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IJST Contact Information:
P.O. Box 2793 Amman 11953 Jordan
Tel. +962796543469
E-mails: info@ijst-jo.com / ijst.jordan@yahoo.com
URL: www.ijst-jo.com

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FORWARD

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Today, we announce a new issue of our journal, that is the first issue from the fourteen volume of IJST, March, 2019.

Finally, I hope that all significant figures of sciences whom joined the editorial board, the researchers, and the readers of our journal will keep IJST between their eyes and contribute in continuing its journey, with their remarks, valuable recommendations and their researching outcomes.

Thanks a lot for all who support IJST.

Editor-in-Chief

IJST

Abdul Jabbar Al- Shammari

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Dr. Moammar Hashim Al-Rawi
Consultant Pediatric Dentist, Qatar Armed Forces. Qatar.

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Effect of some physical factors as mutants on *Proteus* isolated from various medical cases

Shaimaa Mohammed Taher Mehdi Al-Tai and Najwa Ibrahim Khalil

Dept. of Biology/ College of Education for Pure Sciences/ University of Mosul
Republic of Iraq

E- mail: iraqi.quest@gmail.com

ABSTRACT

Three isolates of *Proteus vulgaris* and 13 isolates of *Proteus mirabilis* were used for each isolating species. The use of different temperatures and ultraviolet radiation as physical factors mutant, resulted in a breakthrough in Proteus bacteria in terms of increasing their resistance at the expense of low sensitivity to antibiotics. ($P \leq 0.05$) was the most resistant to *P.mirabilis* when UV was used, with an increase of about 16% compared to the comparison sample (50%). These results were consistent with the results of the T-test in SPSS ver. 15 at a probability level ($P \leq 0.05$).

Keywords: *Proteus*, physical factors, antibiotics, statistical analysis

INTRODUCTION

Proteus was first discovered by Hauser in 1885 (1), by which he called twinkling, due to its feature of fleomorphism. Proteus is a species of streptococcus bacteria, moving by surrounded whips called peritrichous flagella and forms swarming that are appeared as dense growth rings around the pollination point and cover the solid growth media (2). The genus *Proteus* belongs to the intestinal family *Enterobacteriaceae*, which colonizes the human intestine as part of the natural flora with coloniform coliform bacteria, and is considered as a pathogen commonly associated with hospital infection (3). Researchers have become increasingly interested in this species because of the important virulent factors that increase its ability to cause disease, and because it is opportunistic bacteria, so it causes a lot of illnesses when they are not living in their natural habitat (4). *Proteus* can be divided into five species. *P. vulgaris* and *P.mirabilis* are among the most common in clinical cases, causing wounds and burns infections, infections of the gastrointestinal tract, urine, respiratory and septicemia (5,6), and these species can oftenly infect individuals with weak immune action if transmitted (7).

Genetically, *Proteus* contains a single chromosome with DNA molecules with a ring structure known as plasmids carrying donor genes for the phenotypic pattern of certain traits. This in turn justifies its high resistance to various antibiotics, including resistance of *P.mirabiles* against both Gentamycin and Tetracyclin antibiotics (8), in addition of its resistance to β.lactam antibiotics by possessing β-lactamase enzymes that break down the betalactam ring and inhibit the action of the antibiotic Amoxicillin (9).

The study of the physiological principles of the environmental conditions of bacteria is one of the reasons for the adaptation process in which organisms become more fit to live in their habitat and improve their ability to maintain their lives and survival of their species. A striking example of this adaptation is the occurrence of genetic mutations, in genetic information encoded by DNA sequences as a result of a change in the sequence and number of nucleotides by the deletion or insertion of one or more nitrogen bases (10), which results in a change in gene expression and then a change in the appearance morphologies (11), because the apparent growth of any organism comes from its genetic pattern and how it is affected by different environmental conditions in which it lives. Different phenotypes are due to their different genotypes (12). Mutations may occur but rarely, in natural manner because of mechanisms to repair transcription errors in DNA. They may also occur artificially when exposed to so-called irritants such as radiation, high temperatures, hazardous chemicals and some medical agents. The vast majority of these mutations are neutral and do not lead to noticeable changes. However, mutations that alter the proteins produced by gene coding will often be harmful because in one aspect bacteria can resist antibiotics (13). The potential for mutations at the molecular level may vary, which means that some mutations are more common and more likely to be occurred. For example, function loss mutations are much more likely than mutations that produce new genes with full function (14).

From the other hand, genetic mutations that make bacteria resistant to drugs, especially antibiotics had become a source of concern for researchers and physicians due to their inability to produce new antibiotics to fight a new mutation emerges, because those mutations can reduce the strength of antibiotics against them (15). New mutations within the bacteria that promote the development of their resistance to high-level antibiotics as in *Proteus* bacteria that have become more resistant to antibiotics by transmission of resistance genes to different genetic mechanisms or perhaps by chromosomal mutations (16).

Thus, the present study attempted to perform the following goals:

1. Isolation and diagnosis of different types of bacteria from different cases and determine their percentages.
2. Isolation and diagnosis of two strains of *Proteus* bacteria: *P. vulgaris* and *P. mirabilis,* to determine the extent of their resistance or sensitivity to ten selected specific antibiotics.
3. Use of temperature and ultraviolet radiation as mutant physiological factors for these strains.
4. Investigate the effect of these factors on the resistance of the two selected strains of *Proteus* to antibiotics, determine the most effective against them, and compare them with the effectiveness of antibiotics selected for this study.
5. The application of statistical analysis to interpret the results more accurately and to indicate which mutant had the greatest impact on the resistance of *Proteus* bacteria to antibiotics.

MATERIALS AND METHODS

Under complete sterilization conditions, *Proteus vulgaris, Proteus mirabilis, Escherichia coli, Pseudomonas aeuroginosa, Staphylococcus aureus* were cultivated on the Nutrient Agar medium, and in rich, selective cultures such as Blood Agar, MacConkey Agar, and Mannitol Salt Agar by using streaking method for single colonies. All Petri dishes were incubated at 37 ° C for 24 hrs. All pure colonies were kept on the feeders in refrigerator and were renewed monthly.

Identification:

The cultivated samples were studied in terms of size, shape, color and height of the developing bacterial colonies, as well as their abilities to decompose blood in Blood Agar, form the

swarming phenomenon and its fermentation of lactose glutamate. Thin swabs of pure colonies and chromatin dyes were obtained and dyed with gram dye to observe the shape of cells and their susceptibility to the dye. Then, they were taken and examined with a light microscope using a 10X micro-power lens and a 100X power lens.

Biochemical tests:

Biochemical tests were carried out on isolated bacteria from various pathogens according to methods described by different references (17-25).

1- IMViC test group: which included the following tests:
1-1 Indol Test.
1-2 Mythyl Red Test.
1-3 Voges Proskaure Test.
1-4 Citrate Utilization Test.
2- Cytochrome Oxidase effectiveness test.
3- Catalase Test.
4- Gelatin Liquefaction Test.
5- Urease Test.
6- Motility Test.
7- Phenylalanine Deaminase Test.
8- Different Sugar Fermentation Test.

Anti-microbial Sensitivity Testing:

Baur *et al* method (26) was followed to test the sensitivity of *Proteus* bacteria, both *P. vulgaris* and *P.mirabilis*, against 10 types of antibiotics (Table 1) by transferring (3-4) young colonies of these bacteria into a sterilized test tube (5) cm^3 from Nutrient broth, and then placed in the incubator at 37 ° C for 24 hrs. The bacterial residue were precipitated using the centrifuge at 100 cycle /min for 3 minutes, then diluted by normal saline with concentration (0.9%) and compared with the standard control tube (McFarland), which is equivalent to 10^8 cells / cm^3 (20). An amount of (0.1) cm^3 was spread on the center of the Muller-Hinton Agar in sterile petri-dishes, using sterile swab to cover the entire surface of the petri-dish, and then left for 10-30 minutes for the purpose of ensuring sinking and dry surface. Then, discs were distributed among petri-dishes by 5 discs in each petri-dish, with pressure applied to each disc gently to ensure adhesion of the disc to the media surface. Then incubated at 37 ° C for 24 hrs. Results were obtained the day after by measuring the diameter of the inhibition zone in millimeter unit around the antibiotic disc based on what is used in the laboratory of public health that based on the World Health Organization tests (27).

Table (1): Types and concentrations of antibiotics processed by the Turkish company Bioanalyse

Antibiotics	Code	Concentration (mg/ disc)
Ciprofloxacin	CIP	10
Gentamycin	GN	10
Penicillin	P	10
Ampicillin	AMC	30
Trimethoprim	TMP	10
Erythromycin	E	15
Vancomycin	VA	10
Tetracycline	TE	10
Streptomycin	S	25
Nalidixic acid	NA	30

Mutants' physical factors on the growth of *Proteus*:

1-The effect of different temperatures: Test tubes containing 5 cm^3 of Nutrient broth were vaccinated by *Proteus* isolates, *P.Vulgaris* and *P.Mirabilis* and then incubated at three different temperature degrees (4, 28, 43 ° C) for 24 hrs., where the disturbance of the nutrient medium indicated the growth of bacteria, which was measured using the Spectrophotometer at the wavelength of 590 nm and compared with the control sample (the vaccinated and incubated medium at 37 ° C not treated with different degrees of temperature (24, 28).

2- Ultra Violet (UV) effect: This experiment was conducted using Honbarrier's method (29) by preparing a bacterial suspension from *P. vulgaris* and *P. mirabilis* separately, after incubation for 24 hrs. at temperature of (37) ° C. An amount of 10 cm^3 suspensions were transferred by sterilized pipettes into sterilized and empty petri-dishes, and under complete sterilization conditions, petri-dishes were opened and subjected to UV light at wavelength 254 nanometer for three periods (30,45, 60 seconds). Then, petri-dishes were covered by foil layers and kept in dark conditions for 2 hrs. Amount of 0.5 cm^3 were taken from each petri-dish and transferred into vials containing 5 cm^3 nutrient media, then incubated at temperature 37 ° C for 24 hrs. The occurrence of growth was detected by measuring the disturbance of nutrient media using Spectrophotometer at wavelength 590 nanometer, where then compared with control group (not being treated by UV light).

Statistical Analysis:

Data were statistically analyzed using T-test in SPSS ver. 15 at P <0.05 level to determine if any significant differences between the mean diameter of the *Proteus vulgaris* and *Proteus mirabillis* protease inhibitors and the effect of both temperature and ultraviolet radiation as mutagenic factors.

RESULTS AND DISCUSSION

A total of 200 samples were collected from different patients attended the Republican Hospital and Ibn-Sina Medical Hospital in Mosul city/ Iraq between July and December 2013, taking into account patients were not using antibiotics before three days from taking samples.

Isolation and diagnosis of bacterial groups:

Bacterial species were initially identified based on the characteristics of the growing colonies on the selective cultivating media and in the form of bacterial cells and their susceptibility to gram dye.
It was found that some of these isolates belonged to the *Proteus* genus and their growing colonies appeared on the MacConkey agar in single colonies of pale colors with a medium-sized smooth bristle not fermented with lactose sugar as well as the smell of spoiled fish, while their growth was characterized by nutrient agar and blood agar in the swarming movement (Figure 1).

Figure (1): Swarming phenomena for *Proteus*

It was difficult to identify individual colonies because of their creeping spread covering the surface of the cultivating medium with a homogeneous layer of growth. This phenomena is unique to *Proteus* species from other of the family genotypes *Enterobacteriaceae* (30), while the results of microscopic examination of these colonies appeared them as short bacilli or long bacilli in the tissues prepared from young and Gram negative cultures. *Proteus* isolates were assured according to the results of the biochemical tests described in table (2) below:

Table (2): Results of Biochemical tests for *Proteus* diagnosis

Biochemical test	*P.mirabilis*	*P.vulgaris*
Gram stain	-	-
IMViC	-++-	+++-
Oxidase	-	-
Catalase	+	+
Urease	+	+
Gelatinase	+	+
Phenylelenin deaminase	+	+
H_2S	+	+
Motility	+	+
Glucose	+	-
Lactose	-	+
Arabinose	+	+
Mannitol	-	-

(-) negative results , (+) positive results

Sensitivity of *Proteus* to antibiotics:

Sensitivity tests for Proteus species were performed to antibiotics. Results revealed that both *Proteus vulgaris* and *Proteus mirabillis* were found to be sensitive to a group of antibiotics including CIP, GN, TMP, VA and S, but clearly showed resistance to antibiotics P, AMC, E, TE, and NA, with 50% for each species.

Several studies have indicated that Ciprofloxacin (CIP) is a broad spectrum antibiotic that is part of the fluoroquinolone group and is effective against negative bacteria (including the Gram-negative intestinal bacteria, *P.mirabilis*) and Gram positive, in addition to inhibition of enzymes DNA gyrase, Type II topoisomerase, and Topoisomerase IV, which are needed to separate DNA strips during replication and thereby inhibit bacterial cell division (31,32).

These results are in line with what Saito *et al.* (33) pointed out that these two species of bacteria are sensitive to (CIP) antibiotic, as this antibiotic reduces the composition of biofilm, which protects bacteria against external factors, including the effects of antibiotics (34). Gentamycin (GN) was described as a bactericidal agent, belonged to aminoglycosides that affect the process of protein synthesis in Gram-ve bacteria, which is often due to the presence of plasmids that are encoded into proteins specialized in the modified enzymes of the lymphocyte (35). Trimethiprim (TMP) is a broad spectrum antibiotic used to treat intestinal bacteria, including *P.mirabilis*, when infects urinary tract by preventing bacteria from producing folic acid, without which bacteria cannot produce DNA and thus become unable to multiply (36,37).

Vancomycin (VA) inhibits transpeptidation by binding to D-alanyl-D-alanine peptidoglycan, the main component of bacterial cell wall (2000).

Streptomycin (S) is associated with S30 subunits of ribosomes and the formation of ribosomal-mRNA, which is unstable, leading to a genetic mutation that result in protein synthesis and cell death (39).

The results of the current study showed resistance of *Proteus* to Penicillin (P) and Ampicillin (AMP), which belong to beta-lactam blockers, and were in agreement with what Kumar et al. (40) reported that the cause of resistance is mainly due to the possession of *Proteus* isolated from the case of urinary tract infection of the enzyme β-lactamase.

Erythromycin (E) is a broad spectrum antibiotic and is one of the Macrolides antagonist group. The resistance of *Proteus* bacteria to this antibiotic is due to increasing its infusion extracellularly, as well as by the action of methylylase enzymes with restriction of more than 40 registered genes (41).

Furthermore, the chromosomal mutation of *Proteus* prevents the binding of the antibiotic to the target site of bacteria (235 rRNA) (42), while the bacteria resistance mechanism of tetracycline (TE), which is widely used because it is a broad-spectrum and low-toxic antibiotic depends on two basics, firstly, increase the extracellular pump, where the resistance genes of the plasmid are found, and the second is to encode proteins from special chromosomal genes that protect the ribosomes from their association with this antibiotic (43). Chopra *et al* (44) revealed an occurrence of mutation in the *Proteus* bacteria that in turn had led to Tetracycline resistance by the horizontal technique of transferring genes. Nalidixic acid (NA) is used to treat urinary tract infection caused by *E. coli*, *Proteus, Shigella* and *Klebsiella*, which is due to the group of Quinolones, the bacteria resist by reducing the permeability of the cell membrane to prevent its accumulation inside the bacterial cell or increase the outflow (45).

Effect of physical factors as mutagenic agents on the growth of *Proteus* species:

Treatments of *Proteus* species with physical mutations, namely heat and UV, have had significant effects on the growth of these bacteria. Different temperatures had different effects on the growth of *P. vulgaris* and *P.mirabilis* bacteria, as shown in figure (2). It was found that the temperature of 28 ° C had more stimulating effect than the temperature of 43 ° C in the growth of the two isolates with a percentage of 54.5 and 39.7% respectively, while the temperature of 4 ° C had a significant inhibitory effect in the growth of the two isolates compared to the control sample. Xia (46) found that 25 ° C was a suitable temperature for the growth of *P. vulgaris* and towards a marked increase in its growth rate, although the optimum degree of growth was between 37-40 ° C (47).

The treatment of the two isolates of *Proteus* with ultraviolet (UV) at 254 nm and for different time periods of 30, 45 and 60 seconds showed marked increase in the growth of these bacteria with increasing exposure time and minor differences between the two species, as shown in figure (3), with percentages of 67.7, 80.9, 83.6, 58.2, 75.2 and 77.9% for *P. vulgaris* and *P.mirabilis* respectively compared with the control sample. Although Witkin (48) had resulted that UB rays could induct formation of lethal mutations of the bacteria, but it depends on the wavelength absorbed by the bacteria. Peak *et al* (49) found that wavelengths between 234-320 nm could leave bacterial cell alive without any lethal effect on its growth. In addition, Lin and Wang (50) had revealed that bacterial cell stayed alive in 12 strains of *E. coli* when exposed to UV radiation at a wavelength of 254 nanometers, the wavelength used in this study.

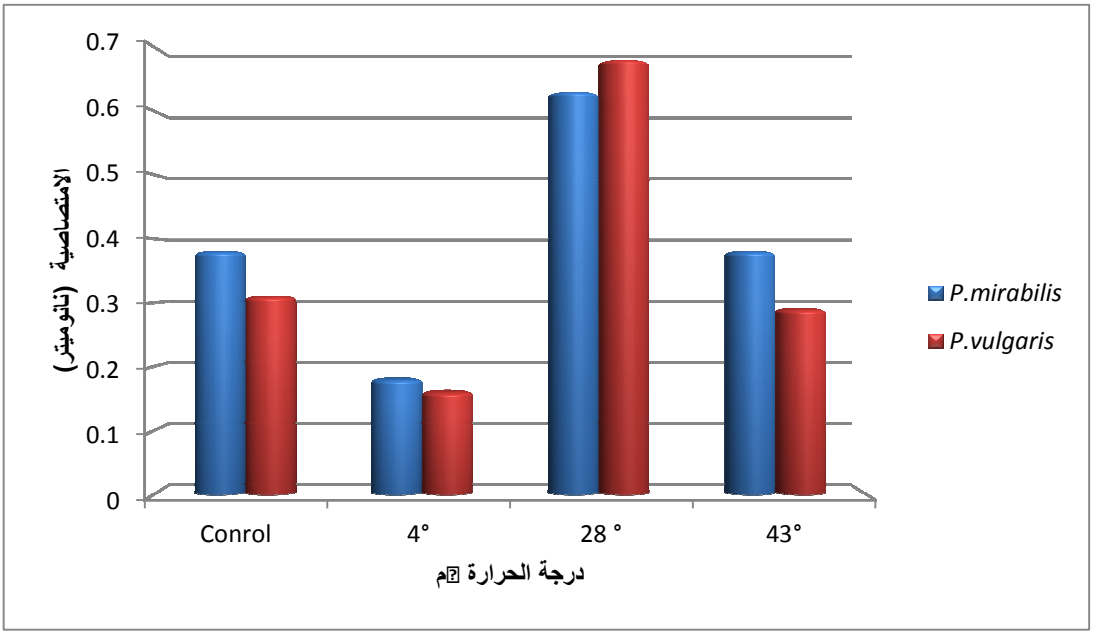

Figure (2): Effect of temperature on the growth of *P.vulgaris* and *P.mirabilis*

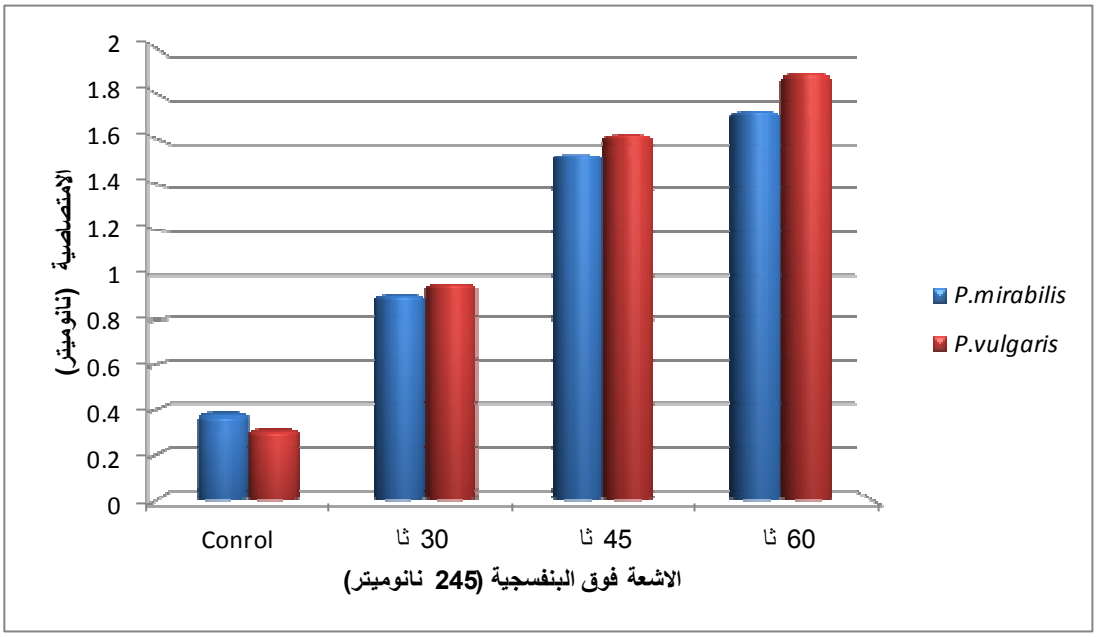

Figure (3): Effect of UV light on the growth of *P.vulgaris* and *P.mirabilis*

Effect of physical factors as mutagenic agents on the sensitivity of *Proteus* bacteria to antibiotics:

Tables (3 and 4) show the effect of temperature on the occurrence of mutation in *Proteus* species in terms of antibiotic sensitivity test. While Proteus both species retained their sensitivity to CIP and S antibiotics in addition to VA antibiotic in *P.mirabilis* and resistance to four antibiotics P, AMC, TE, NA, compared with the control sample (before treatment by heat), there is a mutation in *P. vulgaris,* which had shifted from the sensitive form to the resistor of TMP and VA to resistance to antibiotic E. however, *P. mirabilis* has also got a change in its response to antibiotics after they were mutated at different temperatures, from the sensitive form to the resistor at 4 and 28 ° C, but returned to the sensitivity again at 43 ° C, both GN and TMP resistors from the total antibiotic used. Figure (4) shows Proteus sensitivity for antibiotics after treatment at different temperatures.

Table (3): Effect of different temperatures on the sensitivity and resistance of *P. vulgaris* bacteria to various antibiotics

Antibiotic	Control sample	Inhibition zone diameter (mm)		
		Temperature degrees (°C)		
		4	28	43
CIP	S	S	S	S
GN	S	S	R	M
P	R	R	R	R
AMC	R	R	R	R
TMP	S	R	R	R
E	R	S	S	S
VA	S	R	R	R
TE	R	R	R	R
S	S	S	S	S
NA	R	R	R	R

S = Sensitive, R= Resistance, M= Mean sensitivity

Table (4): Effect of different temperatures on the sensitivity and resistance of *P. mirabilis* bacteria to various antibiotics

Antibiotic	Control sample	Inhibition zone diameter (mm)		
		Temperature degrees (°C)		
		4	28	43
CIP	S	S	S	S
GN	S	R	R	S
P	R	R	R	R
AMC	R	R	R	R
TMP	S	S	R	S
E	R	R	S	R
VA	S	S	S	S
TE	R	R	R	R
S	S	S	S	S
NA	R	R	R	R

S = Sensitive, R= Resistance, M= Mean sensitivity

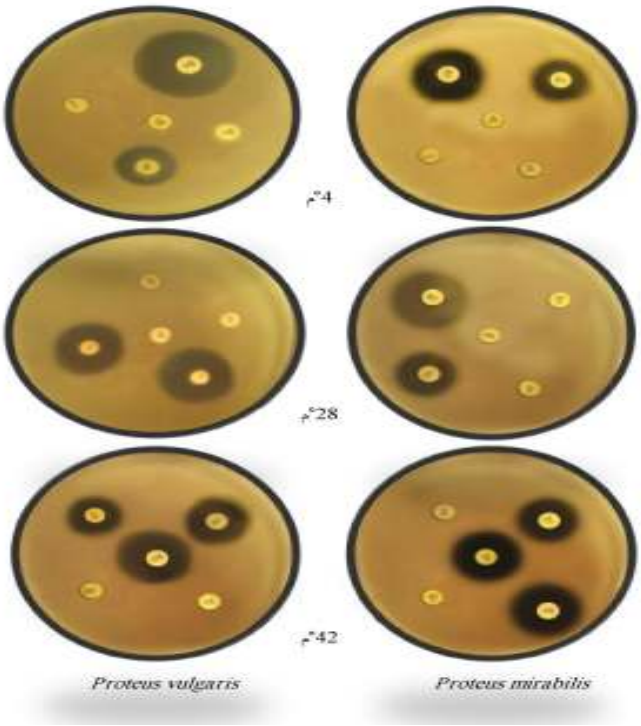

Figure (4): The effect of different temperatures on the sensitivity of *Proteus* species to antibiotics

These results showed that the effect of the mutational effect was to increase the rate of resistance and decrease sensitivity. The total percentages of antibiotic resistance were approximately 56% and 63% and the sensitivity of antibiotics (43 and 36%) in *P.mirabilis* and *P. vulgaris* respectively.

The information mentioned in tables (5 and 6) show that UV radiation had no effect on bacteria behavior towards antibiotics , indicated by maintaining resistance to the four antibiotics P, AMC, TE, NA for both *Proteus* species, and sensitive to the antibodies GN, VA and S in *P. vulgaris* and *P.mirabilis* except for the antibiotic GN, but the mutagenic effect of these rays can be observed on the two types of Proteus bacteria in terms of changing the antibiotic effect on them from sensitivity to resistance, especially for TMP antibiotic as well as for the antibiotic CIP after the exposure of *P. vulgaris* bacteria to UV rays For 60 seconds, it also got a similar emulation J bacteria *P.mirabilis* to antibiotics and CIP GN and TMP. Figure (5) illustrates the sensitivity of these bacteria to antibiotics after treatment with UV light at different periods of time.

Table (5): Effect of UV radiation at different time periods at wavelength 254 nm in sensitivity and resistance of *P. vulgaris* bacteria to antibiotics

Antibiotic	Control sample	Inhibition zone diameter (mm)		
		Periods of time in sec.		
		30	45	60
CIP	S	S	S	R
GN	S	S	S	S
P	R	R	R	R
AMC	R	R	R	R
TMP	S	R	R	R
E	R	S	S	S
VA	S	S	S	S
TE	R	R	R	R
S	S	S	S	S
NA	R	R	R	R

S = Sensitive, R= Resistance, M= Mean sensitivity

Table (6): Effect of UV radiation at different time periods at wavelength 254 nm in sensitivity and resistance of *P.mirabilis* bacteria to antibiotics

Antibiotic	Control sample	Inhibition zone diameter (mm)		
		Periods of time in sec.		
		30	45	60
CIP	S	R	R	R
GN	S	S	R	R
P	R	R	R	R
AMC	R	R	R	R
TMP	S	R	R	R
E	R	S	S	S
VA	S	S	S	S
TE	R	R	R	R
S	S	S	S	S
NA	R	R	R	R

S = Sensitive, R= Resistance, M= Mean sensitivity

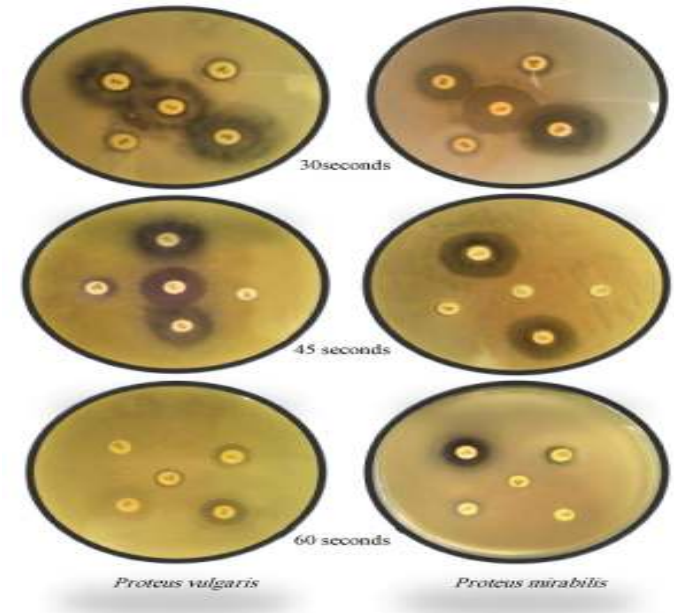

Figure (5): The effect of UV light at different periods of time on the sensitivity of *Proteus* species to antibiotics

The change that had occurred in the influence of antibiotics on bacteria species in comparison of the control sample was due to the effect of UV lights to induce certain mutations in the cellular genome represented by the breakage of the two DNA strips and thus cause default in repair mechanism that in turn had been represented as a damage of the nitrogen bases of DNA due to oxidative stress after irradiation, and this oxidation of the bases do not suffer from the repair of the damage caused during the multiplication and then caused the mutation (51), notifying that the speed rate of DNA molecule repair depends mainly on several factors, including cell life, type, and external environment (49). The structural change caused by mutations in UV-induced plasmid DNA leads to defects in repair mechanism that usually lead to the replacement of Cytocine with Thimine and thus formation of the original DNA sequence in potentially harmful manners (52). Bales *et al* (53) had pointed out the acquisition of K-12 E.coli bacteria of sensitivity to Valine and Rifampin antibiotics after exposure to ultraviolet (UV) radiation, and loss of the gene Rol A, which is responsible for encoding DNA-polymerase. Wang and Lin (50) observed the frequency of UV induced mutations, and the effects of using this radiation at the wavelength of 254 nanometers were detrimental to DNA, especially the genes of Lac Z, Prp B, Omp F, and Amp A on the chromosomes, resistant bacteria to rifampincin and chloramphenicol. It can be said that the percentage of resistance and sensitivity in these bacteria reached about (66, 53%) and (33, 46%) in both *P.mirabilis* and *P. vulgaris* respectively.

Statistical results:

After analysis of data, there were significant differences between the mean diameter of the inhibition zones for the growth of *P. vulgaris* and *P.mirabilis*, for both the temperature at 28 ° and the time period of 45 seconds for the ultraviolet radiation. As for the statistical differences between temperature and ultraviolet radiation, it was revealed that UV light had the more significant effect at $P \leq 0.05$, and this is in consistent with the results obtained, which indicated that the highest resistance in *P.mirabilis* bacteria was when using UV radiation, an increase of about 16% compared to the control sample which was 50%.

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Evaluation the causes of repeating film radiographs in Al-Noor specialized health center of dentistry in Mosul

Atyaf Ghazi Mahmood Al-Dawoodi and Mahdi Mahmood Al-Saleh

Al-Noor Specialist Health Center of Dentistry / Al- Mosul
Republic of Iraq

E –mail: almsafraalwahyd@gmail.com

ABSTRACT

Radiographic examinations are one of the primary diagnostic tools used in dentistry to determine disease states and formulate appropriate treatment. The aim of the current study was to describe the reasons for radiograph rejections through a repeat film analysis. This cross-sectional study performed in Oral Radiology Department in Al-Noor specialized health center of dentistry, Mosul, Iraq, to determine the causes of dental periapical x-ray repetition over a 6 months period. The study was approved by the research ethical and scientific committee of Nineveh health directorate, Iraq. All views were taken by junior dentists, using bisecting angle technique. Radiographs were examined and a faulty radiograph was collected and analyzed. The result was a total of 1675 periapical radiographs were evaluated. Of these, 1260 radiographs (75%) were acceptable; 415 radiographs (25 %) were unacceptable. The most frequent error was filmed misplacement (29.63%), followed by incorrect vertical and horizontal angulation (24.33%), cone cutting (22.16%), while motion blur (10.12 %), processing error (7.22%), and other error (6.5 %). This study concluded that the film misplacement, cone cute, and incorrect angulation were the most frequently occurring error types. Image retake analysis is deemed as a quality indicator and is able to find factors causing retake and useful for designing guidelines to improve the retake rate. The results may be applied to training protocols in both ministries of higher education and ministry of health to reduce public exposure from the source of dental radiographies.

Keywords: repeating film, radiograph, and evaluation.

INTRODUCTION

Radiographic examinations are one of the primary diagnostic tools used in dentistry to determine disease states and formulate appropriate treatment (1). The production of radiographs involves several complex processes (2). Certain errors in the technique or in the radiographic processing, besides being difficult to interpret, lead to a repetition of the radiography (3).
The high incidence of defective X-ray films requires that patients undergo unnecessary and expensive duplicate radiology (4), which was suspected to have a great risk in inducing cancer, even at protracted low-dose exposure (5).
Although dental students had problems in identification of film faults initially, they had the fund of knowledge and competence in the assessment and correction of faults once identified (6).
Reaping of radiographs errors results to waste the time, film and processing solutions as well as to increase the dose to the patient (7). So, the dentist must provide an acceptable radiograph and thereby provide proper clinical services to the patients (8).
The aim of this study was to describe the reasons for radiograph rejections through a repeat film analysis.

MATERIALS AND METHODS

This cross-sectional study performed in Oral Radiology Department in Al-Noor specialized health center of dentistry, Mosul, to determine the causes of dental periapical x-ray repetition over a 6 months period. The study was approved by the research ethical and scientific committee of Nineveh health directorate, Iraq. All views were taken by junior dentists, using bisecting angle technique. The film was a conventional size 2 intra oral films (D-speed, Guangxi YesSar Medical Equipment Co. Ltd, P.R. China) exposed by intra oral X-Ray dental machine (Trophy, France). Radiographs were examined and assessed by seniors of dental x-ray department and faulty radiograph was collected and analyzed.

RESULTS

A total of 1675 periapical radiographs were evaluated. Of these, 1260 radiographs (75%) were acceptable; 415 radiographs (25 %) were unacceptable. The most frequent error was filmed misplacement (29.63%), followed by incorrect vertical and horizontal angulation (24.33%) and cone cutting (22.16%), while motion blur (10.12 %), processing error (7.22%), and other error (6.5 %). The data were entered into the social package of statistical science version 16 for windows. Figure (1) and table (1) show the results of the evaluation. Table (2) shows the faults in repeating film radiograph.

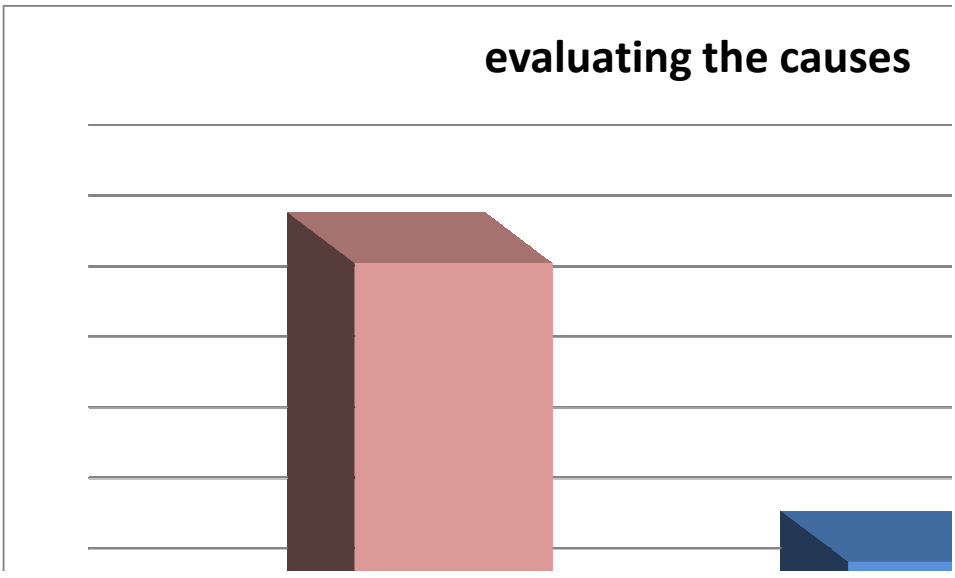

Figure (1): the results of the evaluation –true (accepted radiograph) false (unaccepted radiograph)

Table (1): Category of the evaluation

No.	Case	frequency	%
1	Number Of true	1260	75
2	Number Of Faults(repeat)	415	25
Total Number Of Periapical film Taken		1675	100

Explain the no. of accepted and unaccepted radiographs in relation to total no. of radiographs taken

Table (2): the faults in repeating film radiograph

No.	Types Of Faults	Frequency	%
1	film mas placement	123	29.639
2	incorrect angulation (vertical and horizontal)	101	24.337
3	cone cut	92	22.169
4	motion bluer	42	10.120
5	processing error	30	7.229
6	other	27	6.506
	Sum (total number Of repeated periapical film)	415	100

Explain the frequency and percentage of each fault for total no. of repeated radiographs

DISCUSSION

ALARA (As Low As Reasonably Achievable) holds that exposures to ionizing radiation should be kept as low as reasonably achievable, economic and social factors being taken into account, so that dentists should use every means to reduce unnecessary exposure to their patient and themselves (9).

A principal objective of the quality assurance (QA) program is to ensure the production of good diagnostic quality radiographs, it is vital to monitor image quality on a regular basis. It is recommended that a simple, subjective image quality rating system be used for dental radiographs, as described below:

1- Excellent (No errors of patient preparation, exposure, positioning, processing or film handling).

2- Diagnostically acceptable (Some errors of patient preparation, exposure, positioning, processing or film handling, but which do not detract from the diagnostic utility of the radiograph).

3- Unacceptable (Errors of patient preparation, exposure, positioning, processing, or film handling, which render the radiograph diagnostically unacceptable (15).

A total of 1675 periapical radiographs were evaluated. 1260 radiographs (75%) were acceptable; while 415 radiographs (25 %) with error. A study conducted in India by Elangovan *et al.* show 3538 (26.9 %) were faulty radiograph from total of 13104 intraoral periapical radiographs taken (14).

Our results showed that film misplacement was the most common type of error (29.6%), that is in accordance with the study conducted by Haghnegahdar *et al.* (10), in which improper film placement was reported to be the most frequent error (35.4%). This may be due to the pressure insertion of the patient finger onto the film after placement by operator or gag reflex and unintended resistance of the patient may attribute to misplacement of films.

The frequency of faults of intraoral periapical x-ray because of incorrect vertical and horizontal angulation was 24.33 %. A study was done by Acharya in India reported that the error due to incorrect angulation was 26.1 % (11). Incorrect adjustment of the beam aiming device leading to that error.

The reject film due to cone cut in our study was 22.16 %, while 14.6 % the frequency of repeating radiograph due to cone cut in Zahedan by Masserat *et al.* that come in disagreement with our study (7) Improper adjustment of machine cone to be cover all area of the film which consider the target of exposure causing cone cut.

The study shows the repeating an intraoral periapical radiograph due to motion blur is 10.12% that not agreement with a study carried out in the USA through Alani *et al.* in 2017 on the dental student (0.6 %) (12). If there is any movement of tube head (machine) or patient during exposure time, the result may be motion distortion.

The processing error that causes reject intraoral periapical x-ray was 7.2 % in this study, while study accomplished by Peker *et al.* in 2009 at Gazi university showed that processing error was very low (2.7 %) (13). Lack reading of instruction of constructing company about processing of a film may be causing this error.

This study showed that the other causes of repeating an intraoral periapical radiograph was 6.5 %, that not similar the result of Elangovan *et al.* (2.9 %) (14). Film bending, film aging, finger print, and others may be attributed to that error. The results show that the overall rates were higher than recommended level.

Because of Iraq suffering from hard period during many years ago, that lead to less in training and teaching of dental student, deficiency of dental x-ray equipment and devices, all these affect the knowledge and education of dental students which appeared during clinical training in our dental health center as low image quality.

The little amount of periapical x-ray film present in the department of radiology during the period of study was the most problem.

CONCLUSION

Errors in taking radiographs may increase patient's radiation exposure, and also waste time and money. In this study, film misplacement, cone cute, and incorrect angulation were the most frequently occurring error types. Image retake analysis is deemed as a quality indicator and is able to find factors causing retake and useful for designing guidelines to improve the retake rate. The results may be applied to training protocols in both ministries of higher education and ministry of health to reduce public exposure from the source of dental radiographies.

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Evaluation the quality of multiple radiographic image on one film

Atyaf Ghazi Mahmood Al-Dawoodi and Mahdi Mahmood Al-Saleh

Al-Noor Specialist Health Center of Dentistry / Al- Mosul
Republic of Iraq

E –mail: almsafraalwahyd@gmail.com

ABSTRACT

In a substantial number of cases, a periapical radiograph is used to visualize only one tooth, with a large area of film being unnecessary. In this article we describe technique of a multiple radiographic image on one film. This technique will provide the advantage of change the orientation of film placement, less contamination, time consuming and economic.

Keywords: quality, dental technique, multiple radiograph.

INTRODUCTION

Periapical radiographs ("peri" meaning "around" and apical meaning "apex" or end of tooth root) record images of the outlines, position and mesiodistal extent of the teeth and surrounding tissues (1).
The practicing dentist differs from medical colleagues as he exposes, processes and interprets the radiograph (2).
Intraoral films are packaged with a sheet of lead foil to protect the film from backscatter and secondary irradiation (3).
During the radiographic processing, effluents are generated (developer, fixer, and wash water) posing an environmental threat, as such effluents contain organic and inorganic compounds that are toxic to the environment (4) also presents a potentially serious health risk that warrants attention by industrial hygienists and other occupational health professionals (5).
Radiographers need to be able to deal with advanced technical equipment and be responsive to the continuous technological development within their field (6).
Even with the advent of digital imaging there are a large number of dental offices that use conventional methods to obtain the radiographic images (7).
This technique is useful for patient requiring multiple intraoral periapical radiographs images of x-ray at different angulation on the same site either upper or lower.

TECHNIQUE AND RESULTS

This technique was conducted in Al-Noor specialized health center of dentistry in Mosul, from August 2018 until January 2019. The study was approved by the research ethical and scientific committee of Nineveh health directorate, Iraq. The routine size 2 intraoral periapical film is taken and inserted into prepared (not rigged plastic) billfold, one half of it is covered with the one layer of lead foil which is present in intraoral periapical film packet (Figure 1). Then the billfold film is placed in disposable plastic sleeves and positioned in patient's mouth (in the target area) and exposed as per standard methods. Later the film is removed and rotated so that the exposed site is inserted into film billfold to be covered with lead foil. So that the unexposed site is adjacent to the indicated tooth and a second exposure is tokened at deferent angulation. Following conventional processing, the 2 images taken appeared side by side on the same film (Figure 2). The images are evaluated according to criteria of correct anatomical coverage. In this technique we excluded full mouth edentulous patient, molar region, patient under 12 years, and handicap.

Figure (1): film billfold not fully covered with lead foil

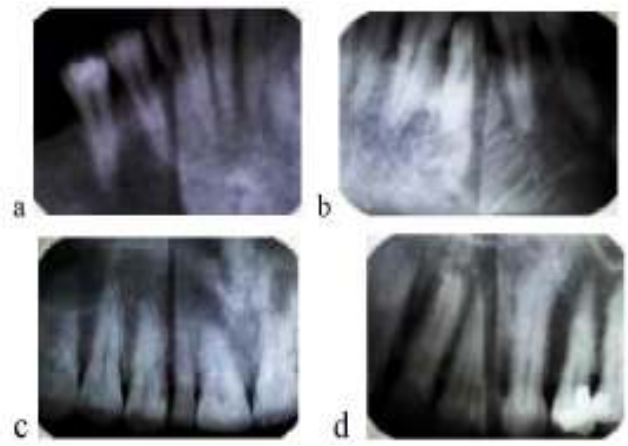

Figure (2): two images taken appeared side by side on the same film. (a), (b) lower centrals in half of film and premolars in second half. (c), (d) upper centrals in half of film and premolars in second half

DISCUSSION

The reasons that encourage us to make this article are that conventional oral radiographic technique is still widely used in our city, in spite of progression in word, also the flexibility of conventional dental film in comparison with rigidity of digital sensor. Conventional oral radiography has never gone out of practice and still plays a pivotal role in dentistry in the era of digitization (8).
A correct execution of the technique is fundamental in order to obtain an adequate radiographic imaging to complement the diagnosis, the planning and the follow-up of the treatments performed (9).
Determination of the adequacy of usual and modified techniques requires application of criteria related to the patient, technician/diagnostician, and technique. Manson-Hing provides eight criteria for choosing a technique. They are: 1. Time expended by personnel, 2. Effort expended by personnel, 3. Radiation dosage patient subjected to, 4. Accuracy of technique, 5. Ability of diagnostician to use the product of the technique, 6. Skill and familiarity of the technician, 7. Patient ability and needs, 8. Available equipment (10).
The choice of sensor size and orientation should be assessed individually and made on the basis of clinical circumstances to give the best image quality

possible rather than by blanket recommendation (11).

In this technique we take two images of two teeth at the same site to be appear on one film which defer from technique of Almeida allows two images of the same endodontically treated tooth (12).

In our technique the images that obtained especially for anterior teeth due to film placement in horizontal instead of vertical orientation come with agreement of correct anatomical coverage (the film should demonstrate all the tooth/teeth of interest (i.e. crown and root[s]), there should be 2-3 mm of periapical bone visible to enable an assessment of apical anatomy) (13). While other criteria of quality stander of periapical radiography including optimal image geometry, Good density and contrast, and Adequate processing usually be maintained.

The length of film prepared area in our technique can occupied the length of target tooth with some exception of cuspid tooth. The study done by Verhoeven et al. in 1979 show that the rang of length of maxillary cuspid tooth was 29.13 mm (men) and 25.75 mm (women), while 28.36 mm (men) and 25.34 mm (women) for mandibular teeth (14). Thus, our technique will provide the facility of imaging anterior teeth by placing the film in a horizontal orientation instead of vertical orientation which is more comfortable for patient because of decreasing the length of the film.

In our technique we need slight bending of the film so V shape or small arch is not recommended, as we need to make excessive bending of film that can cause partial film development. Radiographic noise is the appearance of uneven density of a uniformly exposed radiographic film. The primary causes of noise are radiographic mottle and radiographic artifact. Radiographic artifacts are defects caused by errors in film handling, such as fingerprints or bends in the film (15).

Dentists have a moral and professional responsibility toward the dental as well as general health of patients in their care. This should extend beyond the radiation safety procedures normally adopted within the dental office for specific procedures to a more generalized consideration of the environmental impact of the potentially hazardous waste products from these procedures (3).

Film processing is one of the most important factors influencing patient doses and image quality during x-ray examination (16). In our technique, after processing, the two images will be displayed on one film which differ from traditional technique in which we need to process two film to obtain the same result. So this technique will provide less hazard of processing solution on occupational person. Developer and fixer solutions contain hazardous substances which are known irritants, sensitizers, carcinogens and endocrine disruptors (17). Exposure to radiographic processing chemicals presents a potentially serious health risk that warrants attention by industrial hygienists and other occupational health professionals (5).

As a result of reducing the film number in our technique there will be a less waste product to the half like Lead which is hazardous metal that can contaminated soil and groundwater and it's generated at dental offices in foil from intraoral film packets (18).

our technique provide less cost through reducing the number of the film used and this come with agreement of study done by Patil in 2014 (19).

CONCLUSION

Conventional radiology setup at a dental clinic is always a must and requires a lot of armamentarium such as films and X ray machine along with processing area and solution.

All modifications, adjuncts, and alternatives should be viewed as substitutes rather than equivalents to the usual and customary intraoral film.

This technique will provide the advantage of change the orientation of film placement, less contamination, time consuming and economic.

This technique can be applied on phosphor plate sensor because of the similarity of it with conventional film regarding flexibility properties.

We hope from the company of the film industry to adopt this technique and adding or press land marks like upper (U), lower (L), right (R), and left (LF) resemble raise dote. This will strong this modified technique and made it to be suitable for using in all dental clinical fields including hospital, teaching institutes and others.

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Oilseeds of *Corindrum sativum* as a renewable source of environmentally-friendly polymers in Jordan

Khalid Adel Abu- Hammour

Dept. of Ecology/ Al-Ahlyia Amman University / Jordan

E-mail: ijst.jordan@yahoo.com

ABSTRACT

The aim of this research was to determine of seed contents of Coriander (*Coriandrum sativum*). This plant is wide spread in Jordan and used for medical treatments and as spices and food. Research conducted in the Jordan Valley at 150m below and in Northern Jordan at 200m above sea level. Coriander has hermaphroditic, male and female flowers. Analyses of fertilized seeds showed that they contain 14,9 % Protein while non fertilized ones had a significant lower content of 4.7%. The seeds contain 7.4 % oil, this can vary according to strain. Derivatives or polymers from under-utilized fatty acids such as petroselinic acid, Petroselinic acid (C18:1) can be split to produce C6 (adipic acid) and C12:0 (lauric cid) molecules. Adipic acid is used for the manufacture of a wide range of polymers including high grade engineering plastics. The fatty oil of coriander is of interest because of the high level of petroselinic acid. Petrosilinc acid has potential nonfood applications in oleochemistry. At present, adipic acid is derived from mineral oil by a process of that damage the ozone layer and contributes to which releases gasses such as N2global warming. Petroselinic acid is an isomer of oleic acid and is used as a plastics lubricant, in the manufacture of nylons and for cosmetics. Petroselinic Coriander seed oil is used in the flavor industry, although it is not popular oil in aromatherapy. Other more recent uses include the use as a green vegetable by some ethnic groups, to flavor foods and in the oleo-chemical industry.

Keywords: *Coriandrum sativum* , Petroselinic acid, fertilized seeds and global warming

INTRODUCTION

Coriander (*Coriandrum sativum*) is a culinary and medicinal plant from the Umbelliferae. It is an annual herb originally from the Mediterranean area. Nevertheless, several authors have named *Coriandrum sativum* as a wild plant. Linnaeus (1) reported that *Coriandrum sativum* also occurs as a weed in cereals. Alefeld (2) mentioned that *Coriandrum sativum* was a common weed spread from southeastern Europe to southern Russia. Stoletova (3) also reported on wild *Coriandrum sativum* from Armenia. All parts of the plant have a strong odor, from which the plant takes its name. The cultivation of *Coriandrum sativum* is widespread, but is planted on a small scale only. In Jordan, it is where found in gardens rather than in large fields, in contrast with Germany where are in many landraces of *Coriandrum sativum* (4). It is cultivated as a summer or winter crop.

Description of the Plant:

The plant can reach heights 20 and 80 cm. The stem is more or less erect, branched sometimes with several side branches at the basal node. Each branch finishes with an inflorescence. The color of the more or less ribbed stem is green and sometimes turns to red or violet during the flowering period. The leaves alternate, and the first ones are often gathered in a rosette. The leaves are of two types, lower with leaflets and upper divided into narrow linear segments (4). The *Coriandrum sativum* flower has five irregular-shaped petals, five stamens, five sepals, and two styles. Flowering starts with the primary umbel. The first umbels to bloom have hermaphrodite flowers, with possibly a few staminate ones (4). The inner flowers of umbellets are staminate. The central flowers are circular, with small inflexed petals. The color of the petals is pale pink or sometimes white. The umbels of higher order usually contain more staminate flowers than the first ones, and their flowering period is shorter (4).

In a single flower, the five filaments of the staminate are located between the five petals. After the flower opens, the white filaments are visible between the petals. Under optimum conditions, many different insect species are pollinators or visitors of *Coriandrum sativum* umbels (4). The species of insects that pollinate *Coriandrum sativum* depend on the area of cultivation. Flowering and pollination biology of *Coriandrum sativum* is typically of that for umbelliferous plants, according to Bell (5). Depending on the weather conditions, 2-3 days after opening of the first flower, the pollen sacs open and spread the pollen. McGregor (6) showed that selfing of the *Coriandrum sativum* is impossible, but Glukhov (7) showed that *Coriandrum sativum* is partially self-fertile. He suggested that geitongamy is common and cross is possible. Bees are beneficial to *Coriandrum sativum*, Glukhov (7) reported that when they were excluded only 49.4 percent of the seeds set, but when they were present 68.3 percent of the seeds set. Bogoyavalensell *et al* (8) associated seed yields with greater insect visitation.

Use of *Coriandrum sativum:*

This plant is of economic importance since it has been used as a flavoring agent in food product, perfumes and cosmetics. Moreover the essential oils of the fruits and various extracts from *Coriandrum sativum* have been shown to possess antibacterial (9-11), anticancerous and antimtagenic activities (12). *Coriandrum sativum* has been used in medicine for thousands of years.

In Jordan the primary product is the fresh green herb of *Coriandrum sativum*, used of its specific flavor, which is completely different from that of the ripe fruits. In other countries the fruits are used as a spice and vegetable.

Oleum (13) stated that Russian produces high quality *Coriandrum sativum* oil, with a linalool content of 55%. Bauer (14) found that *Coriandrum sativum* attains its greatest yield of volatile oil (0.9%). The fatty oil of *Coriandrum sativum* is of interest because of the high level of petroselinic acid. Adipic acidic used for the manufacture of a wide range polymers including high grade engineering plastic and has a global marketing.

MATERIALS AND METHODS

Research Sites:

The research was conducted in Jordan at two different locations. The first one is located 150m below sea level. This area is wet with warm temperatures in winter, and dry and hot in summer. The other is located 200m above sea level. It is characterized by rainy cold winter and dry mild summer (Figure 1). The site has produced relatively high biodiversity in Flora and biogeographically units. Jordan valley, which extends down the entire flank of Jordan 50km away from Amman, is the country's most distinctive natural feature. The Jordan valley is located between 22° 40′ 0′′ on latitudes, and 35° 30′ 0′′ on longitudes (Figure 2). The northern segment of the Jordan valley, known in Arabic as the Ghor, is the nation's most fertile region. It contains the Jordan River and extends from the northern border down to the Dead Sea. Several degrees warmer than the rest of the country, its year-round agricultural climate, fertile soils, high winter rainfall and extensive summer irrigation have made the Ghor the food bowl of Jordan. According to MD (15), the mean maximum and minimum temperature is 29.9°C and 16.98°C respectively, with rainfall amount around 77-392mm over 44.84 rainy days yearly. The total rainfall loss is mostly through evaporation, with mean yearly loss approximately. Its mean relative humidity is (72.45%) in winter and 48% in summer since the

last 30 years. The Jordan valley is experienced to ground frost in nearly 2.5 days yearly.

Figure (1): Jordan Valley Overview of Vegetation Covers

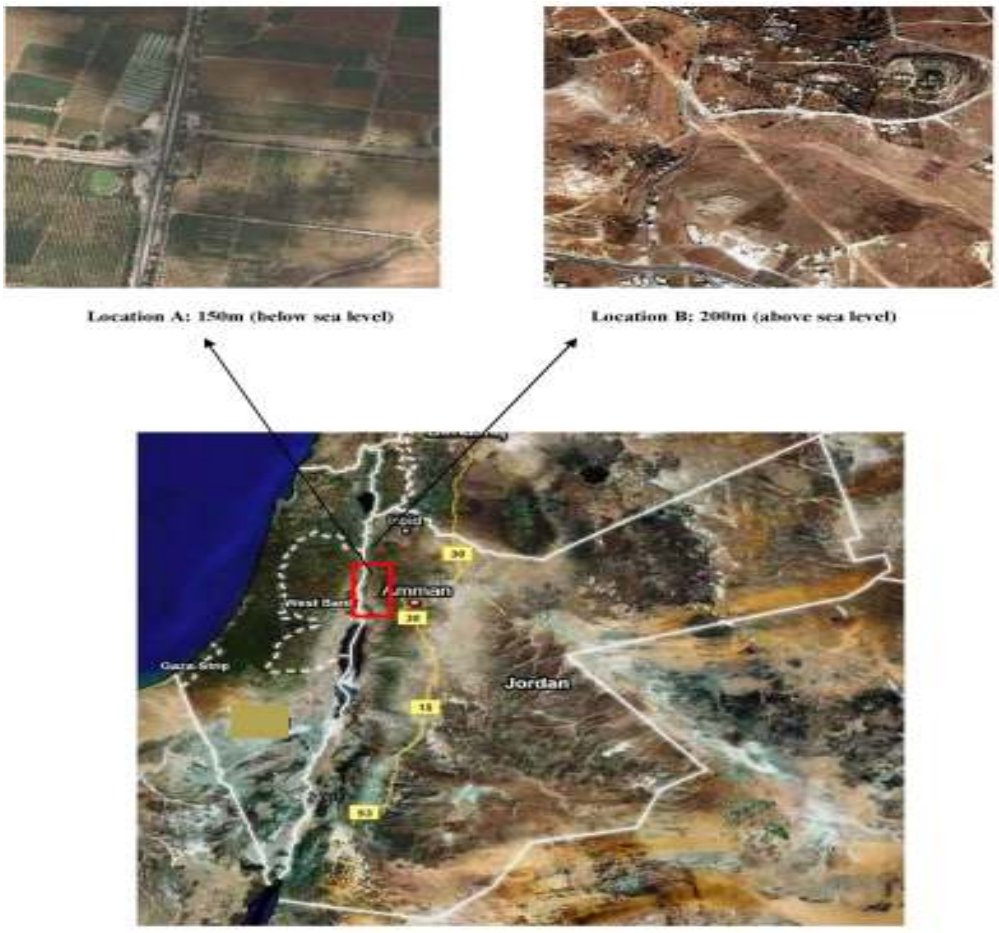

Figure (2): Research Sites (53, 15, 30, 30, are the Main International Highway)

***Coriandrum sativum* plantation:**

Coriandrum sativum was planted in location A and location B. Seeds were obtained from local markets (landraces). The rows were 20m long with 1m between rows. Water was supplied daily by drip irrigation, and extra fertilizers (NPK) were applied. Each two locations were kept weed free by cultivation and hand weeding; because *Coriandrum sativum* is a weak competitor for weed, in spite of the black plastic mulch that was used.

Determination of seed content:

In order to define the medical usages of *Corindrum sativum*, it was necessary to determine the protein content in *Corindrum sativum* seeds. I choose an accredited lab, using international standard methods for high level of accuracy of results, which is National Center for Agricultural Research and Technology Transfer (NCARTT) chemist's lab. I collected (400) gm. of *Corindrum sativum*' seeds. The preparation requirements of seeds for extraction were being applied including: drying of seeds, crushing, and solvent extraction to facilitate the extraction operation. Gas liquid chromatography is the most valuable analytical procedure available for separation and analysis of complex mixtures of volatile organic and inorganic compounds, where the sorbent is a nonvolatile liquid called the stationary-liquid phase, coated as a thin layer on an inert, and selecting the proper to obtain a quantitative estimate of the chain length, the amount of unsaturation, and the types of substitution associated with the fatty-acid chain. The analyzed protein uses (SOP: 130M01-006) the international standard method (Table 1).

Table (1): Analyzed Constituents for *Corindrum sativum*

No.		Standard method used
1. Seed constituent	Ash	SOP: 130M01-009
	Moisture	SOP: 130M01-010
	Oil	SOP: 130M01-001
	Proteins	SOP: 130M01-006
	Trace elements:	
	Mg – Mn – Cu	SOP NO :131M02-005
	Ca – K	SOP NO :131M02-002
2.Oil constituent	Fatty acids	COI/T.20/Doc. No. 24 (2001)
		AOCS Ch 2-91 (1997)

RESULTS

Seed Content:

Seeds tested were chosen from both locations of for fatty acids composition, essential oils and mineral contents. Moisture content was no significantly different among treatments. Protein values were significantly different among pollination treatments and ranged from 13.01% to 15.78%. Oil content was low in all seeds and varied from 5.61% to 7.40%. Ash values ranged between 6.26% and 6.51% (Table 2). Fatty acids composition varied significantly among the seeds of the selected location. Oleic acid was significantly the most concentrated 80.10% (Table 3).

All minerals contents varied significantly among seed samples. Manganese and Cupper were the most prevalent minerals. The linalool acid is the common essential oil in Coriander and was 66.7%. The fatty oil of Coriander is high of interest because of the high level of petroselinic acid, which has potential non-food applications in oleo chemistry. This oleic acid like isomer opens up another potential approach to the manufacture of medium-chain acids, since it can be split into lauric (C 12:0) and adipic (C 6) acids by the oxidative cleavage.

Table (2): Percentage of Constituents for *Corindrum sativum*

Contents	Results	Unit
Moisture	8.31	%
Ash	6.31	%
Protein	15.78	%
Oil	7.40	%
Magnesium	0.34	%
Calcium	0.40	%
Potassium	1.46	%
Manganese	18.8	mg./kg
Cupper	11.6	mg./kg

Table (3): Percentage of Fatty Acid for *Corindrum sativum*

Fatty acid	Trivial name	Systematic name	%
C 14:0	Myristic acid	Tetradecanoic acid	0.10
C 16:0	Palmitic acid	Hexadecanoic acid	3.33
C 16:1	Palmitoleic acid	*cis*-9-Hexadecenoic acid	0.42
C 17:0		Hexadecanoic acid	0.03
C 17:1		Desaturation of *cis*-9-Hexadecenoic acid	0.04
C 18:0	Stearic acid	Octadecanoic acid	0.88
C 18:1, n-7	Vaccenic acid	*cis*-11-Octadecenoic acid	80.10
C 18:2	Linoleic acid	*cis*-9, 12-Octadecadienoic acid	14.63
C 18:3	α –Linolenic acid*cis*-9, 12, 15-Octadecatrienoic acid		0.29
C 20:0	Arachidic acid	Eicosanoic acid	0.09
C 22:0	Behenic acid	Docosanoic acid	0.03
C 20:1	Gadoleic acid*cis*-9-	Eicosenoic acid	0.04
C 24:0	Lignoceric acid	Tetracosanoic acid	0.02

Low percentage of protein in *Corindrum sativum*' seeds:

The protein percent in treated fecundated-seeds for *Corindrum sativum* was 14.9% of the total dry mass but Protein percent in empty embryo was 4.7% of the total dry seed mass. The testing was applied through analyzing fecundated and non-fecundated seeds for *Corindrum sativum* using the international method for protein determination in NCARTT labs, analyzed by Gas Liquid Chromatography. Protein content in fecundated seeds is more than in non-fecundated seeds, explained by the enzymatic activity of protein synthesis in complete fertilized ovary (embryo) versus the empty ovary (gamete) and the cell division needs in the fecundated seeds more protein to complete their division versus the empty ovary (gamete). Many studies were emanated out the seed content in *Corindrum sativum* but not included the percent of protein in fecundated and non-fecundated.

CONCLUSION

Little is known about the metabolic origin of petroselinic acid (18:1), the principal fatty acid of the seed oil of most Umbelliferae species. Petroselinic acid is an unusual fatty acid that occurs primarily in seeds of the Umbelliferae. The structure of petroselinic acid differs from that oleic acid, a common plant fatty acid, in the position of double bond. Petroselinic acid is of potential industrial significance because of the unsaturation at C-6. Through chemical cleavage at its double bond, petroselinic acid can be used as a precursor of both lauric acid , which is a component of detergents and surfactants, and adipic acid, which is the monomeric component of nylon. Monounsaturated fatty acids of plants are typically derived from desaturation of C16 and C18 saturated fatty acids bound either to the acyl carrier protein or to glycerolipids. In this study we have shown that seed contents of coriander have Petroselinic acid (18:1) is the major component of the seed oil of Umbelliferae species. Petroselinic acid comprises as much as 80 % of the totally fatty acid content of Umbelliferae seeds. Petroselinic acid is metabolized and accumulated in the developing endosperm of some Umbelliferae species, including coriander and carrot. Derivatives or polymers from under-utilized fatty acids such as petroselinic acid are regarded as new materials. Adipic acid is used for the manufacture of a wide range of polymers including high grade engineering plastics and has a global market in excess of £2.5 million tones worth over £1 billion. At present, adipic acid is derived from mineral oil by a process which releases gasses such as N2O that damage the ozone layer and contribute to global warming. Petroselinic acid is an isomer of oleic acid and is used as a plastics lubricant, in the manufacture of nylons and cosmetics. Oleic acid (C 18:0) is used in

many industrial processes, and in the food industry. It is a major constituent of salad cream and mayonnaise. Other more recent uses include the use as a green vegetable by some ethic groups and to flavour dishes and foods such as pickles and sauces. In General, the protein percent in treated fecundated and non-fecundated seeds for *Corindrum sativum* was low of the total dry mass.

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The significant activity of some enzymes and their functional roles in tapeworm *Avetillena benedidea* (Cyclophylidea, Anoplocephalidae)

Sundus Natheer Al- Kallak

Dept. of Biophysics/ College of Sciences/ Mosul University
Republic of Iraq

E-mail: alkallaksundus3@yahoo.com

ABSTRACT

The current study aimed to detect the activities of a number of enzymes that the important in the physiology and the development of the Cestoda parasitic worms *Avetillena benedidea*, as a parasitic in intestinal of sheep. The findings of the current study included the enzymes such as Acetyl cholinesterase (AchE), which is a key enzyme in the bio functional, and Adenosine deaminase (ADA) for the role in regulation of cellular growth and differentiation. The current findings also included revealing the enzyme Lactate Dehydrogenase (LDH) for its important enzyme for the production of necessary energy for the parasite, as well as enzyme Glutathion S-transferase (GST) for its mechanism for the sustainability of parasite life host.

the present study showed that the tissues and organs of the cestoda *A. benedidea* have these enzymes activity, and there is a high qualitative activity. The both enzyme activity (AchE) and (LDH), then followed by (GST) enzyme activity, while (ADA) enzyme showed less specific activity in different organs of the cestoda.

conclude from this study that the enzymatic content in different tissues is a sigh of metabolic, physiology activities, absorption of nutrients, development, growth and other activities that ensure the sustainability of parasite life in the host.

Keywords: Specific activity, Enzymes, *Avetillena benedidea*

INTRODUCTION

Enzymes are protein-forming materials and biological agents that help to increase the rate of biochemical reactions within living cells. All enzymes share the structural and functional properties regardless of the interaction they induce (1). In addition, they have an important role in biological and vital processes of parasites such as metabolism, energy production, egg hatching and the process of transition to different stages during their life cycles, as well as their role in decomposing host tissues to facilitate infiltration of the parasite during migration and transmission between host organs and its frequent nutrition and resistance to the host's immune response (2,3). Enzymes play a vital role in musculoskeletal activity, by which parasitic worms can move and stay in their natural habitat on host cells (4-6). The inhibition of enzymes caused by anti-parasite drugs is an important mechanism to stop parasitic cell activity, leading to control of the growth and development of the parasite in its host (2), thereby, enzymes perform core functions in fields of health and disease control. Thus, studies and researches had been targeted the enzymes due to their importance in establishing the basic rules of medical diagnosis and making them a target and key of design and manufacture of therapeutic drugs for control of parasitic diseases (7-9).

In Iraq, particularly in the province of Nineveh, a number of researches and studies have been carried out, revealing the histological structures of enzymes in parasites (10-18), while other studies examined the biochemical aspects of parasites (13,17, 19-26).

Based on the above, it is needed to increase the knowledge and study of parasite enzymes because of their medical, diagnostic and therapeutic importance. In this regard, the present study was aimed to detect the specific efficacy of some of the important enzymes in life cycle of tapeworms which live in the intestines of sheep.

MATERIALS AND METHODS

A number of 6 tapeworms *Avetillena benedidea* were isolated from sheep intestines from Mosul city during December 2011. Samples were washed with distilled water to remove the mucous membrane residue of the intestines and placed in a normal saline with concentration 0.9%. Methods of Knowles and Oaks (27) and Pappas (28) were applied to prepare an extract of tapeworm with a concentration of 100 mg/ cm^3 body weight in Triss-Hcl Buffer solution with a concentration of 0.5 Molar and pH=7.8 which contained 1 cm^3 Ethylen Diamin Tetracetic (EDTA) of 1 m. molar to remove the inorganic substances. A stripping of tapeworms was performed using the homogenizer and crushing was completed using ultrasonic apparatus. The residue was then separated from the sediment for the purpose of deposition of the non-broken cells. The residue was then used to measure the enzymatic efficacy of the following enzymes in the studied tapeworm:

1- Acetylcholin esterase (AchE) (29).
2- Adenosin deaminase (ADA) (30).
3- Lactate dehydrogenase (LDH)(31).
4- Glutathion S- treansferase (GST)(32).

RESULTS AND DISCUSSION

Results of the current study revealed the specific efficacy of a number of enzymes of physiological importance in the survival of *A. benedidae*, including enzymes ACH, GST, LDH, ADA (figure 1).

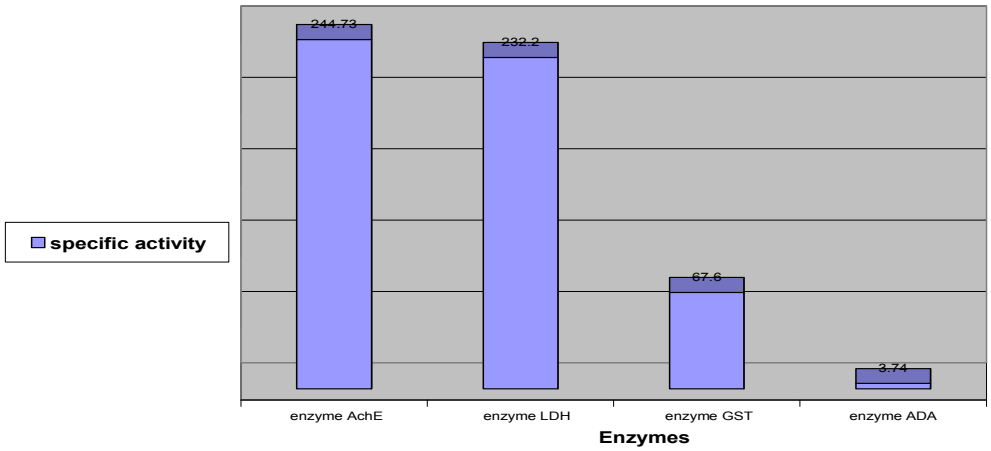

Figure (1): specific efficacy of enzymes on physiological characteristics of *A. benedidae*

Acetyl cholinesterase (AchE E.C. 3.1.1.7):

Results obtained from the current study had shown a significant effectiveness of AchE E.C. 3.1.1.7 enzyme in the studied tapeworm tissues, which recorded 244.73 nanomol / min / mg of protein (figure 1). This finding confirms previous studies of the important enzyme mechanism in the process of transmitting nerve impulses and its ability to analyze the neurotransmitter acetylcholine to choline and uric acid. The enzyme is present in nerve tissue as well as in non-neuronal tissues and plays a role in movement mechanism of the tapeworms (5,6).

In reference to the findings and conclusions of studies (17, 21-24, 26), differences in the specific efficacy of this enzyme for parasites and developmental stages of neurological systems are noted, and this could be due to the maturity of parasites and the mechanism of their nervous system for each species and even within the same one, controlling the vital functions such as nutrition, growth and reproduction and the development of embryonic stages to the matured stages (11,12,14, 15, 16, 17, 26, 33). Neural structures and neural map, which are specialized for each type of parasite (15), are vital markers and reflective mirror of the organism, and a key to its mechanisms and physiological processes, as well as a target for the design of therapeutic drugs to control parasitic diseases, by which this enzyme reduces inflammation in the surrounded areas of tapeworms and thus disguises antibodies, as this enzyme is one of the factors that make tapeworms resistant to antiretroviral drugs (34,35). From the other hand, the role of enzyme in parasitic species differs from that of free-living organisms (36). Edwards *et al.* (37) described this enzyme as a biofixer, allowing the presence or retention of preferred parasites locations as well as its other roles in digesting host tissues and food. The effect of the enzyme on the parasite and its host was found in a polluted environment compared to the pollution-free zones (38). Therefore, further studies are needed to determine the specific efficacy of this enzyme and the neural map of all parasites and their developmental stages even within the same species, as well as the study of the effect of factors such as temperature and pH and compare it with host, which lives in different environments as a result of exposure to pollutants and other factors, where these studies can help in more understanding of this enzyme's vital role.

Lactate Dehydrogenase (LDH E.C. 1.1.1.27):

The results of the present study showed that LDH E.C. 1.1.1.27 enzyme had a specific efficacy of 232.2 micromol / min / mg of protein (figure 1), reflecting its importance in helminths in the reduction of pyruvite to lactate and the formation of two parts of ATP (39), through its role in the available glucose or glycogen oxidation in their tissues to produce the needed energy for staying in their host tissues (4), as appeared in the final step of the glycolysis pathway in the absence of oxygen during muscular activity in the skeletal muscle of the hosts , in which the enzyme is more effective for its functional role (3,4,40).

The reason for different levels of enzyme specific efficacy in the current study results and the results of previous studies (17, 21-25) is due mainly to different parasites in their parasitic locations according to differences of their hosts and the surrounding conditions for producing energy, as well as the stage of maturation of the parasite and its genetic nature, resulting in the estimation of pyrovite in the tissues of the parasites, to be achieved through the knowledge of the specific efficacy of the enzyme, which refers to the metabolism of available glucose and glycogen in the tissues, taking into account the detection of the condition of the infected host if received some non-specialized pharmaceutical doses, as it negatively affects the activity of the parasites and metabolic activities without killing (41). Therefore, the enzyme is a key when designing parasitic anthelmintic drugs (42), because the enzyme mechanism is similar to the mechanism used for pharmacological treatments, or the effect of plant extracts on the inhibition of pyrovate and its conversion to lactate. Therefore, further studies are required to study the functional characterization of the enzyme and to identify its counterparts in the parasite and compare it with its host. Bhandary *et al.* (43) had demonstrated that there are five nucleotide counterparts in a variety of nematodes. The host species and their environment may also play a role in the presence of helminths from vital molecules (25), as well as the contribution of some factors that may be one of the reasons for the availability of the biochemical content in the parasite during the study, including the physiological and seasonal condition of the host with different types of Parasites and infection rate, and the development of the parasite at its host, and the time of dissection of the host is one of the factors affecting the content of the kygogenic in the parasite (44). Therefore, the current study recommends the detection of the enzymatic efficacy of parasites in their developmental stages and in different seasonal stages. This will lead to a better understanding of the metabolic processes and their mechanisms and development between their hosts and their genotypes in order to establish new steps for the pharmacological process and its application with modern biological and pharmaceutical technologies (45). Most of the enzymes in different embryonic stages are unclear, and may be associated with the growth and development of the parasite proliferative system, due to the close relationship between the parasitic LDH enzyme and its host.

Glutathione S-transferase (GST E.C. 2. 5. 1. 18):

The results obtained by the current study demonstrated an enzymetic efficacy in tapeworm tissue with 67.6 μmol / min / mg of protein (figure 1), as this enzyme is a multifunctional protein compound capable of removing the toxicity of internal metabolites and foreign matter entering the body of the organism (46) by transforming water-resistant compounds into water-loving compounds that are easy to secrete out of the body, and by this mechanism, the parasite will be able to secrete out its toxic metabolites to stay parasitic (47-49). The difference in enzymatic efficacy throughout the results of the current study and the results and conclusions of previous studies (9, 21, 24) is based primarily on the quantitative and qualitative protein content of the parasite, which reflects the species and activity of the parasite at its host (13), as the importance of different proteins in each parasite is a reaction to host tissue to form antimicrobial agents, which in turn stimulates the parasite to produce its own immune materials (51). Perhaps the surrounding conditions in the parasite environment give another reason for the difference in enzymatic efficacy. This can be explained by the importance and effectiveness of the enzyme in reducing the effect of chemicals such as drugs, heavy metals and pesticides in the parasite environment, thus facilitating parasite development (48). For this reason, this enzyme is the basic step for the design and manufacture of antiparasitic drugs (52). Therefore, further studies are needed to determine the effect of parasitism on the activity of the host GST enzyme, as well as to detect the enzymatic activity of the parasites and their hosts, with studying some factors such as temperature and pharmacological doses received by the infected host. The current study also recommends the detection of inhibitors, including the heavy elements in parasites found in the tissues and environment of the host (50), which will contribute to the effect on the enzymatic effectiveness of each Parasites and hosts, as parasites work on the overlap of heavy elements and physiological materials, which in themselves have been indicators of pollution, thereby establishing the foundations for the treatment of drugs and control of parasitic diseases.

Adenosine deaminase (ADA E.C.3. 5. 4 .4):

The results of the current study showed that enzymatic efficacy in studied tapeworm tissue was 3.74 nanomol / min / mg of protein (figure 1), reflecting the mechanism of this enzyme in the amino acid removal. ADA is a specialized enzyme in the nucleotide degradation series (1), as well as its role in regulating growth, development and cellular differentiation as well as the regulation of neurotransmitters (42, 53,54). The results of the current study, with the results of other studies (21, 24, 42) found a difference in the specific efficacy of the enzyme, confirming the study in Trott and Balis (56). Enzymatic activity in the living cells, and reported that enzymatic activity is subject to changes depending on the state of the cell and the degree of maturity and effectiveness and whether they are in the case of division and reproduction or not. The results of the current study showed low quality, which clearly explains that the current tapeworm is fully developed, ie, that its cells do not suffer any division. There is another view that the presence of certain elements such as zinc and mercury in the environment and parasite food is another reason to inhibit enzymatic activity (54), given the importance of the enzyme in the vital functions of the body. In this regard, Da Silva *et al,* (42) examined inhibitors qualitative inhibition of the effectiveness of the enzyme in the parasite. Therefore, many studies and advanced technologies of this enzyme, as a key to chemical treatments, as the available biological materials resulting from the parasitic processes are the main objectives and steps for future studies to understand the relationship between the host – parasite.

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