورامفينكول والتراي مثيرين.

اما البكتريا الموجبة كرام فقد تدرجت المقاومة فيها تنازليا وعلى الشكل التالي: كاربنسلين، تراي مثيريم، سيفالكسين، ريفامبين، سيفوتاكسيم، امبسلين، حامض النالدكس، تيتراسايكلين، جنتاميسين، كلورامفينكول ثم نايتروفيورانين.

وبذلك يظهر ان افضل ثلاث مضادات للبكتريا سالبة كرام هي تراي كثيريم، كلورامفينكول وسيفوتاكسيم وللبكتريا موجبة كرام هي النايتروفيورانين، الكلورامفينكول والجنتاميسين.

جدول (6) نسب المقاومة للمضادات الحياتية لعزلات بكتريا التهابات المجاري البولية

العزلات المقاومة للمضاد الحياتي				اسم المضاد الحيوي (تركيزه)		
المجموع	(%)	البكتريا سالبة كرام	البكتريا موجبة كرام	ميكروغرام / قرص		
		العدد	(%)	العدد	(%)	
103	(95.4)	88	(98.8)	15	(78.9)	ريفامبين (20)
102	(94.4)	85	(95.5)	17	(89.5)	سيفالكسين (30)
96	(88.9)	78	(87.6)	18	(94.7)	كاربنسلين (100)
88	(81.5)	76	(85.4)	12	(63.2)	جنتاميسين (10)
87	(80.6)	73	(82.0)	14	(73.7)	امبسلين (25)
85	(78.7)	71	(79.8)	14	(73.7)	حامض النالدكس (30)
84	(77.8)	70	(79.8)	13	(68.4)	تيتراسايكلين (10)
71	(65.7)	56	(62.9)	15	(78.9)	سيفوتاكسيم (30)
70	(64.8)	52	(58.4)	18	(94.7)	تراي مثيريم (1.25)
66	(61.1)	65	(73.0)	1	(5.3)	نايتروفيورانين (200)
64	(59.3)	53	(59.6)	11	(57.9)	كلورامفينكول (30)

: مجموع العزلات الموجبة كرام = 19

: مجموع العزلات السالبة كرام = 89

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من الملاحظ انه خلال الثلاثين سنة الماضية نسب المقاومة لمضاد الستراي مثبريم بصورة سريعة من 3.6% خلال السبعينات الى 36-12% في الثمانينات، وتراوحت نسب المقاومة في الدول النامية بين 38-64% ، أما في جنوب افريقيا فقد بلغت نسبة المقاومة 56.2%⁽¹⁶⁾ و كذلك سجل وجود زيادة في نسبة مقاومة بكتريا *Staphylococcus epidermidis* من 29% عام 1981 الى 43% عام 1987 ، بغض النظر عن طبيعة العينة المرضية المعزولة منها⁽¹⁷⁾ . اشارت احدى الدراسات السريرية في لندن⁽¹⁸⁾ ان كل من مضادى التراي مثبريم و النايتروفيرانتين بشكله البلوري يمثلان افضل علاج لالتهابات المجاري البولية، اضافة الى تأثيرهما الفعال على احياء الامعاء المجهرية والتي تعد مستودعا للبكتريا التي تصيب المجاري البولية.

ان سيادة صفة المقاومة المتعددة للمضادات الحيوية والتي يوضحها الجدول رقم (7) قد ظهرت بين كافة انواع العزلات تقريبا، حيث ان 25% من العزلات قاومت عشرة مضادات في آن واحد. اما نسبة العزلات المقاومة لـ^(9,10,11) مضاد فقد تجاوزت نصف مجموع العزلات المشمولة بالدراسة (53.7%). بينما بلغت نسب العزلات المقاومة لـ^(3,4,5) مضاد اقل من 10%، ولم تعزل أي بكتريا مقاومة لمضاد واحد او اثنين مما يعد مؤشرا خطيرا عن عدم جدوى استخدام بعض هذه المضادات لعلاج التهابات الجهاز البولي. ربما يعود هذا الى المدى الواسع من المقاومة للمضادات الحيوية في احد اسبابه الى الاستعمال العشوائي للمضادات دون الاعتماد على فحص الحساسية مع الزرع البكتيري مما يزيد من فرص تكيف البكتريا المرضية، هذا بالاضافة الى دور بلازميدات المقاومة وبقية العوامل الوراثية المتحركة الحاملة لجينات المقاومة والتي تعمل على نشرها بين البكتريا المحيطة عن طريق الاقتران او التحويل او التوصيل الوراثي⁽¹⁹⁾ .

جدول (7) : مديات مقاومة البكتريا المعزولة من الادرار للمضادات المستخدمة

المجموع	عدد المضادات التي تقاومها البكتريا										انوع البكتريا
	3	4	5	6	7	8	9	10	11		
57	1	2	4	5	8	9	9	15	7	<i>Echerichia coli</i>	
19	-	-	2	2	3	6	3	3	-	<i>Staphylococcus spp</i>	
12	-	-	1	-	2	1	1	4	3	<i>Klebsiella spp</i>	
7	-	-	-	-	-	-	1	2	4	<i>Pseudomonas spp</i>	
6	-	-	-	2	-	1	-	2	1	<i>Enterobacter spp</i>	
4	-	-	-	1	-	1	1	1	-	<i>Proteus spp</i>	
1	-	-	-	1	-	-	-	-	-	<i>Serratia spp.</i>	
1	-	-	-	-	-	-	-	-	1	<i>Acinetobacter spp.</i>	
1	-	-	-	-	1	-	-	-	-	<i>Prividencia spp</i>	
108	1	2	7	8	14	18	15	27	16	المجموع	
	(0.9)	(1.9)	(6.5)	(7.4)	(13.0)	(16.7)	(13.8)	(25)	(14.2)	النسب المئوية	

: لم تعزل بكتريا ذات مقاومة لمضاد حيوي واحد او اثنين

ولو اعتمدنا المقاومة لسبع مضادات كمعدل وسط للمقاومة المتعددة لظهر ان اغلب ايشركيا القولون تقع ضمن مدى المقاومة العالي (48 من مجموع 57 عزلة) و Staphylococcus (15 من مجموع 19 عزلة) وكلبسيلا (11 من مجموع 12 عزلة). اما بكتريا سيدوموناس فقد تميزت عن بقية الاجناس بوقوع جميع عزلاتها في مدى المقاومة العالي وكذلك الحال بالنسبة لبكتريا Acinetobacter، ووقوع (66%) من عزلات Enterobacter و (75%) من بكتريا بروتيويس ضمن هذا المدى العالي للمقاومة.

من خلال الدراسة اتضح ان هناك انخفاض واضح في مدى المقاومة في الدول المتقدمة ولكن تزداد المشكلة سواء في الدول النامية ومنها العراق، الامر الذي يدعو الى اعادة النظر في انواع المضادات المستعملة ووضع نظام صحي جديد منعا للهدر الاقتصادي في استخدامها.

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دراسة مقارنة للاستجابة المناعية الخلطية المتولدة في ارناب ممنعه بمستضدات مختلفة مستخلصة من الجدار الخلوي لجراثيم البروسيلة المالطية

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(استلم بتاريخ ٢٠٠٠/٧/١ : وقيل للنشر في ٢٠٠٠/١١/٢١)

ABSTRACT

The immunological efficiency of isolated antigens from *Brucella melitensis* (local strain) was studied this response was estimated by ELISA and the results obtained were as follows : Outer membrane proteins (OMP) antigen gave the highest immunological response with a titer of > 204800 . LPS aqueous phase gave a hurmoal immune response much higher than the phenol pahse the titers were 25600, 3200 respectively. While LAP-LPS antigen gave higher response than that given by LPS. Further more statistical analysis showed significant variations among the four antigens.

الخلاصة

درست القابلية للمستضدات المحضرة من جراثيم البروسيلية المالطية الذرية المحلية. وقد قيست الاستجابة المناعية الخلطية بمقدار المعيار الحجمي للأجسام المضادة بطريقة اختيار الاليزا حيث اعطى مستضد بروتينات الغشاء الخارجي OMPs اعلى استجابة مناعية وبمعيار حجمي مقداره $204800 <$ كما اعطى مستضد LPS الطور المائي اعلى استجابة مناعية من تلك التي ولدها LPS - الطور الفينولي وهي 3200, 25600 على التوالي اما مستضد LAP-LPS فقد كان اكفاً من مستضد LPS بكلا الطورين في أحداث استجابة مناعية خلطية. كما اظهرت نتائج التحليل الاحصائي وجود فروقات مهمة احصائية بين المستضدات الاربعة.

مقدمة

لاقي استخدام اختبار الاليزا قبولاً كبيراً كاختبار مصلي دقيق في تشخيص داء البروسيلات وذلك لكونه متحسس للاضداد من كافة الاصناف^(١).

كما استخدم كوسيلة تشخيص من قبل العديد من الباحثين بعد مقارنته بالاختبارات المصلية البروتينية والتي تستخدم عادة في التشخيص⁽²⁾. وقد استخدم هذا الاختبار لتقيس الاضداد في الانسان باستخدام المستضد الخلوي الكامل⁽³⁾ وباستخدام LPS⁽⁴⁾.
او باستخدام بروتينات الغشاء الخارجي⁽⁵⁾ وفي هذه الدراسة حاولنا جمع كل تلك المستخلصات من غشاء جرثومة البروسيلة ومقارنة الاستجابة المناعية التي تتولد ضدها في الارنب لتحديد المستضد الاكفأ مناعياً.

المواد وطرائق العمل

الحيوانات : أختيرت ارانب بعمر 6 أشهر تقريباً وقد قسمت الى 6 مجاميع أحتوت كل منها على 5 أرانب..

المستضدات : استخدمت في التمنيع المستضدات التي استخلصت من جراثيم البروسيلة المالطية العزلة المحلية والتي كانت قد استخلصت ودرست مواصفاتها الكيميائية في دراسات سابقة. وهي مستضد بروتينات الغشاء الخارجي OMPs، مستضد LPS بطوربه المائي والفينولي ومستضد البروتين المقترن بـ Lipid-A من LPS (LAP-LPS). مخطط التجربة يبين كل مجموعة والمستضد الذي حقنت به :

- مجموعة (1) : حقنت بمستضد OMPs.
- مجموعة (2) : حقنت بمستضد LPS-الطور الفينولي.
- مجموعة (3) : حقنت بمستضد LPS-الطور المائي.
- مجموعة (4) : حقنت بمستضد LAP-LPS.
- مجموعة (5) : حقنت بمستضد الجرثومة الكاملة المقتولة بالحرارة (سيطرة موجبة).
- مجموعة (6) : حقنت بعامل فروند الكامل الممزوج بذراري الفوسفات (كسيطرة سالبة).

وقد حقنت الحيوانات تحت الجلد بجرعة اولية وجرعتين ممزوجة بعامل فروند وبنسبة 1:1 ولفتره 15 يوم بين جرعة وأخرى.

وقد تم سحب عينات من الدم من المجاميع المختلفة أسبوعين لغرض الحصول على المصول حيث حفظت تلك المصول بدرجة -20 م لحين استعمالها.

ELISA : أعمدت طريقة (Lamb et al., 1979)⁽⁶⁾ وقد استخدم Goat anti rabbit 196 والمجهز من شركة Cappel للاقتران ببروكسيديا ليزا HRP ثم قيست التراكيز المثلى لكل من المستضدات

الاربعة والتي تبين بان تركيز (10, 20, 10, 20 مايكرو غرام / مليلتر) لكل من OMPs , LPS الطور الفينولي LPS الطور الفينولي و LAP-LPS على التوالي. وقد أجري اختبار الاليزا باستخدام المستضد وضده النوعي وللفرات الزمنية المختلفة لغرض مقارنة كفاءة تلك المستضدات باحداث استجابة نوعية خلطية.

اختبار الاليزا المتصالب

أجري هذا الاختبار بتعريض المستضد الى الاضداد اللانوعية والمحضرة ضد المستضدات الاخرى لغرض معرفة مدى العلاقة بين هذه المستضدات.

النتائج

بينت نتائج اختبار الاليزا مدى حساسية الاختبار ودقته في تقدير معيار الاجسام المضادة وأعطت صورة واضحة عن الآلية التي يتفاعل بها جسم الحيوان ضد كل مستضد من المستضدات التي حقنت فيه. وتمثل الاشكال (1 و 2 و 3 و 4) علاقة الاضداد النوعية التي تكونت نتيجة تمنيع الارنب بالمستضدات المختلفة متمثلة بقيم الامتصاص الضوئي مع عامل الزمن الذي استغرقته التجربة وهي عشرة اسابيع حيث يتبين بان OMPs مستضد كفاء مناعياً اذ ولد استجابة مناعية خلطية يعتبر مبكراً جداً وان هنالك زيادة بقيم الامتصاص الضوئي OD مما يدل على وجود ارتباط عال بين قيم OD والتي تعكس كمية الاضداد المرتبطة مع المستضد والفرات الزمنية وهذه القيمة مهمة احصائياً ($p < 0.01$) ولكن هذه المعادلة لاتطبق على نتائج LPS الطور الفينولي حيث ان هذه الفرقات كانت غير مهمة احصائياً ($p > 0.05$) ويوضح الجدول (1) العلاقة بين OD والفرات الزمنية للمستضدات الاربعة، أما جدول (2) فيوضح نتائج الاليزا للمستضدات المختلفة ومصول السيطرة الموجبة والسيطرة السالبة مع نتائج التحليل الاحصائي. أما الجدول (3) فيوضح نتائج الاختبار المتصالب.

وعند اجراء تحليل التباين بين المستضدات المتفاعلة مع ضدها النوعي والاضداد اللانوعية ظهر ان هنالك فرقاً مهمة احصائياً تختلف باختلاف المستضد. ويبين الجدول بان هنالك تفاعلاً تصاليباً بين المصول المضادة اللانوعية والمستضدات المختلفة ولو أنه اتضح نتيجة التحليل الاحصائي بأن الفرقات معنوية بين النتائج التي أعطاها المستضد مع ضده النوعي والنتائج التي سجلت مع الأضداد اللانوعية.

المناقشة

يمكن الاستنتاج بان المستضدات الاربعة عند اجراء الاليزا باستعمال اضعادها النوعية بان أعلى استجابة مناعية تكونت عندما كان المستضد الممنع هو OMPs و LAP-LPS وتتفق هذه النتائج مع ما معروف عن المستضدات البروتينية بكونها مستضدات ذات كفاءة تمنيعية جيدة أفضل من مستضدات LPS اما بالنسبة للمناعة المتكونة ضد LPS بطوريه فقد اختلف كلا المستضدين بفترة ظهور الاضداد، حيث أستجاب الجهاز المناعي للطور المائي لفترة زمنية قصيرة بعد إعطاء الجرعة الاولى بينما لم تظهر هذه الاستجابة مع الطور الفينولي الا بعد إعطاء الجرعة المنشطة الاولى ولكن هذه النتيجة تؤكد بان LPS موجود في كلا الطورين وهذا يتطابق مع ما ذكره^(8,7) فقد ذكرنا بان الاضداد المتولدة في مصول المضيف ضد LAP-LPS تكون ضد الجزء LPS من المستضد وعند اجراء تحليل التباين بين المستضدات المختلفة عند تعريضها الى مصول السيطرة الموجبة والسالبة تبين وجود تباين مهم احصائياً ($p < 0.0001$) وكما مبين في الجدول (1)، ومهما يكن فان المستضدات الاربعة المستخدمة للجراثيم والتي تكون في حالتها الطبيعية في حالة ارتباط قوي مع بعضها وان تداخلاً موجود فيما بينها حيث تمتد جزيئات LPS عبر OM⁽⁹⁾ وترتبط بها البروتينات المكونة لذلك الغشاء والتي تسمى OMPs ارتباطاً قد يكون تساهمياً⁽¹⁰⁾ لا يمكن فصلها بالطرق الفيزيائية او بالترشيح الهلامي أو بالنذ فائق السرعة لذلك يعد منطقياً وجود هذا التفاعل التصالبي بين المستضدات المختلفة.

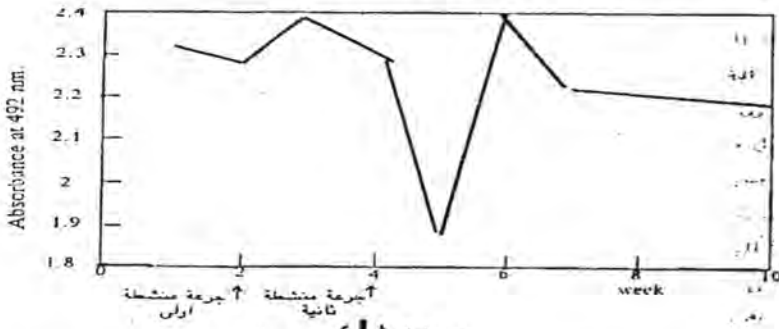
جدول (1) معدل الامتصاص الضوئي \pm الخطأ القياسي لاختبار الاليزا غير المباشرة للمستضدات مع

اضعادها النوعية خلال الفترة الزمنية للتمنيع باستخدام اختبار الاليزا غير المباشر

الفترة الزمنية مقدرة بالاسابيع بعد الحقن								N	المستضد
الاسبوع العاشر	الاسبوع السابع	الاسبوع السادس	الاسبوع الخامس	الاسبوع الرابع	الاسبوع الثالث	الاسبوع الثاني	الاسبوع الاول		
2.1840 0.0186± Λ d	2.2192 0.0176± Λ dc	2.3932 0.0239± Λ a	1.8699 0.0955± Λ b	2.2324 0.029± Λ a	2.3811 0.021± Λ a	2.2738 0.101± Λ a	2.3243* 0.0106± Λ** a*	12	OMP's
1.300 0.150± B bc	0.886 0.147± B cbd	0.794 0.116± B c	1.018 0.198± B b	1.126 0.234± B b	0.5211 0.0683± B a	0.4742 0.0645± B a	0.4020 0.0536± B a	16	-LPS الطور الفينولي
1.063 0.140± C a	1.136 0.151± C a	1.109 0.134± C a	1.044 0.129± BC a	1.29 0.145± BC a	1.121 0.143± C a	1.177 0.146± C a	1.180 0.162± C a	12	- LP S الطور النمائي
1.4952 0.0688± D a	1.3914 0.0425± D b	1.3498 0.0524± D af	1.3132 0.067± D bc	1.3604 0.0612± D bd	1.072 0.157± CD c	1.3512 0.0436± D b	1.5322 0.0367± D a	12	LAP- LPS

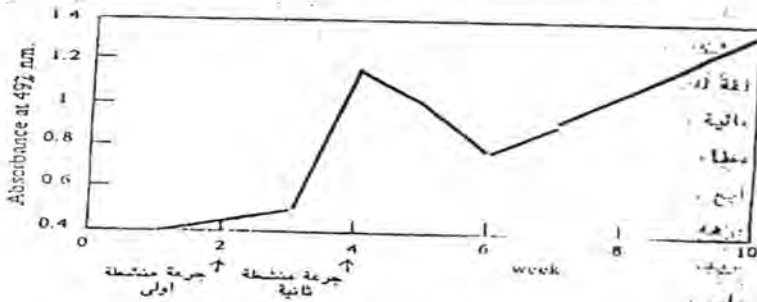
* الفروقات بين المعدلات التي تحمل حروف انكليزية صغيرة مختلفة مهمة احصائياً بنسبة ٥% للمقارنات الافقية.

** الفروقات بين المعدلات التي تحمل حروف انكليزية كبيرة مختلفة مهمة احصائياً بنسبة ٥% للمقارنات العمودي × معدل قيم ١٢ قراءة لـ OD .



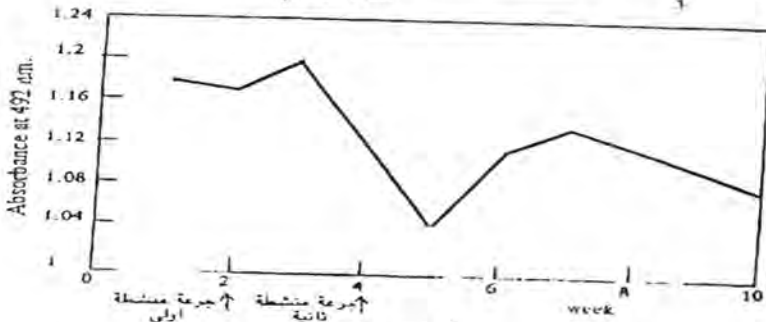
شكل (١)

اختبار ELISA مستخدم بـ OMP المعزول من جراثيم البروسيلا الماطية الذرية الماطية مع ضده النوعي



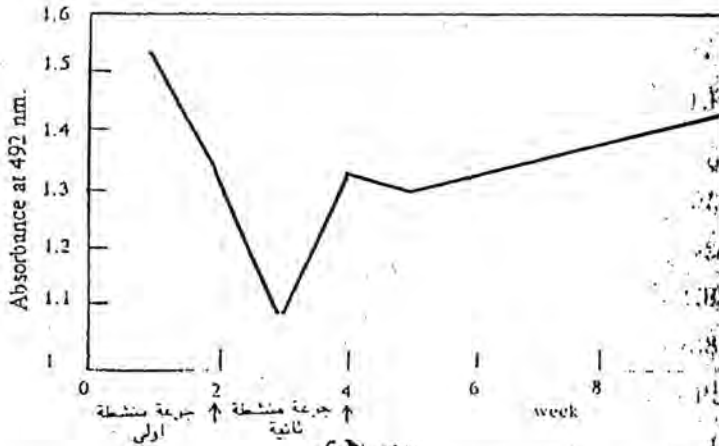
شكل (٢)

اختبار ELISA مستخدم لـ LPS - الطور القينولي المعزول من جراثيم البروسيلا الماطية الذرية الماطية ضده النوعي



شكل (٣)

اختبار ELISA مستخدم لـ LPS - الطور الماشي المعزول من جراثيم البروسيلا الماطية الذرية الماطية مع ضده النوعي



شكل (٤)

الختبار ELISA لمستضد LPS - AP المعزول من جراثيم البروسيلة المالطية الذرية اطحليه مع ضده

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قابلية انواع الجنس بروتيويس المعزولة من التهابات الاذن الوسطى على انتاج الهيمولايسين ومقاومة المضادات الحياتية

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ABSTRACT

A total of 698 ear swabs collected from otitis media patients, from 3 different, hospitals in Baghdad city (61 /males, 39% females), were cultured on routine culture medium. Proteus bacteria were isolated only from 70 patients (67.1% males, 32.9% femals). Patients age ranged from 1 to more than 50 years, divided into six age categories. The age category no. 1 (1-10) years showed the less accumulation of patients and the high accumulations were in the age category no. 3 (21-30) years. The isolated Proteus bacteria divided into (3) species according to their biochemical reactions, the predominant species was Pro. mirabilis (84.3%) which is distributed into (60.1 males and 24.2% females) followed by Proteus vulgaris (12.8%) which is distributed into (7.1% males and 5.7% females) then Pro. Penneri (2.8%) which is distributed into (1.4% males and 1.4 females). The infection with Proteus bacteria appeared to be accumulated into the age categories no. 2 (11-20) years and no. 3 (21-30) years. The antibiotics sensitivity test for Proteus bacteria revealed that the antibiotics Ciprofloxacin Cefotaxime, Chloramphenicol and Carbenicillin were the most effective antibiotics and their sensitivity were 95.7%, 94.3%, 92.9% and 87.1% in accordance, then followed by the antibiotics Tobramycin, Gentamicin, and equal effect of Rifampicin and Ampliclox with the following sewnsitivites 81.4% , 80%, 78.6%. The antibiotics cephalothin and Trimethoprim showed moderate effect (65.7% and 54.3). The less effective antibiotics were Ampicillin, Lincomycin, Erythromycin and tetracycline in a ratios 37.1%, 30%, 25.7% and 18.6%. The Haemolysin was produced by (81.4%) of the isolates. The animals Blood were more favorable to show the Haemolysin effect than Human Blood. Among the animals blood, the rabbits blood took the first Rank and constitute 37.7%, while the cows and horses blood showed the ratios 64.9% and 5.3% respectively. The Human blood group A, B, AB showed the ratios 8.8%, 28.1% and 21.1% in accordance.

الخلاصة

تم زرع (689) مسخة اذن (ذكور 61% ، اناث 39%) من المصابين بالتهابات الاذن الوسطى من ثلاث مستشفيات في مدينة بغداد. لم يكن نصيب بكتريا بروتيوس منها سوى (70) عزلة (61.1% ذكور، 32.9% اناث). تراوحت اعمار المصابين ما بين (1 - اكثر من 50 سنة) موزعة على ست فئات عمرية، بحيث كانت الفئة العمرية الاولى (1-10) تضم اقل الاعداد والفئة العمرية الثالثة (21-30) تضم اكثر اعداد المصابين. شخصت بكتريا بروتيوس الى ثلاثة انواع هي *Pro.minabilis* وعزلت بنسبة (48.3%) موزعة بنسبة 60.1% عند الذكور و 24.2% عن الاناث ، ثم بكتريا *Pro. Vulgaris* وعزلت بنسبة (12.8%) موزعة بنسبة (7.1%) عند الذكور و (5.7%) عند الاناث واخيراً *Pro.penneri* وعزلت بنسبة (2.8%) موزعة بواقع (1.4%) عند الذكور و (1.4%) عند الاناث تركزت اصابات البروتيوس في الفئتين العمريتين الثانية (20-11) والثالثة (30-21) . وعند اجراء فحص الحساسية للمضادات الحيوية لانواع هذا الجنس باستخدام (14) مضاد، احتلت المضادات التالية موقع الصدارة في التأثير وهي كل من السبروفلوكساسين، سيفوتاكسيم، الكلورامفينيكول ثم الكارباميسين وبنسب حساسية 95.7%، 94.3%، 98.9%، 87.1% حسب التعاقب، تلتها المرتبة الثانية كل من توبراميسين، جنتاميسين وتساوي الريفاميسين والامبلي كلوكس وبنسب التالية : 78.6%، 78.6%، 81.4%، 80% حسب التعاقب، في حين كلن المضادين سيفالوثين وتراي مثيريم متوسطي التأثير وبنسب حساسية 65.7% ، 54.3% وعلى التوالي. اما اقل المضادات تأثيراً فهي الامبسلين ، لنكوميسين، ارثروميسين، تتراسايكلين حيث كانت بالنسب التالية 37.1%، 30%، 25.7%، 18.6%. كان الدم البشري فصيلة (o) ودم الارانب هما الافضل في ظهور تأثير الهيموليسين وبنسب تحلل 78.9%، 73.7% على التوالي، في حين كانت الفصائل الاخرى للدم البشري A, B, AB اقل كفاءة فقد اعطت النسب التالية 8.8%، 2.8%، 21.1% كذلك دم الماعز والخيول وبنسب 64.9%، 5.3% وبصورة عامة ظهر ان 81.4% من العزلات كانت محملة للدم من مجموع العزلات الكلية.

المقدمة

تعد التهابات الاذن الوسطى من المشاكل الطبية واسعة الانتشار وهي في الغالب تنشأ كنتيجة لأحد المضاعفات المتولدة عن التهابات الجهاز التنفسي العلوي كالاصابة بمرض التهاب اللوزتين او الأنفلونزا، ويتميز الالتهاب بوجود الم في الاذن وخروج مادة قيحية (pus)⁽¹⁾ تلعب البكتريا دوراً هاماً في احداث الالتهاب وتنبأين ما بين سالبة وموجبة لصيغة كرام. ومن انواع البكتريا سالبة صبغة كرام المسببة للالتهاب بكتريا الجنس بروتيوس (Proteus)، الذي اصبح

يضم أنواعاً ثلاثة مهمة وهي Pro. mirabilis, Pr. penneri, Pro. vulgaris . تشترك جميعاً في أحداث امراض متعددة في مناطق جسمية مختلفة. يعتبر النوع Pro. Mirabilis من اكثر انواع الجنس انتشاراً وتسبباً في أحداث المرض ومن الملاحظ كذلك ظهور صفة مقاومة المضادات الحياتية في العديد من عزلات هذا الجنس والتي اصبحت واحدة من الاسباب التي تؤدي الى فشل العلاج بالمضادات دون اجراء فحص الحساسية لها لتحديد العزلات المقاومة والحساسة للمضادات المستخدمة⁽²⁾ .

استهدفت الدراسة الحالية استخدام عدد كبير من المضادات الحياتية لتحديد الانواع الافضل استخداماً في العلاج حيث استخدمت مضادات جديدة لم تدخل حيز الاستعمال في العراق الا منذ سنوات قريبة، كمضاد سيفوتاكسيم و السبروفلوكساسين ومقارنة كفاءتها بالعديد من المضادات القديمة شائعة الاستعمال ومدى تباين انواع الجنس في الاستجابة للمضادات المختلفة. تضمنت الدراسة تقصي نشاط عامل الفوعة الهيمولايسين ودراسة تأثيره على انواع مختلفة من الدم البشري ودم الحيوانات لتحديد هوية الدم المناسب لعمله ليتم استخدامه في حالة التحري عن وجود هذا الانزيم.

طرائق العمل

اجريت هذه الدراسة للمدة من ايلول 1997 ولغاية ايلول عام 1999 على 698 مريضاً، اختيروا عشوائياً من بين مراجعي وحدات الأنف والأذن والحنجرة لثلاث مستشفيات في مدينة بغداد (مدينة صدام الطبية والكندي التعليمي و صدام العام في مدينة صدام). دونت المعلومات عن كل مريض والمتضمنة العمر والجنس ومدة الاصابة والتأكد من عدم تناوله للمضادات الحياتية لفترة لا تقل عن ثلاثة ايام قبل مراجعته المستشفى.

استخدمت المسحات (Swabs) النبيذه لاستحصا عينة الخراج (Pus) من داخل الاذن المصابة حيث زرعت مباشرة على وسطي اكار الدم ومكونكي وحضنت هوائياً في درجة حرارة 37 م° لمدة 24 ساعة.

كذلك تم عمل فلمين لكل مسحة احدهما صبغ بصيغة كرام لمشاهدة اشكال البكتريا وتفاعلها في صبغة كرام والاخر كان فلماً رطباً (wet film) لمشاهدة وجود خلايا الخراج عند فحصه مجهرياً وكذلك للتأكد من وجود الاصابات الفطرية من نوع الكانديدا (Candida albicans) .

شخصت المستعمرات النامية على وسط الدم تشخيصاً اولياً لمشاهدة ظاهرة انتشار النمو (swarming) على سطح الطبق الزراعي مع رائحة النمو المميزة (fish smell) ، اما على وسط مكونكي فقد ظهرت المستعمرات صفراء شاحبة لكونها غير مخمرة لسكر اللاكتوز، اما عملية التشخيص النهائي للبكتريا فقد تضمنت الفحوصات البايوكيميائية التالية :-

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١- زرع وسط اليوريا السائل ومشاهدة النتائج بعد فترة الحضانة.

٢- زرع وسط Triple Sugar Iron Agar (TSI) وتدوين النتائج بعد مدة 18-24 ساعة حضانة في 37 م°

٣- اجراء فحص الأندول باستعمال ماء اللبتون الحاوي على الحامض الاميني تريبتوفان، واستخدام كاشف كوفاكس (kovacs) لقراءة النتيجة.

٤- زرع وسط الستريت (Simmon's citrate) وتدوين النتائج بعد فترة الحضانة.

اجري فحص الهيموليسين⁽³⁾ للتحري عن قابلية العزلات على انتاج انزيم الهيموليسين وتحليل سبعة انواع من الدم، اربعة تعود الى الدم البشري بفصائله الاربعة (A, B, AB, O) وثلاث اخرى لدم حيوانات هي الماعز والارانب و الخيول.

وضع الدم بعد سحبه في دوارق زجاجية معقمة حاوية على 3% سترات الصوديوم وذلك باستخدام حجم 1 لكل 10 حجوم من الدم المسحوب، بعدها اجريت عملية النبذ المركزي لجميع عينات الدم باستخدام انابيب نبذ زجاجية معقمة للتخلص من جميع البلازما الموجودة في الدم والحصول على راسب كريات الدم الحمراء والذي غسل مرتين متتاليتين بالمحلول الملحي المعقم (السالين)، بعدها استخدم بنسبة 5% لتحضير اوساط الدم الزرعية الحاوية على مادة الاكار بنسبة 3%، حيث زرعت على هذه الاوساط جميع العزلات ووضعت في الحاضنة بدرجة 36م° لمدة 24 ساعة لملاحظة قابلية العزلات على تحليل الدم.

ولاجراء فحص الحساسية للمضادات الحيوية استخدمت طريقة بور - كيربي (Bauer-Kirby method) باستخدام الوسط الزرعي مولير هنتون (Mueller - Hinton) لاجراء هذا الفحص وباستعمال اقراص المضادات قطر 6.3 ملم المجهزة من شركة Oxoid. زرعت كل عزلة من العزلات في وسط المرق المغذي المحضر في قناني معقمة و بحجم موحد (5 ملم) وذلك باخذ نقلتين من كل مزروع ورجه بصورة جيدة في الوسط الزرعي السائل ومقارنة العكرة المتكونة مع ثابت العكرية (Turbidity standard) الذي حضر باضافة 0.5 مل من كلوريد الباريوم 1.175% الى 99.5 مل من حامض الكبريتيك (1%).

كما وضع ثابت العكرة في نفس نوع القناني الزجاجية المستخدمة للوسط الزرعي السائل وحفظ في الظلام بدرجة الحرارة الغرفة مع احكام الغلق لمنع التبخر.

قورنت العزلات المزروعة في وسط المرق المغذي مع ثابت العكرة للحصول على نفس العكرة.

صبت جميع المحتويات على سطح الوسط الزراعي الصلب مولير هنتون وتركت لمدة 15 دقيقة ثم سكب منه الكمية الزائدة وترك الطبقة ليجمد لمدة ساعة واحدة ثم وضعت عليه اقراص المضادات الحياتية وحضنت في درجة حرارة 37 م⁰ لمدة 24 ساعة لمشاهدة مناطق زوال النمو (Inhibition Zone) حول اقراص المضادات. قورنت النتائج مع النتائج التي تم الحصول عليها باستخدام العزلة القياسية (*Escherichia coli* (ATCC25922) واعتبر قطر منع النمو المساوي او الذي يقل بـ (1) ملم عن قطر منع النمو للعزلة القياسية مساوياً وهذا يعني ان العزلة حساسة للمضاد، اما اذا قل قطر منع النمو عن (2) ملم او اكثر عن العزلة القياسية فتعد العزلة مقاومة للمضاد. استخدمت اقراص المضادات الحياتية التالية وبتركيز المشار اليها الكلورامفينيكول (30)، امبسيلين (25)، تتراسايكلين (10)، جنتاميسين (10)، سيفالكسين (30)، سيفوتاكسيم (30)، ريناميسين (30)، ترائي مثيريم (1.25)، كاربنسلين (100)، امبلي كلوكس (امبسيلين 25) + كلوكساسلين (5)، اريثرومايسين (15)، توبراميسين (30)، لنكوميسين (10)، من شركة UPJOHN، الاموكساسلين (25) سبروفلوكساسين (30).

النتائج والمناقشة

تم زرع (698) مسحة اذن على الاوساط الزرعية المختارة (طرائق العمل)، حيث كانت نتيجة الزرع ظهور انواع متعددة من الاحياء المجهرية، لم يكن نصيب بكتريا بروتيتوس منها الا (10%) (70) عزلة، والعزلات الباقية كانت تعود لاجناس اخرى من البكتريا والفطريات هي ليست بصدد الدراسة الحالية.

توزعت نتائج الزرع المختبري على (61%) (426) عزلة تعود للذكور، و (39%) (272) عزلة تعود للاناث، اما اصابات بكتريا بروتيتوس عند الذكور فقد بلغت (67.1%) (47) اصابة، وعند الاناث (32.9%) (23) اصابة الجدول رقم (1).

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الجدول رقم (1) : اعداد ونسب المسحات موجبة الزرع المختبري وتلك الحاوية على بكتريا بروتينوس
موزعة حسب الجنس

المسحات المزروعة	(%) المجموع	(%) الذكور	(%) الاناث
العدد الكلي	690 (100)	426 (61)	272 (39)
الحاوية على بكتريا بروتينوس	70 (10)*	47(67)	23 (32.9)

* النسبة محسوبة من المجموع الكلي للمسحات

يتضح من الجدول ان نسبة الاصابة ببكتريا بروتينوس عند الذكور مقارنة بالاناث هي بحدود (1:2) جاءت هذه النسبة متوافقة تقريباً مع دراسات اخرى⁽⁵⁾.

اما اعمار المصابين فقد تراوحت ما بين (1 - اكثر من 50 سنة) موزعة على ست فئات عمرية، وان الفئة العمرية الاولى (1-10) كانت من اقل الفئات العمرية في اعداد الاصابة وان الفئة العمرية الثالثة (21-30) كانت اعلى الفئات اصابة. الجدول رقم (2).

يتبين من الجدول ان نسب الاصابة عند الذكور اعلى منها عند الاناث ولجميع الفئات العمرية المدروسة.

الجدول رقم (2) : الفئات العمرية واجناس المصابين بالتهابات الاذن الوسطى

الاجناس	العدد	الفئات العمرية (سنة)					
		1-10(%)	11-20(%)	21-30(%)	31-40(%)	41-50(%)	اكثر من 50(%)
ذكور	426	18(4.2)	72(16.9)	108(25.4)	84(19.7)	64(15.0)	80(18.8)
اناث	272	15(5.5)	54(19.8)	66(24.3)	48(17.6)	57(21.0)	32(11.8)
المجموع	698	33(4.7)	126(18.1)	174(24.9)	132(18.9)	121(17.3)	112(16.1)

بصورة عامة يمكن القول ان الاصابات شملت جميع الفئات العمرية بغض النظر عن الاعداد المحتواة ضمن كل فئة عمرية، وقد اشار⁽⁶⁾ ان البكتريا تكون مسؤولة عن العديد من اصابات الاذن الوسطى ولجميع الفئات العمرية اطفال وبالغين، ولا تقتصر اصاباتها على فئة عمرية محددة.

استخدمت الفحوصات البايوكيميائية التشخيصية لتشخيص انواع الجنس (طرائق العمل) بالاعتماد على النتائج الموجبة في فحص اليوريز وقابلية البكتريا على النمو وتخمير السكريات وانتاج غاز كبريتيد الهايدروجين على وسط T.S.I وتكوين راسب كبريتيد الحديدوز (FES) اسود اللون في قعر الوسط الزرعي، وعدم قابلية البكتريا على تخمير سكر اللاكتوز والسكروز وتكوينها لمستعمرات صفراء شاحبة اللون على وسط مكوني لعدم قدرتها على تخمير سكر اللاكتوز، كذلك اجري فحص الاندول لتفريق النوع السالب من افراد هذا الجنس (*Pro. mirabilis*) عن غيره من الانواع الموجبة لهذا الفحص.

وجد ان النوع (*Pro.mirabilis*) من اكثر انواع الجنس عزلا من النماذج المرضية، حيث شكل نسبة (84.3%) من مجموع اصابات الجنس، موزع بين الذكور (60.1%) والاناث (24.2%) كما مبين في الجدول (3).

اما النوع (*Pro.penneri*) فكان اقل الانواع عزلا، حيث شكل نسبة (2.8%) من المجموع الكلي للعزلات، في حين كانت نسبة عزل النوع (*Pro.vulgaris*) (12.8%).

الجدول رقم (3): انواع البكتريا بروتيووس موزعة حسب الجنس والفئات العمرية

الانواع	الاجناس	المجموع (%)	1-10	11-20	30-21	31-40	41-50	اكثر من 50
<i>Pro.mirabilis</i>	ذكور	(60.1)42	2	16	10	5	5	4
	اناث	(24.2)17	2	6	4	2	2	1
<i>Pro.vulgaris</i>	ذكور	(7.1)5	2	-	1	1	1	-
	اناث	(5.7)4	-	2	1	-	-	1
<i>Pro.penneri</i>	ذكور	(1.4)1	-	1	-	-	-	-
	اناث	(1.4)1	-	-	1	-	-	-
المجموع		(100)70	6	25	17	8	8	6

يتضح من الجدول ان الفئتين العمريتين الثانية والثالثة كانتا اعلى الفئات عرضة للاصابة، وان الفئتين الاولى والاخيرة كانتا اقل الفئات من حيث عدد الاصابات، من هذا يتبين ان الاصابة لا تقتصر

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على فئة عمرية دون سواها وان جميع الفئات عرضة للاصابة، وان تحديد الفئة العمرية لم يخدم الدراسة من أي ناحية، كما تاكد ذلك من دراسات سابقة⁽⁶⁾.

اشارت احد الدراسات⁽⁷⁾ الى ان بكتريا بروتايوس كانت مسؤولة عن (21.8%) من مجموع (1145) اصابة وهي موزعة على نوعين فقط هما *Pro.mirabilis* وبنسبة (21%) و *Pro.vulgaris* وبنسبة (8.0%) ولم تتمكن من عزل أي نوع من انواع الجنس الاخرى.
كذلك كان للمضاد امبيسلين تأثيرا بحدود 35% بينما في دراسة اخرى⁽¹⁶⁾ كان تأثيره على حوالي 50% من العزلات.

الجدول رقم (4) حساسية انواع الجنس بروتايوس للمضادات الحياتية

المجموع		انواع الجنس بروتايوس						اسماء المضادات الحياتية
		<i>Pro.penneri</i>		<i>Pro.vulgaris</i>		<i>Pro.mirabilis</i>		
(م)	(%) (ح)	(م)	(ح)	(م)	(ح)	(م)	(ح)	
3	(95.7)67	-	2	1	8	2	57	سبروفلوكساسين
4	(94.3)66	-	2	2	7	2	57	سيفوتاكسيم
5	(92.9)65	-	2	2	7	3	56	كلورامفينكول
9	(87.1)61	2	-	2	7	5	54	كاربنسيلين
13	(81.4)57	1	1	3	6	9	50	توبراميسين
14	(80)56	-	2	1	8	13	46	جنتاميسين
15	(78.6)55	1	1	3	6	11	48	اميلي كلوكس
15	(78.6)55	-	2	1	8	14	45	ريفامبسين
24	(65.7)46	2	-	5	4	17	43	سيفالوثين
32	(54.3)38	1	1	4	5	27	32	تراي مثيريم
44	(37.1)26	1	1	8	1	35	24	امبيسلين
49	(30)21	2	-	7	2	40	19	لنكوميسين
52	(25.7)18	2	-	8	1	42	17	ارثروميسين
57	(18.6)13	1	1	7	2	49	10	نتراسايكلين

ح = حساسية

م = مقاومة

استخدمت انواع متعددة من الدم لتحديد نشاط انزيم الهيمولايسين منها دم بشري يعود للاصناف الاربعة المعروفة وهي O, AB, B, A ودم حيوانات مختلفة الانواع هي الماعز والخيول والارانب، بطريقة التخطيط (Streaking) على الطبق ومشاهدة قابلية العزلات على تحليل الدم بعد فترة الحضانة بدرجة حرارة ٣٧ °م لمدة 24 ساعة. اتضح من النتائج وجود تباين واضح في مقدرة العزلات على تحليل انواع الدم المختلفة، كما هو موضح في الجدول رقم (5) الذي يبين قابلية عزلات الجنس بروتئوس على انتاج الهيمولايسين على اوساط الدم المختلفة.

فسرت بعض الدراسات⁽¹⁾ قلة عزل النوعين *Pro.vulgaris*, *Pro.penneri* الى كون هذه الانواع اقل ضراوة من النوع *Pro.mirabilis* لذلك تكون نسب عزلها من النماذج المرضية قليلة. تمتلك بكتريا *Pro.mirabilis* عوامل ضراوة متعددة تزيد من قابلية انتشارها وبقائها واحداثها للمرض، هذه العوامل ربما تكون هي المسؤولة عن سبب سيادة هذا النوع من دون غيره من انواع الجنس *Proteus* في النماذج المرضية.

من عوامل الضراوة في هذه البكتريا هي قابليتها على انتاج انزيم اليوريز⁽⁸⁾ وقدرتها على اختراق الخلايا (invasiveness)⁽⁹⁾ ونتاجها للانزيمات المذيبة للبروتينات (proteolytic enzymes) والتي تعمل على شطر الاجسام المضادة من نوع IgG, IgA⁽¹⁰⁾، امتلاكها لبروتينات الغشاء الخارجي (outer membrane proteins)⁽¹¹⁾، وافرازها لانزيم الهيمولايسين (Haemolysin)⁽¹²⁾، تكوينها لظاهرة النمو الغشائي (Swarming phenomenon)⁽¹³⁾، والمقاومة للمصل الطبيعي (Resistance to normal serum)⁽¹⁴⁾، وقابليتها على الالتصاق بالخلايا الطلائية البولية (Adhesion to the uro-epithelium)⁽¹⁵⁾.

عند اجراء فحص الحساسية للمضادات الحياتية للانواع المعزولة، اظهرت هذه الانواع تباينا في مدى حساسيتها لهذه المضادات، كما هو موضح في الجدول (4)، حيث يلاحظ ان اكثر المضادات تأثيرا على نمو انواع هذا الجنس والتي احتلت موقع الصدارة هي كل من سيروفلوكساسين سيفوتاكسيم، كلورامفينيكول ثم الكاربينسلين وبنسب حساسية 92.3%, 94.3%, 95.7% ثم 87.1% حسب التوالي في حين احتل المضادين توبراميسين وجنتاميسين الموقع الثاني في التأثير وبنسب حساسية 81.4% و80%، بينما تساوي كل من المضادين ريفامبين والاميلي كلوكس في تأثيرهما وبنسبة حساسية 78.6% لكل منهما.

اما اقل المضادات تأثيرا على هذه البكتريا والتي اظهرت البكتريا مقاومة تجاهها هي الامبسيلين، لنكوميسين، ارثرومايسين وتتراسايكلين وبنسب حساسية 18.6%, 25.7%, 30%, 37.1% على التوالي اما المضادين سيفالوثين وتراي مثبريم فكانا ذا تأثير متوسط الشدة الا ان نسب العزلات

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الحساسية كان اعلى من المقاومة، حيث بلغت نسب الحساسية لهذين المضادين %65.7, %54.3 حسب التوالي.

ان تحديد حساسية او مقاومة العزلات للمضادات الحيائية، تمت بالاعتماد على قياس قطر زوال النمو بالمليميتر (ملم) حول اقراص المضادات المستخدمة، وقيمت النتائج بالمقارنة مع اقطار زوال النمو حول العزلة القياسية المستخدمة⁽⁴⁾.

ظهر من النتائج ان العديد من المضادات والتراكيز المشار اليها في الاقراص تكون غير مؤثرة على البكتريا، كما ان اختلاف تراكيز المضادات في الاقراص يولد تأثيرا متغيرا على البكتريا، والتلثير لايتناسب طرديا مع تركيز المضادات القرصي، فمضاد تراي مثيريم بالرغم من قلة تركيزه القرصي، الا ان تأثيره كان اعلى بكثير من مضادات اخرى ذات محتوى قرصي عالي كمضادات الارثرومايسين، الامبيسيلين والتتراسايكلين.

اشارت احد الدراسات⁽¹¹⁾ ان للمضاد جنتاماميسين الدور الرائد في التأثير على البكتريا بروتينوس مقارنة بالعديد من المضادات الاخرى المستخدمة كمضادات الكلورامفينيكول، التتراسايكلين وحامض التالدكس، وكذلك اظهر كل من مضادي الكلورامفينيكول وحامض التالدكس تأثيرين متقاربين واحتلا الموقع الثاني في التأثير، بينما في الدراسة الحالية كان للمضاد كلورامفينيكول موقع الصدارة في التأثير. اما بخصوص المضاد تتراسايكلين فكان تأثيره بحدود %18 على مجموع العزلات، في حين كان تأثيره في دراسة اخرى⁽⁶⁾ بحدود %15 اضافة الى تقدم المضاد كاربنسيلين في تأثيره على خمسة انواع من البنسيلينات المختارة واربعة من مجموع السيفالوسبورينات.

تأكدت هذه النتيجة ايضا من خلال النتائج التي تم الحصول عليها حيث احتل المرتبة الاولى في تأثيره.

الجدول رقم (5): قابلية عزلات الجنس بروتيوس على انتاج الهيمولاسين على اوساط الدم المختلفة

انواع الدم واعداد العزلات المحللة							عدد العزلات المحللة للدم	العدد الكلي	انواع جنس بروتيوس
دم	دم	دم	فصائل دم الانسان						
الخيول	الماعز	الارانب	O	AB	B	A			
2	30	35	40	10	15	5	(84.7)55	59	<i>Pro.mirabilis</i>
1	6	6	4	2	1	-	(66.7)6	9	<i>Pro.vulgaris</i>
-	1	1	1	-	-	-	(50)1	2	<i>Pro.penneri</i>
3	37	42	45	12	16	5	(81.4)57	70	(%) المجموع
(5.3)	(64.9)	(73.7)	(87.9)	(21.1)	(28.1)	(8.8)			

- النسب المئوية لتحلل الدم محسوبة على اساس العدد الكلي المحلل للدم (56)

يتضح من الجدول ان (81.4%) من العزلات كانت محللة للدم، وهناك تفاوت واضح في النسب المئوية للتحليل، تختلف باختلاف انواع اوساط الدم المستخدمة، وبصورة عامة فان دم الحيوانات اعطى نتائج افضل من الدم البشري لمشاهدة النشاط الهيمولاسيني للعزلات. كذلك لوحظ وجود تباين في النتائج المستحصل عليها اعتمادا على نوع دم الحيوانات المستخدم، قدم الارانب كان الافضل في الحصول على نسب تحلل عالية (73.37%) تلاه دم الماعز (64.9%) ثم دم الخيول (5.3%).

اشارت احد الدراسات⁽³⁾ الى عدم ظهور صفة تحلل الدم على وسط دم الخيول عند تنمية البكتريا *Pro.mirailis* عليه وان هيمولاسين هذه البكتريا ينتج من قبل (94%) من العزلات ويكون من النوع الذائب الذي يفرز الى الاوساط الزرعية، هذه النتيجة كانت مقاربة لما توصلت اليه الدراسة الحالية وهي ان نسبة العزلات المنتجة للهيمولاسين كانت (84.7%) كذلك فان دم الخيول كان غير مشجعا لظهور تأثير الهيمولاسين حيث لم تظهر صفة التحلل عليه الا بنسبة ضئيلة فقط (5.3%).

تبين ايضا انه عند استخدام الدم البشري، وجود تباين واضح في فعالية الهيمولاسين اعتمادا على فصيلة الدم المستخدمة في تحضير الاوساط الزرعية، حيث اتضح ان فصيلة الدم (O) هي من افضل الفصائل المستخدمة للحصول على نسب تحلل عالية (78.9%) تلتها الفصائل A, AB, B وينسب تحلل (28.1%)، (21.1%) (8.8%) على التوالي وبذلك يمكن القول ان الفصيلة (O) هي الافضل من بين فصائل الدم البشري وكذلك دم الارانب لمشاهدة ظهور فعل انزيم الهيمولاسين وهكذا يمكن القول انه من المفضل استخدام هذين النوعين من الدم لتحضير اطباق وسط الدم للكشف عن نشاط الهيمولاسين.

ربما يمكن تفسير الامر باحتواء هذين النوعين من الدم على مستقبلات اقل تخصصا مما تحتويه بقية الاصناف، او ان الية عمل الهيموليسين هي انزيمية كما هو الحال في هيموليسين المكورات العنقودية، وقد اشار (18) الى وجود مواضع استقبالي (Receptors) على كريات الدم الحمراء تلتصق بها البكتريا ويمكنها ان تلتصق كريات الدم الحمراء للانسان. كذلك فان تركيب الوسط الزراعي يؤثر على القابلية التحليلية للدم حيث ان وجود المصل او الكوليسترول يعملان على تثبيط التحلل مما يؤدي الى عدم الكشف عن الهيموليسين⁽¹⁹⁾ ولهذا حرصت الدراسة الحالية على غسل كريات الدم الحمراء عند تحضير اوساط الزرع (طرائق العمل). ان فعل الهيموليسين يعتمد على عدة عوامل⁽²⁰⁾ منها نوع كريات الدم الحمراء المستعملة ونوع الوسط الزراعي وظروف اجراء الاختبار وان الفضلات الناتجة من العمليات الايضية للبكتريا اثناء نموها تعمل ايضا على تحليل الدم في الاوساط الزرعية، عليه ولتقليل تأثير هذه المواد الى الحد الادنى، يوصى بغسل كريات الدم الحمراء واستعمال راسب الكريات في تحضير اوساط الدم وزيادة الاكار الى حدود 3% للحصول على مستعمرات منفصلة على اوساط الدم والتخلص من ظاهرة النمو الغشائي على الاطباق.

ان الهيموغلوبين المتحرر بفعل الهيموليسين يعمل على تثبيط قابلية كريات الدم البيضاء على التهام البكتريا داخل وخارج جسم المضيف⁽²⁰⁾ وبذلك يزيد من فرصة بقاء البكتريا واحداث المرض. اتضح في الدراسة الحالية ان نسب العزلات المحللة للدم من البكتريا Pro. mirabilis و Pro. vulgaris و Pro. penneri كانت وعلى التوالي (84.7) ، (66.7%) و (50%)، في حين كانت نسب التحلل في دراسة سابقة⁽³⁾ وللانواع الثلاثة حسب التعاقب (94%)، (84%) و (100%)، يلاحظ من هذا وجود تشابه تقريبا في النتائج الى حد ما عدا ما جاء في النوع الاخير، وربما يفسر الامر على اساس قلة عدد العزلات و البالغة اثنتين فقط والتي لا يمكن بناء تفسير جازم حولها.

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قابلية انواع الجنس بروتيوس المعزولة من التهابات الاذن الوسطى على انتاج الهيموليسين ومقاومة المضادات الحياتية
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تأثير التلوث بمياه المجاري على الفطريات المائية في نهر ديالى

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ABSTRACT

Thirteen species belongs to four genera of aquatic fungi were isolated from Diala river and Al-Rustumeyah station, these genera are *Achlya*, *Saprolegnia*, *Dictyuchus* and *Pythium*. Ecological studies of the physical and chemical characters of Diala river showed that, concentration of nutrients of the Rustumeyah station were very high and it elevated the conc. Of Calcium, Sodium, Ammonium and Phospahte in the water of river, it also reduced the dissolved Oxygen. Percentage of fungi isolate, was less at the site near the station compared with other sites. The highest percentage of fungi was recorded at the site before station, the reduction of number of isolate near the station was due to the effect of sewage water which significantly effected the values of dissolved oxygen, Ca^{++} , Na^{++} , NH_4^+ , PO_4^- between this site and other sites.

الخلاصة

تم عزل وتشخيص (13) نوعاً من الفطريات المائية من نهر ديالى ومحطة معالجة المياه الثقيلة (الرستمية)، تعود الى اربع اجناس، ثلاث منها عائد للعائلة السابروليكنيسية Saprolegniaceae هي Pythiaceae و *Pythium* عائد للعائلة البيثيسية *Dictyuchus*, *Saprolegnia*, *Achlya* وعند اجرا الدراسة البيئية للعديد من الخصائص الفيزيائية والكيميائية للنهر ولمياه محطة الرستمية الثقيلة، لوحظ ان المادة العضوية والمغذيات قد وجدت في مياه المجاري بتركيز عالية جداً مما ادى الى رفع تراكيزها في النهر لاسيما الكالسيوم والصوديوم والامونيوم والفوسفات، فضلاً عن انخفاض الاوكسجين المذاب فيها كما انخفضت نسبة الاعداد الفطرية المعزولة من الموقع القريب من المحطة مقارنة بالمواقع النهرية الاخرى، وكانت اعلى نسبة لاعداد هذه الفطريات في الموقع الذي سبق المحطة وذلك عائد الى تأثير الموقع القريب من المحطة بمياه الصرف التي ادت الى فروق معنوية في الاوكسجين المذاب والكالسيوم والصوديوم والامونيوم والفوسفات بين هذا الموقع والمواقع الاخرى.

المقدمة

ان الاخلال بالتوازن نتيجة الاستغلال غير المنظم للمكونات الاساسية للبيئة (التلوث) هو ما يسبب العرقلة في الفعاليات الحيوية لمعظم الكائنات الحية، ويعد التلوث بمياه المجاري من احد الملوثات المهمة للبيئة المائية، فضلاً عن ارتفاع تراكيز العناصر الثقيلة فيها فهي تحتوي على نسبة عالية جداً من المواد العضوية والمغذيات الطبيعية المؤدية الى ظاهرة الاتراء الغذائي (Eutrophication) في البيئة المائية المطروحة اليها، مما تؤدي الى فقدان البيئة للعديد من مقوماتها الاساسية لمعيشة الاحياء (السعد وجماعته، 1997)، وقد لوحظ اثر الظروف البيئية على نسبة تواجد بعض الفطريات المائية في الكثير من الدراسات منها (Czeczuga, 1991; Czeczuga et al., 1998). فعلى سبيل المثال تُعد درجة الحرارة من العوامل المهمة المحددة لانتشار هذه الفطريات.

لاحظ (Mer et al., 1980) ان الفطريات *Saprolegnia* و *Achlya* و *Aphanomyces* و *Dictyuchus* تنتشر في مديات حرارية متوسطة (18-26.8) °م كذلك فإن زيادة الملوحة تُعد عاملاً هاماً آخر، اذ تؤدي الى اختزال نسبة أنبات الأبواغ السابحة للفطر (Padgett 1978a) *Saprolegnia australis*.

ومن المعروف ان للكالسيوم تأثيرات هامة وعديدة على الأحياء المختلفة ولا سيما الفطريات البيضية، فقد لاحظ (Czeczuga 1996) أن نسبة الفطريات المائية المعزولة من بحيرة Biale Scienenskie عالية وقد يعزى هذا الارتفاع الى محتوى البحيرة العالي من الكالسيوم مقارنةً مع البحيرات الاخرى، وكذلك لوحظ بأن للمغنيسيوم تأثيراً مختلفاً على كل من التكاثر الجنسي واللاجنسي للفطريات السابروليكنينية زيا (1985)، كما وأثبت (Domnas et al., 1982) أن التركيز (0.5) مل مول مغنيسيوم يثبط انتاج أبواغ الفطر *Lagenidium aiganteum*.

اما البوتاسيوم والصوديوم فلهما دوراً هاماً في العديد من الفعاليات الحيوية فمثلاً لاحظ (Smith et al. 1990) أنخفاض نمو وانتاج أبواغ الفطريات السابروليكنينية عند انخفاض الجهد المائي من استخدام التراكيز العالية للبوتاسيوم، كما أشار (Boddy 1983) الى انخفاض نسبة نمو عدداً من الفطريات البازيدية في تراكيز الصوديوم العالية وإزدياد هذا النمو بارتفاع تراكيز البوتاسيوم، ويعد الفسفور من العناصر الحياتية المهمة في أنظمة نقل الطاقة ودخوله في تركيب الأغشية والمكونات البروتوبلازمية فضلاً عن وظائفه المهمة الأخرى، فقد اشار (Nolan 1979) الى ان تراكيز الفوسفات المثلى لنمو عزلتين من الفطر *Saprolegnia australis* هي (2.5-12.5) مل مول فوسفات. ومن المعروف ان ارتفاع تركيز النتروجين في المياه عن (1) ملغم / لتر يعتبر دالة للتلوث العضوي (السعدي وجماعته، 1986) والذي سيؤثر سلبياً على معيشة الأحياء المائية الأخرى.

طرائق العمل

- أ- جمع العينات : تم تعيين أربع محطات، شكل (1) لدراسة الخصائص الفيزيائية والكيميائية والبايولوجية لها، وهي :-
- ١- المحطة الاولى : الموقع على نهر ديالى، يبعد مسافة (2) كم قبل محطة الرستمية.
 - ٢- المحطة الثانية : موقع نهري يقع بالقرب من مصبات الرستمية المباشرة الى النهر بمسافة (3-8)م.
 - ٣- المحطة الثالثة : يبعد الموقع بمسافة (2) كم على النهر ما بعد مصبات المحطة.
 - ٤- المحطة الرابعة : تضمنت محطة الرستمية والمشملة على الاحواض الرئيسية التي تتجمع فيها مياه المجاري الخام من شبكة المجاري الرئيسية لمدينة بغداد.

جمعت عينات المياه في قناني زجاجية معتمة ومعقمة فتحت تحت سطح الماء بعمق (20-10)سم، وأغلقت القناني باحكام وهي تحي الماء، ثم نقلت العينات مباشرة الى المختبر.

ب- دراسة الخصائص الفيزيائية والكيميائية

تم قياس درجة حرارة المياه مباشرة في محطات الدراسة باستخدام محرار زئبقي Thermometer مدرج بوحدات (1) م⁰ بعد غمره في الماء لمدة (10-15) دقيقة اما قياس الاس الهيدروجيني فتم اجراءه باستخدام جهاز pH-meter، في حين تم قياس الملوحة بوساطة جهاز قياس التوصيل الكهربائي Conductivity meter اعتماداً على قيم التوصيلية الكهربائية، وقد قيست كمية الاوكسجين المذاب والطلب الكيميائي للاوكسجين، الموضحين من قبل Lind (1979) وفق طريقة وينكلسر واكسدة الكرومات، على التوالي، وحسبت تراكيز كل من العنصرين الكالسيوم والمغنيسيوم بطريقة E.D.T.A. Method الموضحة من قبل المصدر نفسه، كما تم قياس تراكيز الصوديوم والبوتاسيوم باتباع الطريقة المبينة من قبل Moore & Chapman 1986 بوساطة جهاز (Flame photometer) على طول موجي (766) نانوميتر للبوتاسيوم و (589) نانوميتر للصوديوم، وقد اتبعت الطرائق الموضحة من قبل APHA (1975) والمعتمدة على الطريقة اللونية Colorimetric method باستخدام جهاز Auto Analyzer على طول موجي (880 ، 650-660 و 543 و 460) نانوميتر لقياس كل من الفوسفات والامونيوم والنترات والكبريتات على التوالي، في حين اتبعت الطريقة المعتمدة على أكسدة الكرومات لقياس الكلوريدات.

عزل وتشخيص الفطريات قيد الدراسة

تم عزل الفطريات السابرولكينيضية بطريقة الطعوم Baiting Method (Jones, 1971; Al-Rekabi et al., 1996)، رجت عينات الماء بلطف ثم سكبت في اطباق بتري حاوية على بذرة معقمة ومغلية لكل من السمسم والدخن والذرة اضافة للمضاد الحيوي كلورامفينيكول، وحضنت الاطباق الحاضنة بدرجة حرارة (20)°م وفحصت بعد (48) ساعة بواسطة المجهر الضوئي Light Microscope لمراقبة نمو الخيوط الفطرية غير المقسمة المتكونة، ثم نقلت البذور بعد غسلها عدة مرات بالماء بذرة جديدة وتركت بضعة ايام عند درجة الحرارة نفسها حتى تستطيل الخيوط الفطرية وتبدأ بانتاج الحافظات البوغية والبيضية بحيث يمكن فصلها وعمل المزارع النقية منها، اما الاطباق التي لم يظهر فيها نمو نهائياً فقد أهملت بعد مرور شهر بعد التأكد من عدم وجود نمو، وقد اعتمد على كل من المصدرين (1923) Coker و (1970) Seymour لتشخيص الانواع الفطرية المعزولة.

العلاقات الاحصائية

جرى معاملة نتائج القياسات البيئية الفيزيائية والكيميائية بتحليل التباين ثلاثي الاتجاه 3 way analysis of variance لتحديد معنوية الفروق في الخصائص البيئية المقاسة والفروق الموقعية والشهرية بينها جدول (3). ثم اجري اختبار L.S.D على الدراسة التي اوضحت فرقاً معنوياً لتحديد أي من القياسات البيئية يوجد بينها فرق معنوي جدول (4)، جرى الاختبار على مستوى احتمالية 0.01 و 0.05 وثبتت النتائج على شكل معدل حسابي \pm الانحراف المعياري.

النتائج والمناقشة

الفطريات المائية المعزولة

تم عزل (7) أنواع تعود الى الجنس *Achlya* هي : *Achlya klebsiana* و *A. imperfecta* و *A. dubia* و *A. flagellata* و *A. americana* و *A. proliferodes* و *Achlya sp.* (4) أنواع تعود للجنس *Saprolegnia* هي : *Saprolegnia anisospora* و *S. ferax* و *S. diclina* و *Saprolegnia sp.* ، ونوع واحد لكل من الجنسين *Dictyuchus* و *Pythium* هما *Pythium sterile* و *Pythium sp.* جدول (1)، شكل (2) ومن الملاحظ بأن العديد من هذه الفطريات قد تم عزلها سابقاً من شط العرب من قبل (Al-Rekabi & Zia 1989 ; وزيا 1985 Muhsin 1977).

كما عزلت من بعض الترب والمبازل العراقية من قبل الجوهري (1998)، ولاتوجد بحوث منشورة لعزل هذه الفطريات من المسطحات المائية الاخرى في العراق ولاسيما نهر ديالى.

المؤشرات البيئية

درجة الحرارة

تقاربت درجات الحرارة في جميع المواقع المائية المدروسة، جدول (2)، وقد وجدت علاقة ارتباط سالبة بين درجة حرارة المياه واعداد الفطريات المعزولة ($r = 0.7899$) وعلاقة ارتباط سالبة بين درجة الحرارة والانواع الفطريات ($r = 0.6680$)، جدول (1)، وهذا عائد لتأثير عملية انتاج وانتشار الابواغ السابحة لهذه الفطريات باختلاف درجات الحرارة كما لاحظ سابقاً (Muhsin, 1977 ; Ismail et al., 1979) وتوقف درجة حرارة 30°م انتاج السبورات (Raja Shekhar Kaveriappa, 1996).

درجة الاس الهيدروجيني

لوحظ بأن درجة الاس الهيدروجيني متقاربة في المواقع المائية على النهر، أما محطة الرستمية فسجلت فيها قيماً أقل (جدول 2) ، وفي جميع المصادر المائية المدروسة كانت معدلات الاس الهيدروجيني تميل الى القاعدة عموماً (7.2-7.8) مما يجعل هذا العامل ينحرف عن القيمة المثلى لنمو الفطريات السابروليكينيضية (7.3-5.3) كم اشار اليها كل من (Ferre & Dumas, 1977 ; Nolan, 1979).

وتباين قيم هذا العامل في فصل الربيع (شكل 5) قد يعزى الى الكثافة العالية للهائمات النباتية في النهر وبالتالي ازدادت فعالية البناء الضوئي التي تؤدي الى استهلاك غاز CO₂ ورفع درجة الاس الهيدروجيني (Al-Lami, 1998).

الملوحة

تماثلت تراكيز الملوحة في المواقع النهرية لثلاث، أما محطة الرستمية⁽⁴⁾ فقد ارتفعت تراكيز الملوحة فيها (3.4-1.9) غم / لتر، جدول (2)، وهذا عائد الى الارتفاع في نسبة الاملاح المغذية المتسببة عن الفعاليات الايضية للحياة المجهرية وخاصة البكتريا، وقد لوحظ ارتفاع النسبة المئوية لاعداد العزلات الكلية للجنس *Achlya* في محطة الرستمية البالغة (45)% عن النسبة المئوية للجنس *Saprolegnia* (38.8)% ثم يليها الجنس *Dictyuchus* حيث تصل النسبة المئوية (5.5)% كما يوضح الشكل (2 و 3).

سجال عبد الوهاب الركابي و اشواق شنان عبد

وتباين القدرة على تحمل الملوحة بين اجناس السباروليكنيسية عائد الى الاختلاف في الية التنظيم الازموزي لهما Padgett (1978b) وقد يكون لهذا العامل السبب في خفض المجموع الكلي للعلزلات والانواع الفطرية المعزولة في هذه الدراسة من مياه المجاري مقارنة مع مياه نهر ديالى لاسيما الموقعين (1 و 3) ، الجدول (1)، إذ أوضح Padgett (1978) ان ارتفاع التراكيز الملحية تؤدي الى اختزال انبات الابواع السابحة للعائلة السبارولسكنيسية وترفع نسبة موت هذه الابواع.

الاوكسجين المذاب

سجلت اعلى قيم للاوكسجين المذاب في المحطات المائية للنهر في شهر كانون الثاني بسبب وجود علاقة عكسية بين الاوكسجين المذاب ودرجة الحرارة والملوحة، كما يلاحظ من شكل (4 و 5) وهذا متفق مع ما ورد من قبل (الحميم، 1986) إذ ان طرح فضلات المجاري في المياه تؤدي الى تقليل تركيز الاوكسجين المذاب فيها بسبب ظاهرة الاثراء الغذائي والتي تؤدي الى اقلال تركيز الاوكسجين المذاب بسبب عمليات التحلل البيولوجي (بدير، 1986)، وهذا ما يفسر انخفاض قيم هذا العامل في الموقعين الواقعين تحت تأثير مياه الصرف (3,2) عنه في الموقع النهري(1) والبعيد عن المحطة، جدول (4).

وسجلت أوطأ التراكيز للاوكسجين المذاب في مياه الصرف داخل محطة الرستمية بسبب الفعاليات الفيزيائية والكيميائية والحياتية الجارية في هذه المياه إذ يعد التركيز (2) ملغم/ لتر متعباً للاحياء المائية (Lind,1979).

الطلب الكيميائي للأوكسجين

وضح الجدول (2) ارتفاع معدلات الطلب الكيميائي للاوكسجين اعتماداً على طريقة الديكرومات المبينة في (1975) APHA في مياه محطة الرستمية الملوثة بالمواد العضوية والفضلات المنزلية حيث بلغ (10.8) ملغم / لتر وهذا يتوافق مع نتيجة موسى وجماعتها (1986) حيث أشاروا الى ارتفاع قيم هذا الطلب في مياه مجاري محطة الرستمية والتي تعدت قيم المقارنة الموضوعه من قبل وكالة حماية البيئة الامريكية E.P.A وقد انخفض معدل قيم هذا العامل في الموقع النهري⁽¹⁾ الى (1.5) ملغم/ لتر، الا ان معدلاته ارتفعت نسبياً بسبب تأثرهما بمياه الصرف الخارجة من المحطة، أن هذا الارتفاع في معدل الطلب الكيميائي للأوكسجين يعكس ازدياد نسبة المواد العضوية في هذه المياه وهذا يؤثر سلبياً على تواجد معظم الفطريات، وقد أكد Tan & Lim (1984) على ذلك.

الكالسيوم والمغنيسيوم

انخفضت تراكيز الكالسيوم في بعض اشهر السنة في المواقع⁽¹⁾ عنه في كل من الموقعين^(3,2)، كما يوضح ذلك الجدولين (3,2) وهذا عائد لتأثرهما بمياه الصرف المطروحة من محطة الرستمية والحاملة لتراكيز عالية من الكالسيوم (115-2.5) ملغم / لتر .
أما بالنسبة للمغنيسيوم فكانت قيمة أوطأ من الكالسيوم في معظم اشهر السنة وتقاربت معدلات تراكيزه في المواقع النهرية الثلاث في حين ارتفعت قيمه في مياه مجاري الرستمية. وتعتبر نسب كل من الكالسيوم والمغنيسيوم في نهر دبالى مرتفعة عند مقارنتها مع روافد نهر دجلة الأخرى التي لم يتجاوز مدى تراكيز الكالسيوم فيها عن (33-71) ملغم/ لتر، وتراكيز المغنيسيوم فيها عن (28-61) ملغم / لتر (الربيعي، 1982, 1997, Al-nimma).

البوتاسيوم والصوديوم

ارتفعت تراكيز الأيونين بصورة كبيرة في مياه محطة الرستمية عنها في المحطات النهرية^(3,2,1) لارتفاع نسب الملوثات العضوية والكيميائية فيها، وكان البوتاسيوم اقل الأيونات الموجبة تركيزاً، جدول (2). كما وجدت زيادة معنوية في قيم هذه التراكيز عند المواقع النهرية الواقعة تحت تأثير مياه الصرف، الموقعين (2 و 3) مقارنة مع الموقع (1) البعيد عن تأثير هذه المياه لاسيما فيما يخص أيون الصوديوم، جدول (3).

الفوسفات

تأثر الموقعين النهرين (2 و 3) بمياه الصرف الخارجة من محطة الرستمية الغنية بهذا الجذر⁽⁴⁾ (SO_4)، إذ بلغ معدله في مياه المجاري الخام (10.5) ملغم/ لتر، إن ارتفاع تراكيز الفوسفات في هذه البيئة المائية يشجع ظاهرة الاثراء الغذائي (الحميم، 1986)، والتي تؤثر سلباً على سائر الأحياء المجهرية ومنها الفطريات المائية المدروسة، جدول (2 و 3).

الأمونيوم والنترات

سجلت الدراسة ارتفاعاً معنوياً في تراكيز الأمونيوم في الموقع النهرى (3) مقارنة مع الموقعين النهرين الآخرين، كذلك هو الحال بالنسبة للنترات، إلا أن الارتفاع كان نسبياً وليس معنوياً، كما سجلت أعلى التراكيز لهذين المركبين في مياه محطة الرستمية، جدول (2 و 3)، ويعود هذا الارتفاع لكون مركبات النتروجين (الأمونيوم والنترات) من المكونات المهمة الداخلة في تركيب المواد العضوية،

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ويوجدان بتركيز ضئيلة في المياه بصورة عامة، وأن أي ارتفاع في قيمها عن (1) ملغم/ لتر سيكون دالة لتلوث هذا المصدر المائي بالمواد العضوية وبالتالي سيهدده بظاهرة الإثراء الغذائي (السعدي وجماعته، 1986)، وهذا ما سجل في جدول (2) في الموقع (3) .

الكلوريدات والكبريتات

سجلت قيم الكلوريدات والكبريتات مستويات متماثلة في كل من المواقع النهرية الثلاث، في حين اقتصت مياه المجاري في محطة الرستمية بالمستويات العالية جداً للمركيبين لأنهما من النواتج الأيضية المهمة لعمليات التحلل التي تقوم بها الأحياء المجهرية، وهما دالتين للتلوث، ولهذين الايونين التأثيرات المهمة في المؤلفات الكيميائية إذ توجد علاقة ارتباط موجبة بينهما وبين الملوحة وبعض العناصر الأخرى (Al-Lami, 1998).

التأثيرات البيئية على تواجد الفطريات المائية

لوحظ ارتفاع اعداد العزلات الفطرية في المحطة النهرية⁽¹⁾ التي تسبق مصبات تصريف مشروع الرستمية مقارنة مع سائر المحطات النهرية المدروسة، جدول (1) ، لاسيما عند المحطة النهرية (2) الواقعة بالقرب من مصبات التصريف، وقد يعود هذا الى طرح كميات كبيرة من المواد المعقمة مع مياه الصرف الى النهر، فضلاً عن الظروف البيئية المذكورة سابقاً.

قد يعزى انخفاض اعداد العزلات الفطرية في المحطة (3) الى تأثرها بارتفاع نسب المغذيات المذكورة سابقاً وانخفاض قيم الاوكسجين المذاب بسبب ارتفاع شدة التلوث العضوي في مياه النهر والذي يؤثر سلباً على تواجد معظم الفطريات المائية.

ولم يلاحظ أي تباين في مجموع الأنواع الفطرية المعزولة بين المحطات النهرية باستثناء المحطة (2) التي انعدم فيها التنوع تقريباً للسبب المذكور سابقاً، اما التنوع في العزلات الفطرية من مياه محطة الرستمية فقد انخفض الى النصف تقريباً لما تحمله هذه المياه من تراكيز عالية في العناصر المغذية والثقيلة (شكل 2 و 3)، فضلاً عن انخفاض الاوكسجين المذاب (2) ملغم / لتر وامتد هذا التأثير الى الهائمات النباتية المعزولة من محطة نهر ديالى المتأثرة بهذه الحياة (Kassim et al, 1996).

جدول (1) الاعداد والنسب المئوية لتوزيع وانتشار الفطريات المائية المعزولة من المحطات قيد الدراسة خلال اشهر السنة

الانواع	الاشهر	كانون (2)	شبا ط	اذار	نيسان	ايار	حزيران	تموز	اب	ايلول	تشرين (1)
Achlya klebsiana		3	-	-	2	-	-	-	-	-	-
A. imperfecta		1	-	-	-	-	-	-	-	-	-
A. dubia		1	-	-	-	-	-	-	-	-	-
A. flagellata		-	-	-	-	-	-	-	-	-	1
A. americana		-	-	-	-	-	-	-	-	-	2
A. Proliferoides		3	-	3	-	2	-	-	-	-	-
Achlya sp.		-	-	2	2	4	-	3	2	2	1
Saprolegnia anisospora		-	-	2	-	-	-	-	-	1	-
S. diclina		2	-	-	-	-	-	-	-	-	-
S. ferax		5	2	-	4	-	-	-	-	-	-
Saprolegnia sp.		8	6	1	2	-	-	-	-	2	-
Dicylichus sterile		1	2	-	-	-	-	1	1	-	-
Pythium sp.		2	-	1	1	-	2	-	-	2	1
المجموع الكلي للعزلات		26	10	9	11	6	٢	4	3	7	5
المجموع الكلي للأنواع		9	3	5	5	2	١	2	2	4	4

- * المحطات على نهر ديالى (1) قبل محطة الرستمية (2) قرب المحطة (3) بعد المحطة -لا يوجد

تابع جدول (١)

م محطة الرستمية	نهر ديالى *			النسبة المئوية للتردد	المجموع الكلي للعزلات	(1)كانون	(2)تشرين	الاشهر	الانواع
	3	2	1						
	3	2	1						
	2	-	3	4.9	5	-	-	Achlya klebsiana	
	1	-	-	0.9	1	-	-	A. imperfecta	
	-	-	1	0.9	1	-	-	A. dubia	
1	1	-	3	4.8	5	-	4	A. flagellata	
-	-	-	4	3.8	4	2	-	A. americana	
2	3	-	3	7.7	8	-	-	A. Proliferoides	
5	9	-	6	19.4	20	3	1	Achlya sp.	
-	3	-	4	6.7	7	1	3	Saprolegnia anisospora	
-	-	-	2	1.9	2	-	-	S. diclina	
2	4	-	7	12.6	13	-	2	S. ferax	
5	6	-	8	18.4	19	-	-	Saprolegnia sp.	
1	3	-	3	6.7	7	1	1	Dicylichus sterile	
2	1	6	2	10.6	11	2	-	Pythium sp.	
18	33	6	46	100	103	9	11	المجموع الكلي للعزلات	
7	10	1	12		47	5	5	المجموع الكلي للأنواع	

جدول (2) المدى والمعدل \pm الانحراف المعياري للخصائص الفيزيائية والكيميائية للمياه في نهر ديالى ومحطة الرستمية

محطة الرستمية	نهر ديالى *			المواقع الخاصية
	(4)	(3)	(2)	
(29.5-15) 24.8 \pm 5	(29-13) 23 \pm 5.6	(29-15) 23 \pm 5	(29-14) 23 \pm 5	درجة الحرارة (م)
(8.1-6.4) 7.2 \pm 0.5	(8.4-7.3) 7.7 \pm 0.4	(8.4-6.5) 7.6 \pm 0.4	(8.3-7.1) 7.8 \pm 0.4	درجة الأس الهيدروجيني
(3.4-1.9) 2.6 \pm 0.3	(2.5-1.1) 1.6 \pm 0.4	(2.5-0.6) 1.5 \pm 0.4	(2.3-0.6) 1.4 \pm 0.4	الملوحة (غم/لتر)
(3-1.5) 2 \pm 0.5	(7.7-4.3) 5.7 \pm 0.9	(11.2-4.9) 6.5 \pm 1.6	(11.1-6.4) 7.1 \pm 1.4	الاوكتجين المذاب (ملغم/لتر)
(23-3) 10.8 \pm 6.2	(6-11) 2.2 \pm 1.2	(5.6-1) 1.9 \pm 1.2	(2.8-0.6) 1.5 \pm 0.5	الطلب للأوكسجين الكيميائي (ملغم/لتر)
(205-115) 150.4 \pm 29	(144-64) 104 \pm 20.9	(146-64) 103.6 \pm 21.8	(146-72) 97.2 \pm 21.4	الكالسيوم (ملغم /لتر) **
(120-83.5) 97.9 \pm 24.3	(107-49) 71.8 \pm 21	(101-27.8) 71.4 \pm 19.5	(112-27.8) 67.9 \pm 21.6	المغنيسيوم (ملغم/لتر)
(19.2-10.1) 13.8 \pm 3	(4.5-3.2) 4 \pm 0.5	(4.4-2.8) 3.5 \pm 0.6	(4.1-2.6) 2.9 \pm 0.4	البوتاسيوم (ملغم/لتر)
(301-202) 253.6 \pm 40.3	(246-152) 193.7 \pm 40.7	(246-151) 193.7 \pm 32.6	(239-115) 178.6 \pm 38.4	الصوديوم (ملغم/لتر)
(14.0-5.2) 10.5 \pm 6.4	(1.1-0.2) 0.6 \pm 0.2	(0.9-0.18) 0.5 \pm 0.2	(1.1-0.1) 0.3 \pm 0.2	الفوسفات (ملغم/لتر)
(26.0-5.3) 15.9 \pm 6.4	(2.8-0.5) 1.6 \pm 0.6	(2.9-0.2) 1.0 \pm 0.7	(1.1-0.2) 0.6 \pm 0.3	الأمونيوم (ملغم/لتر)
(25.5-1.9) 11.3 \pm 10.6	(9.1-0.9) 6.5 \pm 4.8	(7.8-0.9) 4.8 \pm 1.9	(8-0.8) 5 \pm 2.1	النترات (ملغم/لتر)
(415-150) 295.5 \pm 85.8	(293-60) 198.6 \pm 66.9	(251-67) 182 \pm 57	(288-133) 186.3 \pm 64.9	الكلوريدات (ملغم/لتر)
(624-204) 461.6 \pm 130.6	(575-127) 357.6 \pm 110	(585-290) 340 \pm 108.3	(519-140) 354 \pm 105.8	الكبريتات (ملغم/لتر)

* المحطات على نهر ديالى (1) قبل محطة الرستمية، (2) قرب المحطة ، (3) بعد المحطة
 التركيز (ملغم/لتر)
 ** التركيز (مل مول) = $\frac{\text{الوزن الجزيئي للعنصر او المركب}}{\text{التركيز (ملغم/لتر)}}$

جدول (3) قيم "F" لتحليل التباين (ثلاثي الاتجاه) بين الخصائص المقاسة (A) والفروق الشهرية (B) والموقعية (C) والتفاعل بينهما

C x B	C x A	B x A	C	B	A	مصدر التباين
1.86**	4.71**	6.70**	36.70**	19.11**	921.96**	قيم F

* معنوي عند مستوى $P < 0.05$

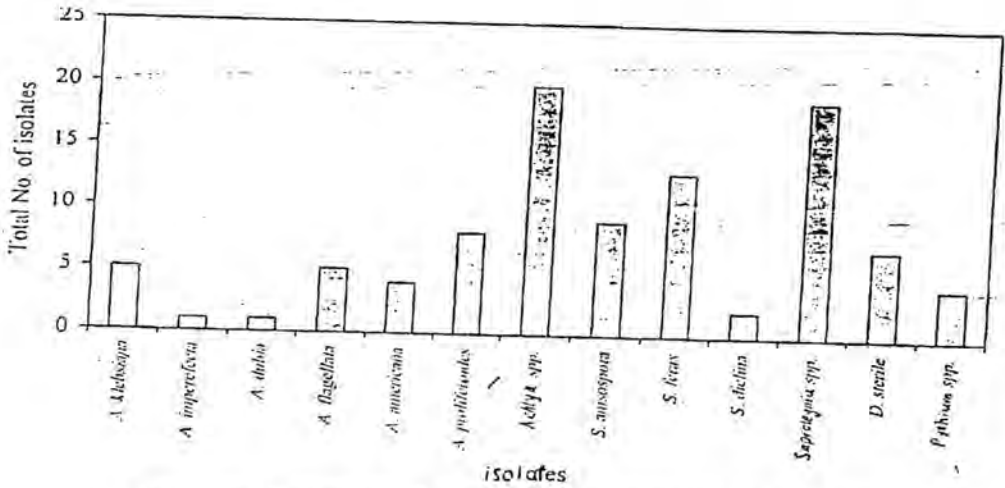
** معنوي عند مستوى $P < 0.01$

جدول (4) : اختبار L.S.D لمقارنة الخصائص البيئية المقاسة بين محطات الدراسة

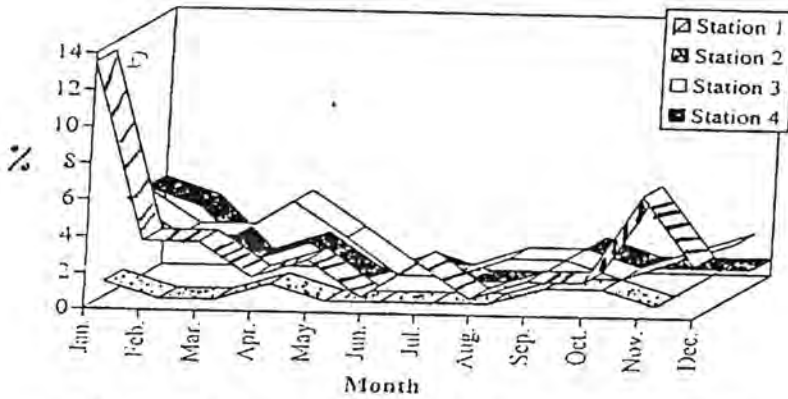
محطة الرستمية		نهر ديالى*		المحطة
(4)	(3)	(2)	(1)	الخاصية
جـ 2	ب 5.7	ب 6.5	أ 7.1	الأوكسجين المذاب
جـ 10.8	ب 2.2	أب 1.9	أ 1.5	الطلب الكيميائي للأوكسجين
جـ 150.4	ب 104	أب 103.6	أ 97.2	الكالسيوم
ب 97.9	أ 71.8	أ 71.4	أ 67.9	المغنيسيوم
ب 13.8	أ 4	أ 3.5	أ 2.9	البوتاسيوم
جـ 253.6	ب 193.7	ب 193.7	أ 178.6	الصوديوم
جـ 10.5	ب 0.6	أ 0.5	أ 0.3	الفوسفات
جـ 15.9	ب 1.6	أ 1.0	أ 0.6	الأمونيوم
ب 11.3	أ 6.5	أ 4.8	أ 5	النترات
ب 295	أ 198	أ 182	أ 186	الكلوريدات
ب 461	أ 357	أ 340	أ 354	الكبريتات

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المحطات التي تحمل أحرف متماثلة لأبوجد بينها فرق معنوي.



شكل (2) تواجد الأنواع الفطرية في المحطات قيد الدراسة وعلى مدار السنة
(كانون ثاني- كانون أول، 1998)



شكل (3) النسبة المئوية لتواجد لفطريات المائية المعزولة من نهر ديالى
(محطة 1 و 2 و 3) ومحطة الرستمية (4)

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دراسة تجريبية لسرعة انتشار البلازما كدالة لشدة الشعاع الليزري وضغط الاستئصال

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الخلاصة

لقد تمت في هذا البحث دراسة تجريبية لسرعة انتشار البلازما v_p المنتجة بليزر النيادميوم - زجاج كدالة لشدة الشعاع I والقيمة العظمى لضغط الاستئصال P_{Amax} للمعادن (Cu, Al, Zn) وذلك باستخدام كاشف PVDF. فوجد ان سرعة الانتشار تتناسب طردياً مع كل من شدة الشعاع وضغط الاستئصال، وتأكيداً اعتمادها على طبيعة المعدن، ووفقاً للمعادلات التجريبية الآتية: $V_p = AI$ ، $V_p = B P_{Amax}$. لقد تم استنتاج قيم الثوابت A و B لكل معدن باستخدام طريقة ترهيم المربع الاصغر .Least Squar Fit

المقدمة

تعرف البلازما بانها غاز شبه متعادل ذو جسيمات متعادلة واخرى مشحونة وتظهر سلوكاً جماعياً يعتمد على درجة حرارتها وكثافتها الالكترونية. وتقسّم البلازما من حيث درجة حرارتها الى صنفين :- بلازما حارة (درجة حرارتها $100000 K^0$)، بلازما باردة (درجة حرارتها $10000 K^0$). لقد اثبتت التجارب والدراسات النظرية اعتماد كل من سرعة انتشار البلازما وضغط الاستئصال على طبيعة المعدن وشدة الشعاع الليزري، وان معظم هذه الدراسات اثبتت الاستئصال على طبيعة المعدن وشدة الشعاع الليزري، وان معظم هذه الدراسات اثبتت التناسب الطردي بين كل من سرعة انتشار البلازما v_p وضغط الاستئصال P_{Amax} وشدة الشعاع الليزري I (11,10,9,8,7,6,5,4,3,2,1). فعند تسليط الشعاع الليزري على المعدن فان طاقته ستقتسم ما بين طاقة لصهر المعدن وتحرير الايونات و طاقة حركية لهذه الايونات المحررة فعليه يبدو واضحاً اعتماد سرعة انتشار البلازما (والتي تعتبر مقياس لدرجة حرارة البلازما) على شدة الشعاع الليزري وعلى طبيعة المعدن المشع.

ومن المحتم وجود علاقة، اعتماداً على طبيعة المعدن المشع وعلى شدة الشعاع الليزري، ما بين سرعة انتشار البلازما وضغط الاستئصال للمعدن فمن المعروف ان الضغط ووفقاً لقانون نيوتن الثاني يعرف على انه القوة لوحدة المساحة وقانوناً :-

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$$P = \frac{F}{A}$$

والقوة تعرف على انها معدل التغير الزمني للزخم عندها سيساوي الضغط الى :-

$$P = \frac{Mv}{A\Delta t}$$

طريقة العمل

لقد تم في هذا البحث استخدام الكاشف من نوع PVDF لقياس ضغط الاستئصال للهدف المثبت عليه. لقد استخدمت عدسة لامة ذات بعد بؤري 15 cm لتجميع اشعة الليزر ذو الطول الموجي 1.06 μm وبعد اسقاط الشعاع على الهدف ونتاج البلازما سيقوم كاشف الاجهاد الكهربائي بتحويل الفعل الميكانيكي الى اشارة جهد كهربائي وتنقل هذه الاشارة عن طريق سلك الى راسمة ذبذبات نوع Iwatsu Electric ومنها استطعنا حساب قيمة الضغط المسلط في اية لحظة وذلك بتطبيق المعادلة :-

$$P_{\Lambda\text{max}} = V (C_D + C_L) / A dt$$

V تمثل الفولتية المسجلة والناجمة عن تحويل الفعل الميكانيكي الى نبضة جهد، A مساحة بقعة الليزر و C_D تمثل سعة الكاشف ومساوية الى 2.7 nf و C_L تمثل سعة الحمل (وحيث $C_D \gg C_L$)، اما dt فيمثل ثابت الاجهاد ومساوي الى $22 \times 10^{-12} \left(\frac{P_{\text{column}}}{N} \right)$.

ولغرض قياس سرعة انتشار البلازما المنتجة فقد تم استعمال كاشفين من نوع PVDF، ووضع احدهما شاقولياً خلف الهدف وملاصقاً له لقياس $P_{\Lambda\text{max}}$ وفقاً لما تم شرحه اعلاه، اما الثاني فالغرض منه قياس سرعة انتشار البلازما v_p مما استوجب وضعه بنفس جهة جهاز الليزر كي سيتقبل البلازما المنتجة وصانعاً زاوية 45° مع الاول كي لايعيق الشعاع الليزري وكانت المسافة بين مركز الكاشف الثاني ومركز الهدف مساوية الى 1cm. فعند اسقاط الشعاع الليزري وبشدة معينة على الهدف سيسجل كل منهما نبضة ضغطية ولكن بفارق زمني t_d وبقسمة المسافة بين الهدف والكاشف الثاني على الفارق الزمني نحصل على سرعة انتشار البلازما في الهواء. وبتغير شدة الشعاع الليزري الساقط على الهدف يتغير الفارق الزمني t بين نبضتي الكاشفين مما يدل على تغير سرعة انتشار البلازما. وبتحويل نبضة الكاشف الاول الى نبضة ضغط، وفقاً لما شرح اعلاه سيكون بالامكان رسم سرعة انتشار البلازما كدالة لشدة الشعاع مرة وكدالة للقيمة العظمى لضغط الاستئصال مرة اخرى.

النتائج والمناقشة

لقد تمت دراسة سرعة انتشار البلازما v_p مع تغير شدة الشعاع الليزر وذلك باسقاط شعاع ليزر نياديوميوم - زجاج ($\lambda = 1.06 \mu\text{m}$) على ثلاثة معادن هي $(0.39\text{mm})\text{Zn}$ ، $(0.31\text{mm})\text{Al}$ ، $(0.08\text{mm})\text{Cu}$ في الهواء وبشدة تراوحت قيمها بين $(1.05 \times 10^9 - 1.47 \times 10^{10} \text{ W/cm}^2)$ وتم حساب سرعة انتشار البلازما والقيمة العظمى لضغط الاستئصال في كل حالة ودونت النتائج في الجدول (1).

الجدول (1) ضغط الاستئصال P_{Amax} وسرعة انتشار البلازما v_p الناتجة من تشعيع الخارصين

والألومنيوم والنحاس بشعاع ليزر نياديوميوم - زجاج ولشدة مختلفة

شدة الشعاع الليزري W/cm^2	معدن الخارصين		معدن الألومنيوم		معدن النحاس	
	P_{Amax} (bar)	v_p cm/sec	P_{Amax} (bar)	v_p cm/sec	P_{Amax} (bar)	v_p cm/sec
10.5×10^9	95	2.5×10^{-4}	75.77	0.58×10^4	80.72	0.998×10^4
5.5×10^9	520	12.5×10^4	437.64	3.33×10^4	425.8	5.2×10^4
9.5×10^9	833.16	20×10^4	862.34	6.6×10^4	808	10×10^4
1.1×10^{10}	853.16	21×10^4	998.49	7.64×10^4	935.57	11.57×10^4
1.37×10^{10}	1201.5	28.8×10^4	1050.77	8.33×10^4	1257.2	16×10^4
1.47×10^{10}	1319.98	35×10^4	---	---	---	---

يوضح الشكلان (1) و (2) سرعة انتشار البلازما كدالة لكل من شدة الشعاع الليزري I والقيمة

العظمى لضغط الاستئصال P_{Amax} على التوالي. ومن الشكل (1) نلاحظ ان سرعة انتشار البلازما تزداد

خطياً بزيادة الشدة. وان معدل الزيادة يعتمد على طبيعة الهدف. وقد تم استنتاج العلاقات التجريبية الآتية

$$v_p = 2.145 \times 10^{-5} I \quad \dots\dots\dots \text{For Zn}$$

$$v_p = 0.583 \times 10^{-5} I \quad \dots\dots\dots \text{For Al}$$

$$v_p = 1 \times 10^{-5} I \quad \dots\dots\dots \text{For Cu}$$

وبحكم العلاقة الخطية بين القيمة العظمى لضغط الاستئصال وشدة الشعاع الليزري⁽¹²⁾ نجد ان

العلاقة الخطية بين v_p و I قد انعكست على العلاقة بين v_p والقيمة العظمى لضغط الاستئصال P_{Amax}

والمتمثلة متحنياته بالعلاقات الآتية :-

$$v_p = 250 P_{Amax} \quad \dots\dots\dots \text{For Zn}$$

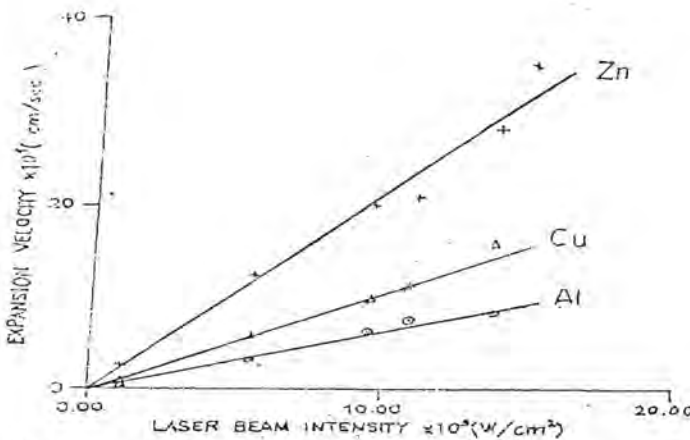
$$v_p = 76.4 P_{Amax} \quad \dots\dots\dots \text{For Al}$$

$$v_p = 125 P_{Amax} \quad \dots\dots\dots \text{For Cu}$$

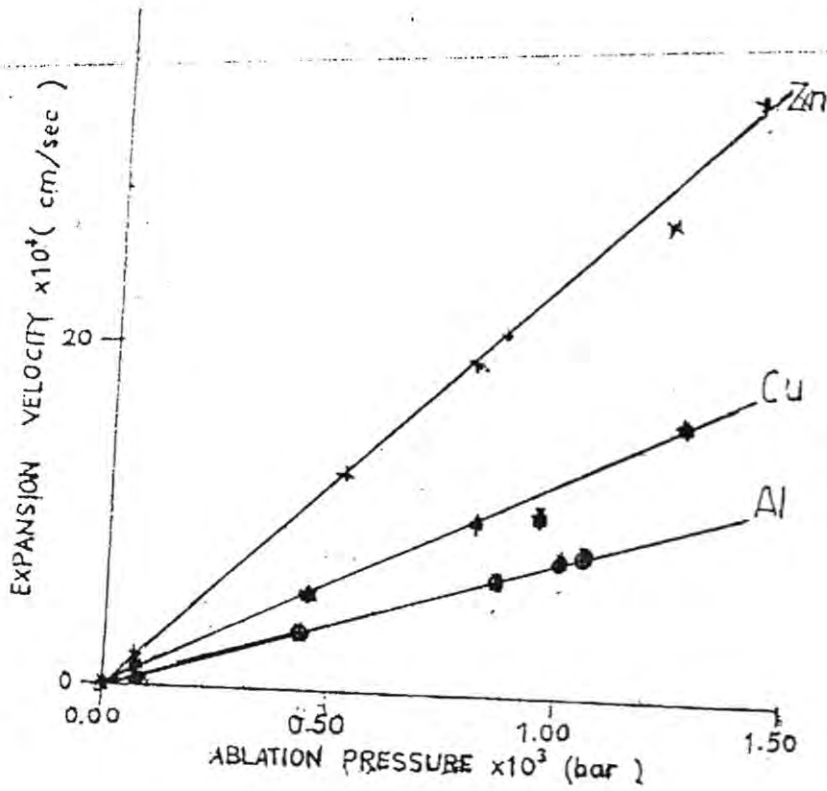
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لقد فسر اختلاف ميل المنحنيات في الاشكال (1) و (2) بكونه يعزى الى اختلاف طبيعة الاهداف المعدنية نتيجة اختلاف التوصيلية الحرارية لكل منها، حيث ان الخارصين ذو التوصيلية القليلة ($1.16W/cm.K$) يملك ميلاً لمنحنيه اعلى من ميل منحني الالمنيوم ذو التوصيلية ($2.37W/cm.K$). وهذا يدل على انه عندما يكون الهدف المعدني ذو توصيلية واطنة فان معظم طاقة الشعاع ستجتمع عند سقوطها على الهدف مما تعمل على صهره وتبخيره ورفس جزيئات ذلك الهدف وقذفها بزخم عالي مما يعني ان سرعتها عالية في حين ان الهدف ذو التوصيلية العالية فان جزء كبير من طاقة الشعاع الليزري ستبتدد بالتوصيل الحراري داخل الهدف والجزء المتبقي سيعمل على صهر وتبخير الهدف وبالتالي ستكون الطاقة الحركية للجزيئات المتطايرة قليلة مما يعني سرعتها قليلة.

ومن الضروري ان نذكر ان السمك في هذه الدراسة كان (0.08 mm) النحاس بينما سمك الالمنيوم (0.31 mm) وهذا يجعل ميل منحني الهدف النحاسي اكبر من ميل منحني الالمنيوم بالرغم من توصيلية الاول اكبر من توصيلية الثاني طبقاً لتأثير السمك على ضغط الاستئصال حيث ان السمك العالي يعمل على جعل الموجة الصدمية تقطع مسافة اكبر وتبعاً لذلك سيقل تأثيرها مع الكاشف مما يؤدي ذلك الى نقصان ضغط الاستئصال وبالتالي سرعة الجزيئات المقذوفة وتتفق هذه النتائج مع نتائج Grun وجماعته⁽⁷⁾. لقد اضطررت لاستعمال هذه الاهداف المختلفة بسمكها لعدم تمكني من العثور على اهداف متساوية السمك.



شكل (1) سرعة انتشار البلازما في الهواء للمعادن (Zn, Al, Cu) كدالة لشدة الشعاع الليزري.



شكل (2) سرعة انتشار البلازما في الهواء للمعادن (Zn, Al, Cu) كدالة للقيمة العظمى لضغط الاستئصال .

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