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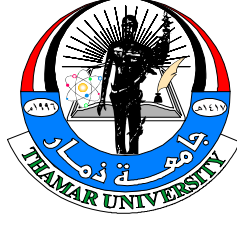
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Thamar University Journal of Natural & Applied Sciences (TUJNAS) is published twice a year by Thamar University, Thamar, Yemen. It aims at publishing original research contributions covering pure science, Agriculture, Engineering, Medicine, Environment and Computer science. In order to reach the widest possible readership, the Journal publishes contributions written in English or Arabic, thus it has two parts: Part A papers in English and Part B in Arabic.

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Three types of paper will be published:

- (a) *Normal-length papers (Articles)*: Comprehensive description and discussion of original research investigations. The experimental techniques must be described in detail. It should contribute new knowledge to the field of interest.
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(ii) Manuscripts must be written in English or Arabic with summaries and arranged as follows:

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2. **Books** : [2] Andrade, J. D. (ed), (1988), *Polymer Surface Dynamics*, Plenum Press, New York, pp, 1633-1646.
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Dear reader

It is with great pride that Thamar University publishes the first issue of *Thamar University Journal of Natural and Applied Sciences*. This reflects the interest of the university and its firm belief that Yemen in progress depends on the amount of progress in scientific research and development of qualitative and quantitative study, universities and educational institutions. To publish a scientific magazine specializing in scientific research and holding an international publication number (ISSN) means the implementation of the program of the President of the Republic on the development of scientific research.

In the past, scientific research on natural and applied science was published in the Journal of the University of Thamar, alternating with the research in the humanities. We are by this humble beginning we hope that thus research published in this journal will reach the global level, which could the national, regional and global levels benefit.

The editorial board calls for academics at the University of **Thamar, the Yemeni** universities and researchers in the Arab and international universities to send their research for publication in the journal, which will referees by experts after ascertaining their conformity with the standards for publication and their compliance with the journal terms and conditions.

The journal welcomes any views or constructive criticism that would contribute to any improvement of the journal.

Last but **not** least I can only give thanks to the patron of the university and its activities, President Ali Abdullah Saleh for his support and monitoring for the development and progress of universities.

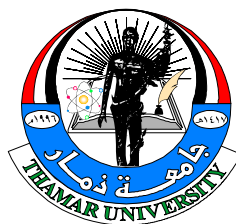
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Part A
Papers in English
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Detection of Extracellular Enzymes Produced by Fungi Isolated from Dried Fruits

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ABSTRACT

Screening of 57 fungal isolates isolated from dried fruits for the production of lipase, cellulase, invertase, and protease showed that there is a variation in enzyme production not only among the different genera and species, but also among the different isolates in the same species. Thirty two of tested fungi (66%) belonging to *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *A. tamarii*, *Cochliobolus spicifer*, *Humicola insolens*, *F. oxysporium*, *P. glaprum*, *P. oxalicum*, *P. stekii*, *P. variabile* and *Phoma sp.* had a high ability to produce lipase. Ten of tested isolates representing 24.39% of tested fungi had a moderate ability to produce cellulase enzyme. These isolates were belonging to *Aspergillus flavus*, *A. niger*, *A. parasiticus*, *A. versicolor*, *Curvularia lunata*, *Penicillium griseofulvum*, *P. oxalicum*, *P. stekii*, *P. variabile* and *Ulocladium atrum*. Twenty seven isolates representing 37.64 % of tested isolates belonging to *A. flavus*, *A. fumigatus*, *A. niger*, *A. parasiticus*, *A. terreus*, *A. versicolor*, *Fusarium oxysporium*, *Mucor fuscus*, *P. glaprum*, *P. griseofulvum*, *P. oxalicum*, *P. variabile*, *Phoma sp.*, and *U. atrum* were moderate invertase producers. Sixteen fungal isolates representing 28.07 % of tested isolates were moderate protease producers, these isolates were belonging to *A. flavus*, *A. niger*, *A. terreus*, *A. tamarii*, *A. versicolor*, *C. lunata*, *F. oxysporium*, *H. insolens*, *P. corylophilum*, *P. griseofulvum*, *P. oxalicum*, *P. stekii*, *P. vinaceum*, *Rhizopus stolonifer*.

INTRODUCTION

In recent years, the new potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated a renewed interest in the exploration of extracellular enzymatic activity in food grade yeasts (Bilinski and Stewart, 1990; Burden and Eveleigh, 1990; De Mot, 1990; Ratledge and Tan, 1990).

Fungi secrete proteins which are protective in function to the fungi themselves or which can be exploited for the welfare of mankind (Ng, 2004). Lipases (tricylglycerol hydrolyses EC 3.1.1.3) are serine hydrolyases of considerable physiological significance and industrial potential, which



catalyze the hydrolysis of triglycerides at the oil water interface. They are produced by animals, plants and microorganisms (Sztajer, *et al.*, 1988; Aires-Barros, *et al.*, 1994; Ionita, *et al.*, 1997; Ueda, *et al.*, 2002). To date, a large number of lipases produced by filamentous fungi has been extensively studied, both from the biochemical and genetic point of view. The most productive species belong to the genera *Geotrichum*, *Penicillium*, *Aspergillus* and *Rhizomucor* (Stocklein, *et al.*, 1993 and Miura and Yamane, 1997). Cellulose is the most abundant component of all photosynthetic land plants and thus represents the main organic food source for heterotrophic decomposers. On land, the major biological agents of cellulose breakdown are fungi, with aerobic and anaerobic cellulolytic bacteria playing a minor role (Swift, 1982). Invertase (1, 2- β -D-fructofuranosidase fructohydrolase, EC3.2.1.26), an important enzyme used in food industry, is usually synthesized constitutively by yeasts (Costaglioli, *et al.*, 1997) while, in certain filamentous fungi, it is inducible (Chen 1996 and Romero-Gomez, *et al.*, 2000). Many phytopathogenic fungi are known to produce extracellular proteinases (Kalashnikova, *et al.*, 2003) and Sara and Heale, (1990), suggested that proteinases play an active role in the development of plant diseases. Proteases with different molecular masses, optimum pH values and optimum temperatures are produced by different fungal species (Chou, *et al.* 2001; Paoletti, *et al.* 2001; Pekkarinen, *et al.* 2002; Poza *et al.* 2001).

MATERIALS AND METHODS

Fifty seven fungal isolates isolated from dried fruits were screened for their ability to produce extracellular enzymes on solid and liquid media. The following fungal organisms were tested: *Aspergillus flavus*, *A. tamaritii*, *A. niger*, *A. terreus*, *A. versicolor*, *A. parasiticus*, *A. fumigatus*, *Cochliobolus spicifer*, *Curvularia lunata*, *Emericella quadriline*, *Fusarium oxysporium*, *F. verticillioides*, *Hemicola insolens*, *Mucor fuscus*, *Myrothecium roridum*, *Penicillium corylophilum*, *P. expansum*, *P. glaprum*, *P. griseofulvum*, *P. oxalicum*, *P. variable*, *P. stekii*, *P. vinaceum*, *Phoma sp.*, *Rhizopus stolonifer*, *Ulocladium atrum* and *U. botrytis*.

1. Lipase production:

Lipase production was measured according to Ulman and Blasins, (1974). The basal medium was composed of: Peptone, 10.0 g; Magnesium sulphate, 2.0 g; Calcium chloride, 0.2 g; 1% Tween 20, 10.0 ml.; Agar, 15.0 g and Distilled Water 1000.0 ml, pH 6.0. The medium was sterilized by autoclaving at 121 °C for 30 minutes. The Tween 20 was autoclaved separately and 10.0 ml. was added to 1000.0 ml. of cooled basal medium. The isolated fungi were separately inoculated on the surface of agar basal medium and incubated at 28 °C for 10 days. Occurrence of a visible precipitate due to the formation of calcium salt crystals of the oleic acid liberated by enzyme indicates a positive lipolytic production as shown in plate 1.a.

2. Cellulase production:

Cellulase production was screened using CMC agar (carboxymethylcellulose agar medium) which was composed of: Carboxy methyl cellulose (a soluble form of cellulose) 5.0 g, Sodium nitrate 1.0 g, Potassium dihydrogen phosphate 1.0 g, Potassium chloride 1.0 g, Magnesium sulphate 0.5 g, Yeast extract 0.5 g, Agar 17.0 g, Distilled water 1000.0 ml. The medium was sterilized by autoclaving at 121 °C for 30 minutes. The isolated fungi were inoculated on the surface of agar medium and incubated at 28 °C for 10 days. All

plates of our isolates were flooded with Congo red solution (1.0 mg of Congo red per 1.0 ml of water) for 15 minutes, and then de-stained with salt solution (1M Sodium chloride) for 10-15 minutes. Unstained areas indicate where the CMC has been broken down to β 1-4 glucans which contains seven or fewer glucose residues as shown in plate 1.b. The diameter of the clear zone was measured (Teather and Wood, 1982).

3. Invertase (sucrase) production:

Sucrose hydrolysis by fungal isolates was tested on 20% sucrose Czapek's liquid medium (Sucrose 200.0 g, Sodium nitrate 3.0 g, Potassium dihydrogen phosphate 1.0 g, Magnesium sulphate 0.5 g, Potassium chloride 0.5 g, Ferrous sulphate 0.01g and Distilled Water 1000.0 ml. at pH 4.5). The isolates were inoculated and incubated at 28 °C for 10 days. The sucrase (invertase) production was determined in the culture medium filtrate using Benedict solution method. About 0.5 ml. of Benedict solution was added to culture filtrate and heated in water bath at temp. 100 °C, a positive result was indicated by a yellow, green, or brown precipitate as shown in plate 1.c. (Abdel-Sater and Saber, 1999).

4. Protease production:

The fungal proteolytic producer was tested by using a casein hydrolysis medium (Paterson and Bridge, 1994). This medium was intended for presumptive protease production, and contains skim milk, which gives an opaque of final medium. Hydrolysis of the casein results as a clear zone around the colony.

The composition of the medium was: Potassium dihydrogen phosphate, 1.0 g; Potassium chloride, 0.5 g; Magnesium sulphate, 0.2 g; Calcium chloride, 0.1 g; 15% skim milk, 25.0 ml; Glucose, 10.0 g; Agar, 12.0 g and Distilled water 1000.0 ml. The cooled medium was poured into 9 cm Petri-dishes (about 20 ml. for each). The tested fungi were separately inoculated in the centre of Petri-dishes and incubated at 28 °C for a week. After incubation, complete degradation of milk protein was seen as a clear zone in a some what opaque agar around colonies indicating of protease production.

RESULTS

1. Lipase production:

Fifty isolates comprising 87.71 % of tested fungi were recorded as lipase producers (Table, 1). Thirty two of tested fungi (66%) belonging to *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *A. tamarii*, *C. spicifer*, *H. insolens*, *F. oxysporium*, *P. glaprum*, *P. oxalicum*, *P. stekii*, *P. variabile* and *Phoma sp.* had a high ability to produce lipase. Eleven isolates representing 24% of tested isolates were recorded as moderate lipase producers. These fungal isolates were *A. flavus*, *A. niger*, *A. terreus*, *A. tamarii*, *E. quadriline*, *F. oxysporium*, *M. fuscus*, *P. corylophilum*, *P. glaprum*, *P. verrucosum* and *P. vinaceum*. Five isolates comprising 10% of tested fungi were recorded as weak lipase producers. These fungi were *A. flavus*, *F. verticillioids*, *P. expansum*, *P. griseofulvum* and *R. stolonifer*.

2. Cellulase production:

Forty one fungal isolates representing 71.93% of tested isolates were cellulase producers (Table 1). Five isolates (12.19%) had a high ability to produce cellulase. These isolates were *A. parasiticus*, *A. terreus*, *P. expansum*, *P. glaprum* and *P. stekii*. Ten of tested isolates representing 24.39% of tested fungi had a moderate ability to produce cellulase

enzyme. These isolates were belonging to *A. flavus*, *A. niger*, *A. parasiticus*, *A. versicolor*, *C. lunata*, *P. griseofulvum*, *P. oxalicum*, *P. stekii*, *P. variabile* and *U. atrum*. Twenty six of fungal isolates representing 63.41 % belonging to *A. niger*, *A. terreus*, *A. tamaritii*, *H. insolens*, *M. fuscus*, *P. glaprum*, *Phoma sp.*, and *R. stolonifer* were a weak cellulase producers.

2. Invertase production:

The ability of fungal isolates to produce invertase (sucrase) enzyme in liquid medium were studied. It was observed that there is a variation in enzyme production not only among the different genera and species, but also among the different isolates in the same species presented in Table (1). Out of the 57 isolates studied, there are 56 (98.24%) were able to produce invertase enzyme. From these producing isolates, only one isolate had a high degree of invertase production (*A. tamaritii*). Twenty seven isolates representing 37.64 % of tested isolates belonging to *A. flavus*, *A. fumigatus*, *A. niger*, *A. parasiticus*, *A. terreus*, *A. versicolor*, *F. oxysporium*, *M. fuscus*, *P. glaprum*, *P. griseofulvum*, *P. oxalicum*, *P. variabile*, *Phoma sp.*, and *U. atrum* had a moderate degree of invertase production. Twenty three fungal isolates belonging to *A. flavus*, *A. niger*, *A. parasiticus*, *A. tamaritii*, *C. spicifer*, *C. lunata*, *E. quadriline*, *F. oxysporium*, *F. verticillioids*, *H. insolens*, *M. roridum*, *P. corylophilum*, *P. stekii*, *P. verrucosum*, *P. vinaceum* and *R. stolonifer* were a weak invertase producers. Only one isolate was non invertase producer, this isolate was *P. expansum*.

4. Protease production:

Twenty three of fungal isolates were tested for their ability to produce protease enzyme and recorded as protease producers (Table 1). These isolates belonging to *A. flavus*, *A. niger*, *A. terreus*, *A. tamaritii*, *A. versicolor*, *C. lunata*, *F. oxysporium*, *H. insolens*, *P. corylophilum*, *P. griseofulvum*, *P. oxalicum*, *P. stekii*, *P. variabile*, *P. vinaceum*, *Phoma sp.*, *R. stolonifer* and *U. atrum*. Sixteen fungal isolates representing 28.07 % of tested isolates were moderate protease producers, these isolates were belonging to *A. flavus*, *A. niger*, *A. terreus*, *A. tamaritii*, *A. versicolor*, *C. lunata*, *F. oxysporium*, *H. insolens*, *P. corylophilum*, *P. griseofulvum*, *P. oxalicum*, *P. stekii*, *P. vinaceum* and *R. stolonifer* whereas, seven fungal isolates belonging to *A. flavus*, *A. terreus*, *P. stekii*, *P. variabile*, *Phoma sp.*, and *U. atrum* were weak protease producers.

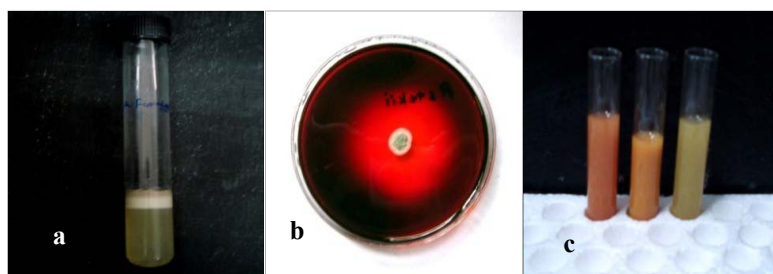


Plate (1): a. White precipitate due to the formation of crystals of calcium salt of the oleic acid liberated by lipase enzyme.
 b. Unstained area indicates CMC degradation by cellulase to β 1- 4 glucan.
 c. Formation of different color precipitates indicating an invertase production.

Table (1): Enzymatic activity of fungal isolates isolated from dried fruit samples.

Organisms	NTI	Lipase			Cellulase			Invertase			Protease						
		NPI	Degree of production			NPI	Degree of production			NPI	Degree of production						
			H	M	W		H	M	W		H	M	W				
<i>Aspergillus flavus</i>	13	11	9	1	1	10	0	1	9	13	0	8	5	5	0	3	2
<i>A. fumigatus</i>	1	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0
<i>A. niger</i>	10	10	8	2	0	10	0	0	10	10	0	5	5	1	0	1	0
<i>A. parasiticus</i>	2	2	0	0	2	2	1	1	0	2	0	1	1	0	0	0	0
<i>A. terreus</i>	2	2	1	1	0	2	1	0	1	2	0	2	0	2	0	1	1
<i>A. tamaraii</i>	3	3	3	0	0	1	0	0	1	3	1	0	2	1	0	1	0
<i>A. versicolor</i>	1	0	0	0	0	1	0	1	0	1	0	1	0	1	0	1	0
<i>Cochliobolus spicifer</i>	1	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0
<i>Curvularia lunata</i>	1	0	0	0	0	1	0	1	0	1	0	0	1	1	0	1	0
<i>Emericella quadriline</i>	1	1	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0
<i>Fusarium oxysporum</i>	3	3	2	1	0	0	0	0	0	3	0	1	2	1	0	1	0
<i>F. verticillioides</i>	1	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0
<i>Humicola insolens</i>	1	1	1	0	0	1	0	0	1	1	0	0	1	1	0	1	0
<i>Mucor fuscus</i>	1	1	0	1	0	1	0	0	1	1	0	1	0	0	0	0	0
<i>Myrothecium roridum</i>	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
<i>Penicillium corylophilum</i>	1	1	0	1	0	0	0	0	0	1	0	0	1	1	0	1	0
<i>P. expansum</i>	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
<i>P. glaprum</i>	2	2	1	1	0	2	1	0	1	2	0	2	0	0	0	0	0
<i>P. griseofulvum</i>	1	1	0	0	1	1	0	1	0	1	0	1	0	1	0	1	0
<i>P. oxalicum</i>	1	1	1	0	0	1	0	1	0	1	0	1	0	1	0	1	0
<i>P. stekii</i>	3	2	2	0	0	3	1	2	0	3	0	0	3	2	0	1	1
<i>P. variable</i>	1	1	1	0	0	1	0	1	0	1	0	1	0	1	0	0	1
<i>P. verrucosum</i>	1	1	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0
<i>P. vinaceum</i>	1	1	0	1	0	0	0	0	0	1	0	0	1	1	0	1	0
<i>Phoma sp.</i>	1	1	1	0	0	1	0	0	1	1	0	1	0	1	0	0	1
<i>Rhizopus stolonifer</i>	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	1	0
<i>Ulocladium atrum</i>	1	0	0	0	0	1	0	1	0	1	0	1	0	1	0	0	1
Total Isolates	57	50	32	11	7	41	5	10	26	56	1	27	28	23	0	16	7

NPI: Number of positive isolates and NTI: Number of tested isolates. W: Weak <0.5; M: Moderate 0.5- 0.9; H: High ≥ 10 m

DISCUSSION

The data in Table (1) clearly showed that 50 of tested isolates were lipase producers. Buzzini and Martini (2002) screened 196 strains of ascomycetes, 155 of basidiomycetes, and 46 of yeast-like organisms for their ability to produce extracellular enzymes; they found that about 60.7%, 43.5% and 13.5% of ascomycetes, yeast-like organisms and basidiomycetes respectively, were lipase producers. Cardenas *et al.* (2001b), screened 960 microorganisms isolated from soil samples, including yeast (100 strains) and filamentous fungi (860 strains) for their ability to produce lipase. They reported that 440 microorganisms produced a clear halo around them in plates containing tributyrin, whereas only 92 microorganisms showed hydrolysis on the olive oil plates. Shatter, (2004), screened 68 fungal isolates for their lipolytic activity, and reported that about 87.88 % of their isolates were lipase producers. Mohammed and Hussein (2004) screened 54 isolates of fungi isolated from luncheon meat for their lipolytic ability; of these isolates, 81.5% were able to produce lipase enzyme. Fotedar and Al-Hedaithy (2005) tested 87 isolates of *Candida dubliniensis* and 52 isolates of *C. albicans* for their ability to produce phospholipase. None of the 87 isolates of *C. dubliniensis* were phospholipase producers whereas, in contrast all the 52 *C. albicans* isolates showed varying degree of phospholipase activity, with 35 of them eliciting a higher phospholipase activity.

Another result presented in Table (1) showed that 41 of the tested isolates were reported as cellulase producers. Schlegel (1996) reported that species of the genera *Fusarium* and *Chaetomium* are prominent. Others known to be cellulolytic are *Aspergillus fumigatus*, *A. nidulans*, *Botrytis cinerea*, *Rhizoctonia solani*, *Trichoderma viride*, *Chaetomium globosum* and *Myrothecium verrucaria*. Strauss *et al.* (2001) screened 245 yeast isolates isolated from four wine production regions of the Western Cape, South Africa, for their cellulolytic activity. They found that only 11 isolates of *Candida stellata*, *C. pulcherrima* and *Kloeckera apiculata* showed some cellulase activity, but they reported that *C. pulcherrima* showed only activity on medium containing glucose only.

Also, results in Table (1) showed that 98.24% of the studied isolates were able to produce invertase enzyme. This result agrees with Abdel-Sater and Ismail, (1993), where they found that all their studied isolates were able to produce invertase. Abdel-Sater and Saber (1999) reported that 86.9% of their tested isolates isolated from dried fruits could produce invertase enzyme.

Finally, data in Table (1) showed that 40.35% of the tested isolates were able to produce protease. This result agrees with Buzzini and Martini (2002) who found that about 3.6, 15.2 and 31.0% of ascomycetes, yeast-like organisms and basidiomycetes, respectively were protease producers. Mohammed and Hussein (2004) screened 54 isolates of fungi for their proteolytic ability; of these isolates, 72.2% of isolates were able to produce protease enzyme. Shatter (2004) screened 68 fungal isolates for their proteolytic activity, and found that about 85.42% of isolates were protease producers.

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التحري عن الانزيمات الخلوية الخارجية المنتجة بواسطة الفطريات المعزولة من الفواكه المجففة

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ملخص

تم في هذه الدراسة الكشف عن قدرة ٥٧ عزلة فطرية تم عزلها من الفواكه المجففة للكشف عن قدرتها على انتاج انزيمات اللايباز، السيلوليز، انفرتيز والبروتيز. حيث أظهرت هذه الدراسة تنوعاً للعزلات في انتاجها لهذه الانزيمات ليس خلال الأنواع و الأجناس المختلفة فحسب ، بل من خلال السلالات من النوع نفسه. أظهرت الدراسة ان ٣٢ عزلة فطرية كان لها قدرة عالية على انتاج انزيم الليباز (٦٦٪) تنتمي الى: اسبرجلس فلافس، اسبرجلس فوميقاتس، اسبرجلس نيجر، اسبرجلس تيرريس، اسبرجلس تاماري، كوتشيلوبولس سبيسفير، فيوزاريم اوكسيسبوريوم، هيوميكولا انسولنس، بنسيليوم قلابرم، بنسيليوم اوكساليم، بنسيليوم فاريايبل، بنسيليوم ستيكي و فاوما كان لها قدرة عالية على انتاج انزيم الليباز. كما ظهرت ١٠ عزلات تمثل ٢٤,٣٩٪ من الفطريات المختبرة لها قدرة متوسطة على انتاج انزيم السيلوليز، وهذه العزلات تنتمي الى الأنواع التالية: اسبرجلس فلافس، اسبرجلس نيجر، اسبرجلس باراسينكس، اسبرجلس فيرسيكولور، كرفيولاريا لوناتا، بنسيليوم قريسوفولم، بنسيليوم اوكساليم، بنسيليوم ستيكي، بنسيليوم فاريايبل والوكلاذ يوم اترم. وجد ان ٢٧ عزلة تمثل ٣٧,٦٤٪ من العزلات التي تم اختبارها تملك قدرة متوسطة لانتاج انزيم الانفرتيزو تنتمي الى: اسبرجلس فلافس، اسبرجلس فوميقاتس، اسبرجلس نيجر، اسبرجلس باراسينكس، اسبرجلس تيرريس، اسبرجلس فيرسيكولور، فيوزاريم اوكسيسبوريوم، ميوكر فاسكس، بنسيليوم قلابرم، بنسيليوم قريسوفولم، بنسيليوم اوكساليم، بنسيليوم فاريايبل، فاوما والوكلاذيوم اترم تملك قدرة متوسطة لانتاج انزيم الانفرتيز. واخيراً بالنسبة لانزيم البروتيز فقد ظهرت ١٦ عزلة فطرية ممثلة ٢٨,٠٧٪ من العزلات المختبرة كانت متوسطة الانتاج لانزيم البروتيز، هذه العزلات تنتمي الى: اسبرجلس فلافس، اسبرجلس نيجر، اسبرجلس تاماري، اسبرجلس تيرريس، اسبرجلس فيرسيكولور، كرفيولاريا لوناتا، فيوزاريم اوكسيسبوريوم، هيوميكولا انسولنس، بنسيليوم كوريلوفيلوم، بنسيليوم قريسوفولم، بنسيليوم اوكساليم، بنسيليوم ستيكي، بنسيليوم فيناسيم و رايزوبس ستولونيغير.

Mycoflora and Total Aflatoxin Isolated from Dry Dates in Sana'a, Yemen

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ABSTRACT

This study is designed to record the mycoflora and total aflatoxin isolated from dry dates in Yemen Republic. Thirty four date samples collected from different shops and markets in Sana'a city were analyzed mycologically for the presence of fungi cultivated on three types of media. Eight species belonging to 7 genera were isolated from analyzed date samples on the three cultural media. *Aspergillus* genera was the most dominant fungi and grew well on the three type of media of which *A. niger* is the most common species, *A. flavus* was isolated in rare, low and rare frequency on 1% and 20% sucrose Czapek's and Sabouraud dextrose agar media. *Rhizopus stolonifer* was isolated in rare, low and low frequency on 1, and 20% sucrose Czapek's and Sabouraud dextrose agar media. The date samples were analyzed for the presence of total aflatoxin using ELISA technique which revealed that 4 out of 5 date samples were contaminated with aflatoxin ranged from 3059.89-7585.96 ppt (ng Kg⁻¹).

Keyword: Dry dates, Yemen, fungi, mycoflora, aflatoxin.

INTRODUCTION

Date fruit (*Phoenix dactylifera*) is an important food commodity consumed in large amounts particularly in Islamic countries (Shenasi *et al.*, 2002). Mycotoxigenic fungi, particularly aflatoxigenic *Aspergilli*, have been associated with dates and date products (Emam *et al.*, 1994; Aidoo *et al.*, 1996; Ahmed *et al.*, 1997; and Ragab *et al.*, 2001). Aflatoxins are highly toxic, mutagenic and carcinogenic secondary metabolites predominantly produced by *Aspergillus flavus* and *A. parasiticus*. Both fungal species infect several agricultural products like cereals, cereals hay, straw, corn, oily seeds, tree nuts, drained fruits and spices. Thus food and feed which is contaminated by aflatoxin producing fungi is a serious problem, not only due to the economic losses resulting from significant yield reduction and low quality of food, but mainly due to the serious worldwide health hazard to both human and livestock (Smith, 1997 and Chu, 2002).



MATERIALS AND METHODS

1. Collection of samples:

Thirty four samples (250 g of each) of dates were collected from different markets and shops at Sana'a city, Yemen Republic during 2005. Kind, number, and source of product of the collected samples are shown in Table (1). Each sample was put in a sterile polyethylene bag, sealed, and put in another polyethylene bag, and transferred to the laboratory (Biology Department, Faculty of Science, Sana'a University) for mycoflora and aflatoxin analysis.

2. Isolation and identification of fungi:

Fungi were isolated by using the dilution plate method as described by Johnson and Curel (1972). Twenty-five g. of each sample were suspended into 250 ml. of sterile physiological solutions 0.85% NaCl in a sterile conical flask of 500 ml. volume. The flasks were shaken, using a mechanical shaker, for 20-30 minutes. Dilutions from 10^{-1} to 10^{-3} were made under aseptic conditions. One ml of appropriate dilution was transferred into Petri dish plat, from each sample, nine Petri dishes were used (three for every medium). Twenty ml. of melted agar media were added after being cooled to 45-50° C. The Petri dishes were incubated for 7-14 days at 28° C.

Table (1): List of dates kind, source and number of samples collected.

Kind of dates	Source of dates	No. of samples tested
White dates	Yemen	10
	Saudi Arabia	16
	Iraq	2
Black dates	Yemen	1
	Saudi Arabia	4
Brown dates	Yemen	1
Total		34

The three culture media used for growing fungi were 1% sucrose Czapek's agar, 20% sucrose Czapek's agar, and Sabouraud dextrose agar. 1% sucrose Czapek's agar medium (Sucrose 10.00 g., sodium nitrate 3.00 g., potassium dihydrogen phosphate 1.00 g., magnesium sulphate 0.50 g, potassium chloride 0.50 g, ferrous sulphate 0.01g and agar 20.00 g l⁻¹), 20% sucrose Czapek's agar medium (Sucrose 200.00 g, sodium nitrate 3.00 g., potassium dihydrogen phosphate 1.00 g, magnesium sulphate 0.50 g., potassium chloride 0.50 g., ferrous sulphate 0.01g. and agar 20.00 g l⁻¹) and Sabouraud dextrose agar medium (Dextrose 40 g., Peptone 10 g. and agar 20 g l⁻¹). The pH of all media was adjusted to 5.5. These media were sterilized by autoclaving at 121° C and 1.5 bar for 30 minutes. Cloramphenicol (500 mg l⁻¹) was added to the medium after sterilizing as bacteriostatic agent. The growing fungi were counted, identified, and isolated.

The isolated fungi were identified up to genus and species level based on macro- and microscopic characteristics. The identification of fungal genera and species was made depending on the following references:

Booth (1971), for the genus *Fusarium*, Ellis (1971), for dematiaceous hyphomycetes, Raper and Fennell (1977), for the genus *Aspergillus*, Pitt (1979), for the genus *Penicillium*, Moubasher (1993) and Samson *et al.* (1995), for other isolates.

Potato dextrose agar medium contain: Potato 200 g., dextrose 20 g. and agar 20 g l⁻¹ was used for purification of fungi. The purified fungi were transferred to slants of the same medium for the good sporulation, and kept in refrigerator at 4° C.

3. Determination of total aflatoxins in dried fruit samples:

Five date samples were analyzed to determine their content of Total aflatoxins by enzyme-linked immunosorbent assay (ELISA) technique using R-biopharm-Germany kits (RIDASCREEN FAST). The samples were analyzed in Laboratory of Molecular Neurology and Functional Neuroproteomics, Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland.

RESULTS

1. Fungi isolated from dates on 1 % sucrose Czapek's agar:

Data in Table (2) shows that 8 fungi species belonging to 6 genera were isolated from date samples on 1 % sucrose Czapek's agar medium at 28° C. The total count of fungi was 40745 g⁻¹ in all samples. *Aspergillus* was the most frequently isolated genus. It was occurred in 97% of the samples comprising 99.74 % of total fungi in dried dates. *A. niger* was the prevalent species. The remaining species were isolated in rare frequency. *Rhizopus* sp., *Penicillium* sp., *Cochliobolus sativus*, *Phoma* sp. sterile mycelium and *Scopulariopsis candida* were found in rare frequency.

2. Fungi isolated from dates on 20 % sucrose Czapek's agar:

Data in Table (2) shows that 2 genera and 6 species were isolated from date samples on 20 % sucrose Czapek's agar medium. The total count of fungi was 343210 g⁻¹ in all samples. *Aspergillus* was the highest frequent genera representing 99.645 % (341990 g⁻¹) of total count. It occurred in 97% of the samples. *A. niger* was the most prevalent species, which isolated in high frequency representing 61.361 % of total isolates. *A. flavus* species was found in low frequency. Other species of *Aspergillus*, which were recovered from date samples, were *A. terreus*, *A. fumigatus* and *A. parasiticus*. *Rhizopus stolonifer* was isolated in low frequency.

3. Fungi isolated from date samples on Sabouraud dextrose agar:

Data in Table (2) shows that 6 fungal species belonging to 4 genera were isolated from date samples on Sabouraud dextrose agar. The total count of fungi was 326720 per g in all samples. *Aspergillus* was the most common genus isolated from date samples representing 99.25 % of total count g⁻¹ of date in all samples. It occurred in 97 % of the date samples and the total count of *Aspergillus* was 324290. *A. niger* was the most common species recovered from date samples. It was found in high frequency representing 63.6538 % of total count. *A. flavus*, *A. terreus* and *A. parasiticus* were occurred in rare frequency.

Rhizopus stolonifer was found in low frequency. It's occurred in 17.64 % of date samples. *Chrysosporium* sp. and *Mucor fuscus* were recovered in rare frequency.

4. Contamination of date samples by Aflatoxin:

Data in Table (3) shows that 4 out of 5 date samples were contaminated by aflatoxin with total aflatoxin ranged from 3059.89-7585.96 ppt (ng Kg⁻¹).

Table (2): Total counts (TC calculated X 10 g⁻¹ of date sample), number of cases of isolation (NCI) and occurrence remarks (OR) of fungal genera and species recovered from 34 date samples on 1 and 20 % sucrose Czapek's and Sabouraud dextrose agar medium at 28°C.

Fungi	1% sucrose Czapek's agar			20% sucrose Czapek's agar			Sabouraud dextrose agar		
	TC	NCI	OR	TC	NCI	OR	TC	NCI	OR
<i>Aspergillus</i>	40639	33	H	34199	33	H	32429	32	H
<i>A. flavus</i>	11	2	R	133	5	L	30	2	R
<i>A. fumigatus</i>	1	1	R	1	1	R	-	-	-
<i>A. niger</i>	25726	33	H	21060	32	H	20797	32	H
<i>A. parasiticus</i>	1	1	R	1	1	R	1	1	R
<i>A. terreus</i>	14900	1	R	13004	2	R	11601	2	R
<i>Chrysosporium sp</i>	-	-	-	-	-	-	1	1	R
<i>Cochliobolus. sativus</i>	1	1	R	-	-	-	-	-	-
<i>Mucor fuscus</i>	-	-	-	-	-	-	1	1	R
<i>Penicillium sp.</i>	3	1	R	-	-	-	-	-	-
<i>Phoma sp.</i>	2	1	R	-	-	-	-	-	-
<i>Rhizopus</i>	97	2	R	122	5	L	241	6	L
<i>R. stolonifer</i>	11	2	R	122	5	L	241	6	L
<i>Rhizopus sp.</i>	86	1	R	-	-	-	-	-	-
<i>Scopulariopsis candida</i>	1	1	R	-	-	-	-	-	-
Sterile mycelium	2	1	R	-	-	-	-	-	-
Total Count	40745	-	-	68520	-	-	32672	-	-
Number of Genera	6	-	-	2	-	-	4	-	-
Number of Species	8	-	-	6	-	-	6	-	-

Table (3): Total aflatoxin content of date samples.

Sample No.	Sample source	Total Aflatoxins ppt. (ng. Kg ⁻¹)
1	Saudia Arabia	0
2	Yemen	3059.89
3	Saudia Arabia	5665.11
4	Saudia Arabia	7585.96
5	Yemen	4018.37

DISCUSSION

Data in Table (2) showed that *Aspergillus* was the most predominant genus isolated from dates on 1 and 20% sucrose Czapek's and sabouraud dextrose agar media from date samples representing 99.74, 99.64 and 99.25% of total count of fungi. Abdel-Sater and Saber (1999) found that *Aspergillus* was isolated in high frequency, whereas *Eurotium* and *Penicillium* were isolated in moderate frequency from date samples. Alghalibi and Shater (2004) found that *Aspergillus*, *Eurotium* and *Penicillium* were the most common genera isolated from date samples. The deterioration of dates is associated with the growth of lactic acid bacteria and yeasts (Bolin *et al.*, 1972, Salik *et al.*, 1979 and Nussinovitch *et al.*, 1989). Yeasts and moulds were detected in pre-packed dates in Greater Glasgow, United Kingdom. Potential aflatoxin producer's *A. flavus* and *A. parasiticus* were found in only four samples (Aidoo, *et al.*, 1996). Lozada (1995) reported that a fruit contaminated by different moulds occurs during preharvesting, harvesting and grape processing. During these periods, temperature and humidity which they are important factors in mycelial growth and conidia germination. The composition of the fruit influences the likely type of spoilage. Because most fruits are somewhat acid, dry at the surface, and deficient in B vitamins and molds are the most common causes of spoilage. The composition, too, must determine the particular kinds of molds most likely to grow; thus, some kinds of fruits support a large variety of spoilage organisms and other kinds comparatively few (Frazier and Westhoff, 2000). Wareing *et al.* (2001) reported that mould growth is worst when drying times are extended during the rainy season. This may be a result of increased relative humidity during the rainy season, or that products take longer to dry if rewetted. In this study, 4 out of 5 date samples were contaminated with total aflatoxins at levels of 3059.89-7585.96 ppt. (ng kg⁻¹). Abdel-Sater and Saber (1999) analyzed date samples for the presence of aflatoxins by chromatographic analysis, aflatoxin B₁ was detected in dates (two samples, 300-390 µg. kg⁻¹). Alghalibi and Shater (2004) analyzed the presence of mycotoxins in date samples. They found that 2 of date samples were contaminated with aflatoxin B₁ and the concentrations of aflatoxin ranged between 110-180 µg kg⁻¹. Ioannou-Kakouri *et al.* (2004) found that dates analyzed from 1997-2000 were almost negative to aflatoxins.

The highest concentrations of aflatoxins are produced as a result of post-harvest spoilage of commodities stored under warm moist conditions; significant concentrations may also be produced in the field before harvest. This arises from endophytic association between these moulds and plants, such as maize and groundnut (Hill, *et al.*, 1985). The toxigenic (aflatoxin-producing) strains of *Aspergillus flavus* are distributed worldwide in soil and air which have been reported to contaminate a variety of foods and feeds (Bilgrami, 1984 and Mahmoud, 1993). Firm and ripe fruits show little contamination when

they are dried immediately. Microbiological investigations revealed the presence of aflatoxin-producing strains of *A. flavus* and *A. parasiticus* (Steiner, *et al.*, 1988). Sales *et al.* (2005) found that the presence of *A. flavus* on food-contact surfaces and in the air surrounding the production area for dried Cavendish bananas is indicative of high probability for Philippine dried Cavendish banana chips to be contaminated with aflatoxigenic fungi and aflatoxins.

The Yemeni limit standard for total aflatoxin is 20 $\mu\text{g. g}^{-1}$ for various foodstuffs (Yemeni standard limits, 2001). World Health Organization (WHO) standards for aflatoxin B₁ in various foodstuffs is 5 ng. g^{-1} and the total aflatoxin level cannot exceed 10 ng. g^{-1} . Germany, Switzerland, USA, and Hungary limit 4, 5, 20 and 5 ng. g^{-1} for various foodstuffs (Papp, *et al.*, 2002). Current legislation limits 4 $\mu\text{g kg}^{-1}$ for total aflatoxins in dried fruits for direct human consumption and 10 $\mu\text{g. kg}^{-1}$ to be subjected to sorting or other physical treatment before consumption or use as an ingredient in foodstuff (Commission Regulation, 2003).

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المحتوى الفطري و كمية سموم الافلاتوكسين الكلي للتمر المجفف في اليمن

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ملخص

تم في هذه الدراسة تشخيص المحتوى الفطري و كمية سموم الافلاتوكسين الكلي للتمر المجفف في الجمهورية اليمنية حيث تم جمع اربعة وثلاثون عينة تمر مجفف من مختلف المحلات و الأسواق في مدينة صنعاء من مختلف الأنواع و المصادر، وتم عزل الفطريات المتواجدة عليها باستخدام ثلاثة أوساط غذائية. تم عزل ثمانية أنواع فطرية تنتمي إلى 7 أجناس من التمر المجفف في الأوساط الغذائية الثلاثة، و كان جنس *Aspergillus* أكثر الأجناس شيوعاً بينما كان النوع *Aspergillus niger* أكثر الأنواع تواجداً من هذا الجنس. تم عزل *Aspergillus flavus* بشكل نادر في وسط 1% سكروز شابك آجار و بمعدل منخفض في وسط 20% سكروز شابك آجار بينما عزل بشكل نادر في الوسط الغذائي سابرود دكستروز آجار. تم تحليل عينات التمر المجفف لتواجد سموم الافلاتوكسين بواسطة تحديد الكم الكلي للافلاتوكسين باستخدام طريقة الاليزا والتي تم بواسطتها التأكد من تلوث 4 عينات من اصل 5 عينات تمر مجففة بسموم الافلاتوكسين بمعدل يتراوح بين 3059.89 و 7585.96 جزء من التريليون (نانو جرام لكل كيلو جرام) من التمر المجفف.

Synthesis and Characterization of Some New Uracil Derivatives and their Biological Activity

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ABSTRACT

This research includes the synthesis of thirty derivatives of uracil. Nucleophilic substitution reaction (thiol, alkylthio, hydrazino, thioacid,.. etc) of 4-chlorouracil afforded the corresponding derivatives. The 4-(2'-thiol-1',3',4'-oxadiazole-5'-thiomethyl) uracil (12), (N'-phenyl-2'-thio-1',3',4'-triazole-5'-methyl)-(2,6-dihydroxy-4-pyrimidinyl) sulphide (14) and 5-(2',6'-dihydroxy-4'-pyrimidinyl thioacetamido)-1,3,4-thiadiazole-2-thiol (15), were synthesized by treatment of 4-thioacetichydrazide uracil (11) with carbon disulfide in the presence of a base or phenylisothiocyanate in the presence of base. Mannich bases of alkynyl thio derivativeis (3a & 10) were synthesized by the reaction of paraformaldehyde and secondary amine in the presence of CuCl (19_{a,b} & 20_{a,b}).

These compounds were characterized by spectroscopic methods (IR & UV), by their elemental analysis (C,H,N) and R_f values.

Biological activity of the synthesized compounds was determined.

Keywords: 4-chloro uracil, 2,6-dihydroxy-4-pyrimidinyl thioacetic acid, Biological activity.

INTRODUCTION

Pyrimidines are among those molecules that make life possible as being some of the building of nucleic acids (DNA & RNA)¹. Pyrimidines are an interesting class of heterocycles which possess numerous biological and pharmacological effects. The pyrimidine ring system is found in many pharmaceuticals, herbicides and fungicides².

Various analogues of thiopyrimidines, aminothiopyrimidines, and hydroxy thiopyrimidines have been synthesized due to their interesting biological activities such as anti-bacterial, anti-fungal and anti-viral activities. They also showed significant chemotherapeutical activities³.

In this paper, we report new results regarding a selective nucleophilic substitution reaction at 4-chlorouracil or by cyclization of the uracil derivatives to obtain new thirty derivatives of uracil by different routes. The resulting uracil derivatives were characterized by spectroscopic methods (IR



and UV), by their elemental analysis (C,H,N) and determination of R_f values by using two systems.

Finally, the biological activity of the synthesized compounds was determined using: Staphylococcus, Klebsiella sp, Candida, Escherichia coli, Pseudomonas, Streptococcus, Salmonella and Proteus.

EXPERIMENTAL

Melting points were determined on an electrothermal melting point apparatus. IR Spectra were recorded using the KBr disc on Unicam sp3-1000-spectrophotometer. The UV and visible absorption were determined in ethanol 95% using Hitachi U2000 spectrophotometer. Elemental analysis (C,H,N) were performed on an elemental analysis system. Chromatography paper chromatograms were developed by the ascending technique, the solvent system being: [Propanol (6) : conc. ammonium (3) ; water (1)], and [1-pentanol (5): acetic acid (2): water (3)]

4-Mercapto uracil (2)

The mixture of 4-chloro uracil (0.003 mol, 0.75gm) in ethanol (20ml), and thiourea (0.004 mol, 0.35gm) in ethanol (15ml), was refluxed for three hours with stirring. The excess of ethanol was removed, and then a concentrated solution of sodium carbonate in water was added; on cooling, the product was formed, recrystallized from aqueous ethanol yielded the compound (2) as yellow precipitate (Yield 70%, m.p. 235 °C (decomp.). physical properties (Table 1).

4-Alkylthio Uracil (3a-c)

4-Mercaptouracil (0.001 mol, 0.144 gm), was dissolved in crushed ethanol (15ml), and crushed potassium hydroxide (0.001 mol, 0.04gm), was added to the solution, alkyl halide (0.001 mol) was added dropwise to the mixture which refluxed for two hours, the solution was acidified with hydrochloric acid until the product was obtained, filtered off and recrystallized from suitable solvent (Table 1).

Also the method was used for alkylation N^1 , N^3 and SH of compound (2) by using three moles of alkyl halide, yielded the pyrimidine (3c) as yellow needles (yield 75%, m.p. 68-70 °C), Physical properties (Table 1).

4-Hydrazinouracil (4)

4-chlorouracil (0.005 mol, 1gm) was dissolved in methanol (95%) (15 ml), and hydrazine hydrate (0.005 mol, 0.35 gm) was added slowly with stirring to the solution, the whole was refluxed for three hours on steam bath. On cooling, the product was collected as a yellow precipitate, recrystallized from ethanol to give the compound (4). Physical properties (Table 1).

Schiff's Bases (5a-e)

Schiff's bases were prepared from (0.001 mol, 0.2gm) of compound (4) with aldehyde or ketone (0.001 mol) in ethanol (15ml). The resulting mixture was refluxed for three hours, and then cooled. The product was filtered off and recrystallized from ethanol, The Physical properties (Table 1).

Preparation of 4-(3',5'-Dioxo-2',3',4',5'-tetrahydropyrazol) uracil and 4-(3',5'-Dimethyl pyrazol) uracil (6,7).

A mixture of compound (4) (0.0015 mol, 0.21gm) and ethylmalonate or acetylacetone (0.0015 mol, 0.24 gm or 0.15 gm) was refluxed in dioxane (20ml) for five hours. The reaction mixture was allowed to cool, poured into cold water (60 ml). The solid product so produced was filtered off and recrystallized from ethanol to produce compound (6) and (7) respectively (yield 61%), Physical properties (Table 1).

2,6-Dihydroxy-4-pyrimidinylthioacetic acid (8)

Compound (1) (0.01 mol, 2gm) was dissolved in ethanol (15 ml), and a solution of mercaptoacetic acid (0.01 mol, 0.92 gm) in aqueous sodium hydroxide (10%) (10ml) was added gradually. The resulting mixture was refluxed for four hours, then cooled and acidified with concentrated hydrochloric acid until the yellow precipitate was formed, filtered off, dried and recrystallized from aqueous ethanol to obtain compound (8), Physical properties (Table 1).

Ethyl (2,6-Dihydroxy-4-pyrimidinyl) thioacetate & Propynyl (2,6-dihydroxy-4-pyrimidinyl) thioacetate (9,10)

The solution of compound (8) (0.005 mol, 1gm) in excess of thionyl chloride (10 ml), was refluxed gently on a water bath with stirring for three hours. The excess of thionyl chloride was removed under vacuum to give the corresponding acid chloride. Ethanol or propargyl alcohol was added (6 ml) and refluxed for two hours, the mixture was extracted with (10 ml) of benzene or chloroform, then (15 ml) of sodium carbonate solution, dried over anhydrous magnesium sulfate, filtered off and removed the solvent to give the compound (9) or (10) respectively, Physical properties (Table 1).

4-Thio acetic hydrazide Uracil (11)

The solution of compound (8) (0.002 mol, 0.4 gm) in thionyl chloride (6ml) was refluxed for three hours, and the excess of thionyl chloride was removed under vacuum to give acid chloride, pyridine (8ml) was added, then hydrazine hydrate (1ml) added dropwise to the mixture with cooling and stirring, left the stirring overnight at room temperature. The mixture was refluxed for two hours at 80 °C, and then cooled, the excess of pyridine was removed under vacuum, and the hydrazine derivative (11) was obtained, physical properties (Table 1).

4-(2'-Thio-1',3',4'-oxadiazol-5'-thiomethyl) Uracil (12)

Hydrazide compound (11) (0.01 mol, 1.8 gm) was dissolved in ethanol 95% (50 ml), sodium carbonate (0.01 mol) was added in (1 ml) of water. After a solution was occurred slightly then carbon disulphide (0.01 mol, 0.76 gm) was added and the mixture refluxed for three hours, after the mixture was concentrated by evaporating the excess of ethanol under vacuum to a small volume. A precipitate was obtained by adding the solution to ice containing hydrochloric acid, the solid was filtered off and dried, recrystallized from benzene yielded compound (12) (yield 45%, m.p. 225°C decomp.), physical properties (Table 1).

1-(4'-Uracil thioacetyl)-4-phenylthiosemicarazide (13)

To a solution of compound (11) (0.01 mol, 1.8 gm) in ethanol (30 ml), phenylisothiocyanate (0.01 mol, 0.27 gm) was added, and the reaction mixture was refluxed for one hour, the excess of the solvent was removed under vacuum. The product was extracted with ethylacetate and dried over anhydrous sodium sulphate to give compound (13) as oil in a good yield.

N'-Phenyl-2'-thio-1',3',4'-triazole-5-methyl-(2,6-dihydroxy-4-pyrimidyl) sulphide (14)

The mixture of compound (13) (0.015 mol, 3.53 gm) in 2N sodium hydroxide (20 ml) was refluxed for three hours, cooled and acidified with hydrochloric acid (10%) to give the compound (14), recrystallized from ethanol (yield 80%, m.p. 178-181 °C), (Table 1).

5-[2',6'-Dihydroxy-4-pyrimidinyl thioacetamido]-1,3,4-thiadiazol-2-thiol (15)

A solution of compound (8) (0.005 mol, 1 gm) in excess of thionyl chloride (8 ml) was heated under reflux on water bath with stirring for three hours, then the excess of thionyl chloride was removed under vacuum to give the acid chloride derivative. Then a solution of 5-Amino-1,3,4-thiadiazole-2-thiol (0.011 mol, 1.33 gm) in tetrahydrofuran, (50 ml) and triethyl amine (0.015 mol, 0.15 gm) was added, the resulting mixture was refluxed with stirring for four hours. Filtered off and the filtrate was concentrated to a small volume by evaporating the excess of the solvent, acidified with concentrated hydrochloric acid to give the precipitate which was filtered off and recrystallized from ethanol, physical properties (Table 1).

General procedure for preparation of compounds (16,17 & 18)

A solution of compound (12 or 14 or 15) (0.001 mol, 2.02 gm) and tri ethyl amine (0.01 mol, 1.091 gm) in ethanol (50 ml), was heated slightly, and alkyl halide (propargyl bromide, benzyl bromide or 2,4-dinitrochloro benzene) (0.01 mol, 1.19 gm) was added, the heating was continuous for four hours. Cooled, the precipitate was formed after addition of ice-water, filtered off and recrystallized from ethanol, physical properties (Table 1).

General procedure for preparation of Mannich bases compounds (19 & 20)

The mixture of acetylenic derivative (3a or 10) (0.002 mol) and paraformaldehyde (0.002 mol, 0.06 gm) in isopropyl alcohol (15 ml) was heated slightly, cuprous chloride (0.07 gm) was added and appropriate secondary amine (diethyl amine, dicyclohexyl amine or morphine) (0.002 mol) was added, the resulting mixture was refluxed for three hours then filtered off. The filtrate was poured onto crushed ice and extracted by chloroform, dried over anhydrous magnesium sulphate, filtered and the solvent was removed to obtain the product (19 & 20) physical properties (Table 1), (Scheme 3).

Table (1): Physical properties, yield, molecular formula and elemental analysis of the synthesized compounds.

Co. No	Molecular weight & Molecular Formula	Yield %	m.p. °C	Crystallization solvent	colour	C,H,N C/(F)
1	(C ₄ H ₃ N ₂ O ₂ Cl) 146.53	90	300 dec.	EtOH+H ₂ O	White	
2	(C ₄ H ₄ N ₂ O ₂ S) 144.15	70	235 dec.	EtOH+H ₂ O	Yellow	
3a	(C ₇ H ₆ N ₂ O ₂ S) 182.20	41	oil	Benzene	Red	46.15 (46.03) 3.29 (3.16) 15.38 (13.01)
3b	(C ₁₀ H ₅ N ₃ O ₃ Cl ₂) 318.14	60	150 dec.	EtOH	Red	37.7 (37.29) 1.57 (1.14) 13.20 (13.01)
3c	(C ₂₂ H ₁₀ N ₈ O ₁₄ S) 642.43	75	68-70	EtOH	Yellow	
4	(C ₄ H ₆ N ₄ O ₂) 142.12	51	270 dec.	EtOH	White	
5a	(C ₉ H ₈ N ₄ O ₃) 220.18	80	240 dec.	EtOH	Brown	
5b	(C ₁₁ H ₁₀ N ₄ O ₃) 246.22	75	231 dec.	EtOH	Orange	
5c	(C ₁₂ H ₉ N ₅ O ₃) 271	80	188-190	EtOH	Red	53.13 (53.02) 3.32 (3.21) 25.83 (25.24)
5d	(C ₉ H ₁₂ N ₄ O ₂) 208	75	115-118	EtOH	Red	
5e	(C ₁₄ H ₁₀ N ₄ O ₃) 282	60	150 dec.	EtOH	Black	
6	(C ₇ H ₆ N ₄ O ₄) 210	60	120 dec.	Chloroform	Gray	40.0 (39.25) 2.85 (2.54) 26.6 (26.13)
7	(C ₉ H ₁₀ N ₄ O ₂) 206	60	110 dec.	Benzene	Gray	
8	(C ₆ H ₆ N ₂ O ₄ S) 202	85	190 dec.	EtOH	Yellow	35.64 (35.12) 2.97 (2.53) 13.86 (13.49)
9	(C ₈ H ₁₀ N ₂ O ₄ S) 230	80	>300 dec.	EtOH	Brown	
10	(C ₉ H ₈ N ₂ O ₄ S) 240	40	oil	chloroform	Brown	45.0 (44.64) 3.33 (3.06) 11.6 (11.19)
11	(C ₆ H ₈ N ₄ O ₃ S) 188	60	Oil	chloroform	Red	33.33 (33.01) 3.7 (3.21) 25.9 (25.34)
12	(C ₇ H ₆ N ₄ O ₃ S ₂) 260	45	225 dec.	Benzene	Yellow	32.5 (32.21) 2.32 (2.16) 21.7 (21.36)
13	(C ₁₃ H ₁₃ N ₅ O ₃ S ₂) 353	90	150 dec.	EtOH	Brown	
14	(C ₁₃ H ₁₁ N ₅ O ₂ S ₂)333	80	179-181	EtOH	Green	46.84 (46.42) 3.30 (3.12) 21.02 (20.89)
15	(C ₈ H ₇ O ₃ N ₅ S ₃) 317	82	Oil	Chloroform	Black	30.6 (30.26) 2.18 (2.03) 20.43 (20.13)
16a	(C ₁₄ H ₁₂ N ₄ O ₃ S ₂)348	62	290 dec.	Methanol	Yellow	
16b	(C ₁₃ H ₈ N ₆ O ₇ S ₂)424	66	210 dec.	Methanol	Yellow	
17	(C ₁₆ H ₁₃ N ₅ O ₂ S ₂)371	60	Oil	EtOH	Brown	
18	(C ₁₁ H ₉ N ₅ O ₃ S ₃)335	50	Oil	Chloroform	Black	
19a	(C ₁₀ H ₁₃ N ₃ O ₂ S)239	40	Oil	Benzene	Red	
19b	(C ₁₂ H ₁₅ N ₃ O ₃ S)281	50	Oil	Chloroform	Dark brown	
20a	(C ₁₂ H ₁₅ N ₃ O ₄ S)297	40	Oil	Chloroform		
20b	(C ₂₂ H ₃₁ N ₃ O ₄ S)433.5	42	Oil	Benzene		

Table (2): Spectral data for synthesized compounds (IR cm⁻¹), (λ nm).

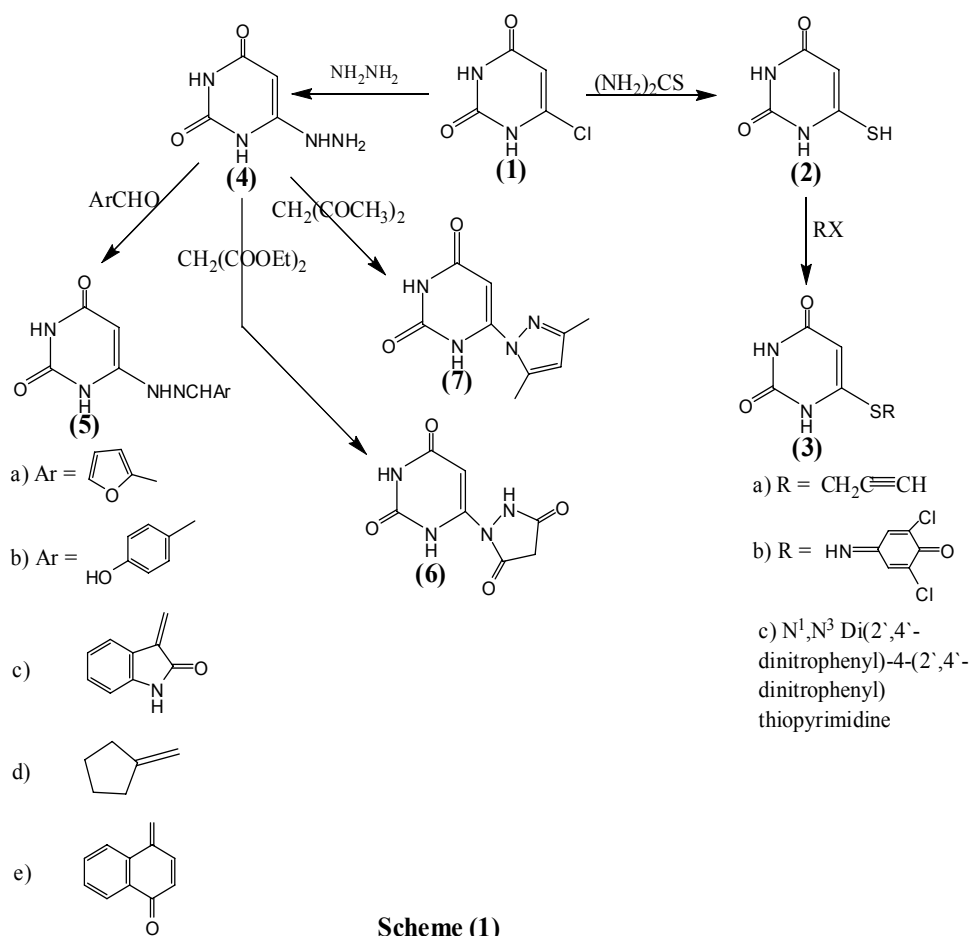
Co. No.	γ - OH	γ N-H	γ C=N	γ C=O	γ C=C arom	γ C-H aliph.	γ C-S	γ other	λ max EtOH (95%), 10 ⁻³			
1	3600	3350	1640	1710	1590	--	--	γ C-Cl 760	247	303	354	
2	3620	3250	1650	1710	1500	--	780	γ S-H 2250	255	280	370	
3a	3500	3200	1600	1700	1500	2850	780	γ C≡CH 3200-3250	244	295	303	372
3b									246	372	383	
3c									245	260	280	372
4	3650	3350	1650	1700	1570	--	--	γ NH ₂ 3365	344	371	454	
5a	3510	3250	1650	1640	1620	2850	--	γ C-O 1150	245	319	372	381
5b	3600	3200	1680	1710	1550	--	--	γ C-H Ar 3100				
5c	3600	3220	1600	1700	1600	--	--	C-H Ar 3100	244	267	374	413
5d	3600	3200	1600	1700	--	2850	--	--				
5e									242	373	382	419
6									241	299	333	373
7									243	267	323	372
8									244	304	354	371
9									240	300	349	370
10	3500	3200	1600	1710	1550	2850, 2900	790	γ C≡C 2100, C≡CH 3250	210	250	272	371
11									242	310	340	371
12	3600	3200	1620	1690	1600	2950	780	C-O-C 1100, SH 2250	244	267	310	329
13									246	300	312	372
14	3500	3150	1600	1610	1550	2900	780	γ C-H Ar 3100, SH 2250	246	325	372	383
15	3600	3200	1600	1700	1500	2850	780	γ C=O Amide 1680	244	268	300	370
16a	3600	3150	1600	1650	1610	2850	780	γ C-O-C 1150 CH Ar 3000	245	260	320	340
16b	3600	3150	1600	1680	1600	2900	780	γ c-o-c 1150, NO ₂ 1560				
17	3600	3200	1610	1640	1550	2880	780	γ C≡C 2150, C-H 3000, C≡CH 3500	245	267	311	373
18	3500	3150	1620	1720	1490	2900	780	γ C≡C 2100	246	250	312	371
19a									209	242	269	
19b									208	244	301	389
20a									210	250	272	371
20b									215	248	280	370

Table (3): Chromatographic behaviour of the synthesized compounds.

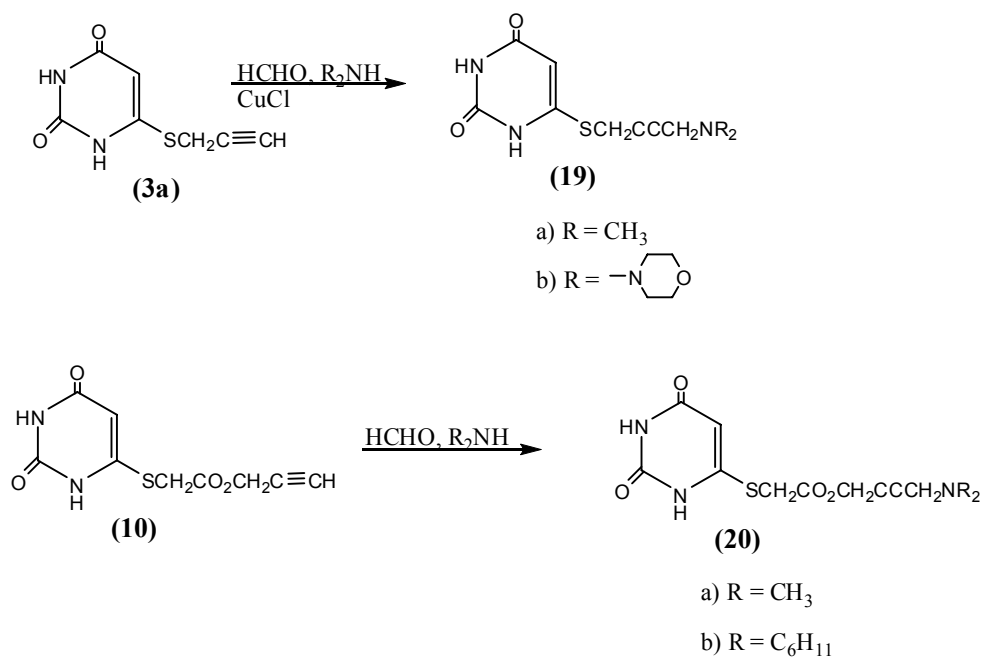
Compound No.	R _f value in system (1)	R _f value in system (2)
1	0.68	0.67
2	0.73	0.75
3a	0.45	0.59
3c	0.54	0.5
4	0.57	0.49
5a	0.56	0.57
5b	0.58	0.60
5c	0.59	0.67
6	0.98	0.92
7	0.95	0.89
8	0.41	0.46
9	0.32	0.35
10	0.73	0.70
11	0.51	0.47
12	0.44	0.41
13	0.31	0.36
14	0.45	0.42
15	0.97	0.99
16a	0.40	0.37
16b	0.38	0.35
17	0.82	0.85
18	0.42	0.45
19a	0.79	0.61
19b	0.66	0.58
20a	0.73	0.70
20b	0.60	0.62

System (1) : Water : ammonia : propanol (1 : 3 : 6)

System (2) : Water : acetic acid : 1-pentanol (3 : 2 : 6)



Scheme (1)



Scheme (3)

RESULTS AND DISCUSSION

The anti-bacterial, anti-viral and anti-cancer activities of various thio, amino and hydroxy pyrimidines have been studied by many workers. In addition, various fused pyrimidines have been reported⁴.

The present work describes the synthesis of some new 4-substituted uracil and also the synthesis of its triazole, oxadiazole and thiadiazole derivatives.

The chloropyrimidines are of a great preparative importance. Thus, 4-chlorouracil⁵ was used as starting material, and the replacement of an active chlorine atom in position (4) of uracil by nucleophilic groups (mercapto, thioalkyl, hydrazino, thioacid,.... Etc.) is normally a convenient route to obtain 4-substituted uracil derivatives⁶.

As shown in scheme 1 heating under reflux a solution of 4-chlorouracil (1) in ethanol with thiourea yield the corresponding 4-mercaptouracil (2)⁷. Its structure was confirmed by physical properties (Table 1), IR spectrum & UV spectrum (Table 2), showed the presence of SH- group stretching (2250-2650 cm⁻¹) and the absence of the stretching band at (760 cm⁻¹) due to C-Cl and R_f values (Table 3).

Thiols behaved as strong nucleophile, therefore undergo the alkylation easily with alkyl halides as SN² mechanism to give the corresponding sulphides^{8,9}. Alkylation of compound (2) with equivalent amount of alkyl halides in potassium hydroxide, while cooling, the mixture after acidification yielded the corresponding 4-alkyl thiouracil (3), but, using propargyl bromide in the presence of triethyl amine in ethanol resulted in the formation of 4-

alkynyl thiouracil (3a). The structure was confirmed by physical properties & C,H,N (table 1) and the IR spectrum (Table 2) showed the presence of ($\equiv\text{CH}$) stretching absorption at (3200 cm^{-1}) and ($\text{C}\equiv\text{C}$) weak stretching absorption at (2100 cm^{-1}) also disappearance of stretching band at ($2550 - 2650\text{ cm}^{-1}$) due to SH group, the UV spectrum (Table 2) and R_f values (Table 3), also the structure of (3_b) was confirmed by physical properties & C,H,N (Table 1), spectral data (Table 2) and R_f values (Table 3).

Treatment of compound (2) with three moles (excess) of 2,4-dinitrochlorobenzene as active aryl halide lead to the formation of the corresponding N^1, N^3 -di(2,4-dinitrophenyl)-4-(2',4'-dinitrophenyl thio) pyrimidine (3c) physical properties (Table 1). IR spectrum, UV spectrum (Table 2) and R_f values (Table 3) of compound (3a-c) are in agreement with assigned structures, (c.f. Experimental and Scheme 1).

The 4-hydrazino uracil (4) was obtained by refluxing compound (1) with equimolar of hydrazine hydrate in ethanolic solution. The structure of compound (4) was identified by IR & UV (Table 2) and R_f values (Table 3). While the reaction of compound (4) with equimolar of selected aromatic or heterocyclic aldehydes or ketone (α -furfural, p-hydroxy benzaldehyde, Isatin, cyclopentanone and 1,4-benzoquinone) in ethanol afforded the corresponding Schiff's bases (5a-e) and their structures were confirmed by physical properties, C,H,N (Table 1) spectra data (Table 2) and R_f values (Table 3).

Recently, there has been a great deal of interest in the synthesis of Uracil derivatives possessing various functional groups by cyclization or by substitution reactions. Refluxing compound (4) with dicarbonyl derivatives (diethylmalonate or acetyl acetone) in dioxane yielded the corresponding compounds (6 and 7) respectively, which have a new ring system¹⁰. The spectroscopic data is in agreement with the signed structures 6 & 7 physical properties and C,H,N (c.f. Experimental and Scheme 1)

Uracil thiocarboxylic acid and their esters can be synthesized by nucleophilic attack to 4-chlorouracil with a mercaptoacetic acid in alkaline solution by using potassium hydroxide in ethanol and refluxing for four hours to give the thio acid (8). Its structure was confirmed by physical properties C,H,N (Table 1), IR & UV spectra (Table 2) and R_f values (Table 3), (Scheme 2)

The importance of synthesizing new acetylenic ester arises from their potential biological activity. Uracil thio acid (8) was treated with thionyl chloride to the corresponding acid chloride which was the reactive reactant in the synthesis of esters (9 & 10) or the acid hydrazide (11) by using ethanol or propargyl alcohol or hydrazine hydrate, their physical properties and C,H,N (Table 1) and spectra data (Table 2) and R_f values (Table 3).

Treatment of the appropriate acid hydrazide derivative (11) with carbon disulphide in alkaline medium caused cyclization by internucleophilic attack to give the corresponding oxadiazole derivatives (12)¹¹. The structure was identified by physical properties C,H,N (Table 1) and spectra data (Table 2) and R_f values (Table 3). While the refluxing of the acid hydrazide (11) with phenylisothiocyanate in ethanol gave the compound (13) and the cyclization was occurred in alkaline solution (2N-NaOH) to give the triazole derivative (14), also the structure was confirmed, physical properties, C,H,N (Table 1), spectra data (Table 2) and R_f values (Table 3).

But the thiadiazole derivatives (16) was synthesized¹² by refluxing the acid chloride derivative of uracil (8) with 2-amino-3H-1,2,4-thiadiazole-2-thiol (15) in the presence of triethyl amine in tetrahydrofuran (THF) for four hours to give the compound (16), its

structure was confirmed by physical properties C,H,N (Table 1), spectra data (Table 2) and R_f values (Table 3).

The alkylation of compounds (12,14,15) with an equivalent amount of halide (2,4-dinitro chloro benzene, benzyl chloride and propargyl) in the presence of triethyl amine in ethanol resulted the compounds (16_{a,b}, 17,18). The structure were identified by physical properties (Table 1), spectra data (Table 2) showed the presence of C-S-C stretching absorption band at 780 cm^{-1} and disappearance of the stretching band of $2250\text{-}2660\text{ cm}^{-1}$ due to SH group.

Consequently the alkynyl derivatives (3_a, 10) which were heated under reflux with paraformaldehyde, appropriate secondary amine (N,N-dimethyl amine, morpholine and N,N-dicyclohexylamine) and catalytic amount of cuprous chloride to increase the nucleophilicity of acetylenic linkage in the isopropyl alcohol were used as solvent to give the corresponding Mannich bases (19_{a,b} and 20_{a,b}), (Scheme 3) the structures were confirmed by physical properties (Table 1); spectra data (Table 2) showed the presence of $\text{-CH}_2\text{-}$ stretching absorption band at $2850\text{-}2900\text{ cm}^{-1}$ and disappearance of the stretching band at 3300 cm^{-1} due to $\text{C}\equiv\text{C-H}$ and R_f values (Table 3).

Finally, the antimicrobial activity of the synthesized compounds were tested in vitro against eight species of bacterio (Escherichia coli, proteus, sp, Salmonella, SPP, Pseudomonas aeruginosa, Strepto coccus, Klebsiella SP, Staphylococcus, and Candida albicans) by using agar diffusion method^{13,14,15}. The results are shown in the following Table 4.

Table (4): Effects of some new compounds on the growth of bacteria (zone of inhibition in mm.).

Co. No.	E. Coli	Proclus SP	Salmonella	Pseud.	stre	kleb	Staphylo	Candida
2	-	-	-	-	-	++	-	+
3b	+++++	++++	+++	+++++	+++++	+	-	-
3c	++++	++++	++++	++	++++	-	-	-
5c	+++++	++	+++++	++++	+++++	++++	++	+
7	-	-	-	-	-	+++	-	+
8	+++++	++	++	++	++	-	-	-
9a	-	-	-	-	-	++	±	±
11	++	+++	+++	++++	+++++	-	-	-
12	++	++	++	±	+	+++	-	-
13	+	++	++	++	++++	++++	++	±
14	++	+	+++	+	+	++	-	-
15	-	-	±	-	-	-	-	±
16a	±	+	-	-	-	++	-	-
17	++	+++	+	+	++	+++	-	±
18	-	+++	++	-	+	++	-	-

0-3 mm 2(-), 6-9 mm (±), 10-14 mm = +, 15-18 mm =(++),19-21 mm =(+++), 22-28 mm =(++++), and 29-35 mm =(+++++)

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تحضير وتشخيص بعض مشتقات اليوراسيل الجديدة وفعاليتها البيولوجية

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ملخص:

في هذا البحث تم تحضير وتشخيص حوالي ثلاثين مشتق جديد لليوراسيل ومنها: تفاعلات الاستبدال النيوكليوفيلية (ثيول، ثيوالكيل، هيدرازينو، حامض ثيوخليك) للمركب 4-كلورو يوراسيل تعطي المشتقات المقابلة (2، 3-c، 4، 5-a، 6، 7، 8)، وكذلك المركبات 4-(2-ثايول-1،3،4-اوكساديازول-5-ثيومثيل) يوراسيل (12) وكبريتيد(ن-فينيل-2-ثايول-1،3،4-ثرايازول-5-مثيل) (2،6)-ثنائي هيدروكسيل -4-بريميديل) 14 وايضا 5-(2،6)-ثنائي هيدروكسيل-4-بريميديل ثايواسيتاميد-1،3،4-ثايوديازول-2-ثايول (15)، ثم تحضيرها بمعاملة المشتق 4-ثايوخليك هيدرازيد يوراسيل (11) مع ثاني كبريتيد الكربون او فنيل ايزوسيانات بوجود القاعدة.

تم تحضير قواعد ماننيخ (19 و 20) و (b، a) للمشتقات الاستيلينية (3 و 10). اثبتت الصيغ التركيبية لهذه المركبات باستخدام الاجهزه الطيفيه (IR, UV) والتحليل الدقيق للعناصر (C,H,N) وقياس قيم R_f ، ثم تم تعيين فعاليتها البيولوجية.

Effect of Silica Fume on the Hydration Kinetics of Atbra Cement Pastes Produced in Sudan

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ABSTRACT

Blended cement pastes were prepared with and without super plasticizer using an initial water/cement ratio of 0.30. Hydration kinetics were followed by determining the non-evaporable water and free lime contents at various hydration times, covering the range from 0.5 hour up to 28 days. The results of hydration kinetics indicated three-stages in the hydration reaction; these are the "dormant", the "accelerations" and the diffusion periods. In this study, Atbra Portland cement pastes were prepared with Silica fume as an admixture.

This work is aimed to evaluate the effect of Silica fume on the effect of this admixture on the hydration reaction of cement pastes at different intervals of time (0.5 hours up to 28days).

Keywords: Portland cement, Silica fume, hydration kinetics.

1. INTRODUCTION

Portland cement is a multi component system. The main factors that affect the rate of cement hydration are cement composition, cement fineness, water-to-cement ratio, curing time, and curing temperature when ordinary Portland cement (opc) is mixed with water; a series of chemical reactions begins to take place. The reactions of cement with water proceed at different rates for the various mineral phases and involve both hydrolysis and hydration processes [1]. Hydration is a chemical process that, from the anhydrous material through several chemical reactions, leads to the formation of hydrates. This complex process has thermodynamic, kinetics and structural features which depend on both chemical and physical parameters [2]. Super plastizers are now widely used in the production of concrete with excellent workability, for easy placement with out reduction in cement content and strength. These admixtures are extremely effective for dispersing cement particles in water. The dispersion mechanism has been described in terms of electrostatic repulsive forces between the cement particles followed by adsorption of charged superplastizer molecules. Several reports have been concerned with the improvement of the strength and development of Portland



cement using admixtures [3-5]. Superplastizers have been used to reduce the water of consistency and to improve the workability of cement pastes and consequently concrete, leading to improvement in mechanical properties and resistance towards environmental deterioration, chemical attack and pastes at early stages.

Silica fume: quartz reduced in an electric arc furnace - some SiO₂ volatilization and oxidation produces largely glassy SiO₂ particles of ≈100 nm diameter. Low density material with 86-95% reactive SiO₂ [6]. In the present paper, Silica fume was used as additive to the blended cement pastes made with an initial water/cement (w/c) ratio of 0.30 by weight as Reported [7]. The effect of this admixture on the hydration kinetics of hardened cement pastes was clarified.

2. EXPERIMENTAL

Materials used in this investigation are Portland cement produced in Sudan, Atbra, which is designated as At for Atbra cement pastes, Chemical oxides composition as determined by using x-ray fluorescence and chemical analysis for the Portland cement is shown in table 1.

Table (1): The chemical oxides composition (%) of the sample of ordinary Atbra Portland cement.

Oxides	CaO	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	MgO	SO ₃	Ign.loss
Atbra cement	63.6	21.6	4.2	3.0	2.4	2.7	1.48

The specific surface area determined was 2777 m²/g. Various cement pastes were prepared by mixing with and with-out additive, with water using a w/c ratio of 0.30 by weight mixing was done for 3 minutes continuously and designated by At for cement without additive and, and (SC) for cement with Silica fume. Hydration kinetics was studied by determining the non-evaporable water contents as well as the free lime contents for various cement pastes, after curing for 0.5, 1, 3, hours and 1, 3, 7 and 28 days. The details about the methods used for the determination of non-evaporable water and free lime contents were fully described in an earlier paper [8]. Non-evaporable water (chemically combined) W_n was determined by heating certain weight of dried sample at 1000°C until constant weight. It was calculated on the heated basis and designated as W_n and corrected with respect to free lime content.

$W_n(\text{corr}) = W_n - (Y \times Z)$, where Y is the free lime content and Z is the ratio of molecular weight of water to CaO (18/56).

Designation:

At = Atbra cement + water.

SC = Atbra cement + silica fume + water

3. RESULTS AND DISCUSSION

Hydration Kinetics

The results of non-evaporable water content (Wn%) and free lime contents(CaO%) for the cement pastes are given in table 2 and 3, and graphically represented as a function of curing in figures 1,2,3, and 4, respectively.

The results of non-evaporable water contents shown in figures 1, 2 indicate a minor increase in the non-evaporable water content from 0.5 hour up to 3 hours of hydration for the neat cement paste (C). During the interval 0.125-1 day hydration, there was a marked increase in the rate of hydration followed by a gradual change of the rate of hydration in the later stages up to 3 days. The values of the combined water contents of the cement pastes mixed with 0.3 by (weight of cement) of superplastizer showed the same trend of hydration as that of the neat cement pastes but there was a slight change in the rate of hydration during the first 3 hours.

Results of free lime contents shown in figures 3 and 4 indicate the same changes as in the non-evaporable water contents. Therefore, gradual changes in the free lime contents were observed during the first 3 hours of hydration, followed by a noticeable increase in the free lime contents in the hydration period of 0.125-3 days. Finally the free lime contents indicate a gradual change with increasing hydration age up to 28 day, again, the addition of superplastizer to the blended cement used in this investigation causes minor changes in the rate release of the free lime with age of hydration.

The results of degree of hydration indicated that the addition of 0.3 % (by weight of content) of superplastizer to the blended cement pastes causes a minor retardation of the rate of hydration especially during the first 3 hours.

The results of hydration kinetics, including non-evaporable water contents, free lime contents of these blended cement pastes made with and without superplastizer indicate a minor increase during the early hydration stages from 0.5 hours up to 3 hours (0.125 days). This initial period represents the formation of the initial coating of hydration products on the cement grains with an almost impervious character, this stage is known as "dormant" period [9,10] since it leads to a retardation of the hydration process.

Hydration characteristics of silica fume reacts relatively fast in the cement system. Pastes require higher water content than silica fume-free ones unless a superplasticiser is added. The silica is consumed in reaction with Ca (OH) lime-rich C-S-H resulting in a paste with lower (or no) Ca (OH)₂ and a C-S-H CaO:SiO₂ ratio (maybe as low as 1.2) [6].

Table (2): Combined Water (Wn %) (Non –evaporable water) of Atbra (Sudani) Cement Pastes with and without Silica fume

Age of hydration	w/c ratio 0.3 At (Atbra cement with silica fume)	w/c ratio SC (Atbra cement without silica fume)
0.021	0.11	0.4
0.041	0.19	0.45
0.125	0.25	0.49
1.000	1.00	3.11
3.000	3.11	3.70
7.000	3.99	8.60
28.000	5.18	10.23

Table (3): Free lime Content (CaO %) of Atbra (Sudani) Cement Pastes with and without Silica fume.

Age of hydration	w/c ratio 0.3 At (Atbra cement without silica fume)	w/c ratio SC (Atbra cement with silica fume)
0.021	0.33	0.28
0.041	0.38	0.32
0.125	0.44	0.35
1.000	2.57	1.67
3.000	3.16	2.85
7.000	3.82	3.00
28.000	5.68	4.40

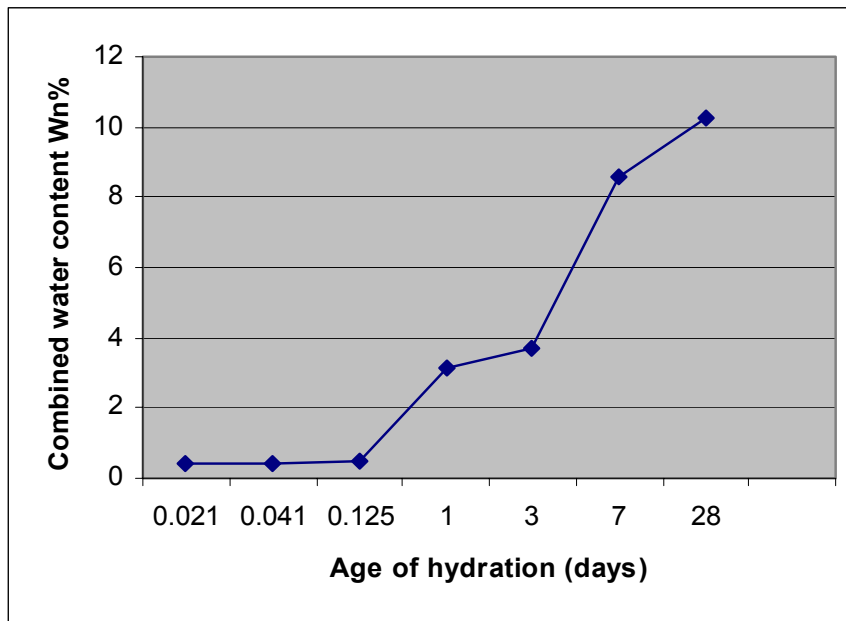


Figure (1): Combined Water (Wn%) (Non –evaporable water) of Atbra (Sudani) Cement Pastes without Silica fume.

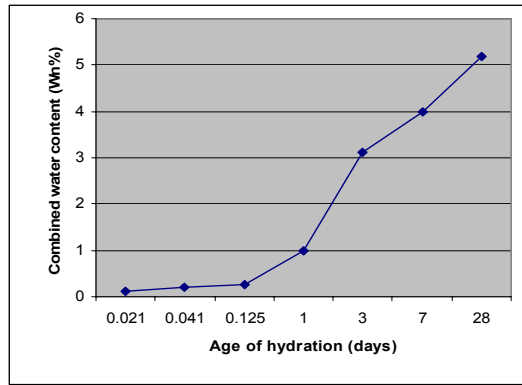


Figure (2): Combined Water (Wn%) (Non –evaporable water) of Atbra (Sudani) Cement Pastes with Silica fume.

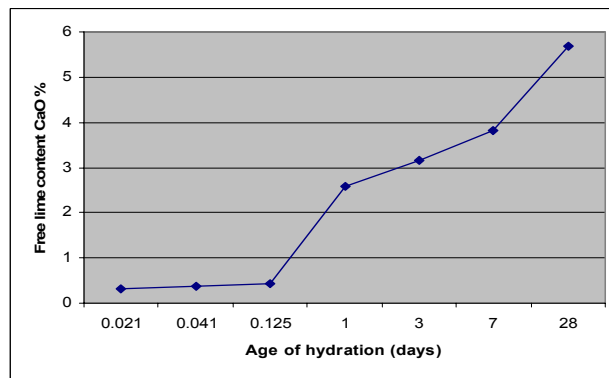


Figure (3): Free lime Content (CaO %) of Atbra (Sudani) Cement Pastes with Out Silica fume.

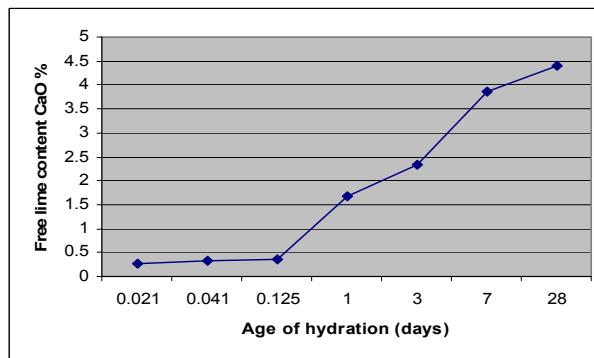


Figure (4): Free lime Content (CaO %) of Atbra (Sudani) Cement Pastes with Silica fume.

In fact, the addition of admixture to the blend cement pastes results in two main opposing effects, which are:

1. A retardation effect on the rate of the hydration of cement which is mainly attributed to the interaction between calcium ions and admixture.

2. A production of a more dense and close textured structure of the resulting blended cement paste including admixture; and this effect leads to an increase in the rate of hydration of the blended particles through activation by the calcium hydroxide liberated during the early hydration stages of the clinker particles; by this way the Ca⁺ ions will find a short diffusion-path between clinker and blended grains due to the more packed structure of the paste.

These two effects operate at the same time in opposite directions and lead finally to either an increase or a decrease in the rate of hydration during the early stages. After 3 hours (0.125 day) hydration, the initially formed impervious coating hydration products were dispersed and/or crystallized leading to an increased accessibility of water through the hydration coating into the unhydrated parts of the cement grains. This period leads to an increase in the rate of hydration with formation of new inner hydrates, located deeper in the cement grains, which is known as the acceleration period [8,9]; it starts from 0.125 day and ends at about 3 days in the hydration of these blended cement pastes. Finally, the non-evaporable water, water contents, free lime contents again showed a gradual change with curing age up to 28 days of hydration. This can be attributed to the accumulation of larger amounts of hydration products within the originally water-filled spaces (pores) of the hardened pastes.

Consequently, the diffusion of water through the dense hydration products into the remaining unhydrated part of cement grains becomes the rate controlling step; this period is called "diffusion" period [8, 11, 12].

The addition of admixtures (0.3%) by weight of cement to the blended cement in this investigation causes a slight change in the rate of hydration, especially during the final hour's hydration. A compensation effect of two parameters, described earlier in this stage may be noticed during the intermediate state of the hydration process.

4. CONCLUSIONS

To summarize, the addition of silica as an admixture to the blended cement pastes results in two opposing effects, namely, retardation of the rate of hydration through the interaction between Ca²⁺ ions and silica as an admixture, and the production of more dense structure of the blended cement pastes, which increases the rate of hydration of the early stages of the clinker particles.

These two effects operate at the same time in opposite directions and eventually lead to either increase or decrease in the rate of hydration during the early stages. A compensation effect of the two parameters may be noticed during the intermediate stages of hydration.

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دراسة تأثير المضافات على عملية الهدرتة للإسمنت المنتج في السودان – اسمنت عطبرة

فاطمة القادري

قسم الكيمياء - كلية العلوم - جامعة صنعاء

ملخص

حضرت عينات من الاسمنت البورتلاندي المنتج في السودان باستخدام المضاف وبدون المضاف ، والمضاف هو السلكا بخلط الاسمنت الجاف مع الماء بنسبة وزنية 0.3%. وسميت العجائن المحضرة من اسمنت السودان بدون مضاف At_0 ومع المضاف sc . ثم أجريت عملية التادرت لجميع العجائن الاسمنتية لفترات زمنية من نصف ساعة إلي 28 يوما، وعند كل زمن من تفاعل التادرت تم إيقاف تفاعل التادرت لكل عجينة. وقد أجريت اختبارات حركية تفاعل التادرت حيث تم دراسة حركية التادرت من خلال تقدير محتوى الماء المتحد كيميائيا الماء غير المتطاير وكذلك تقدير محتوى الجير الحر عند الأزمنة المختلفة من تفاعل التادرت . ومن النتائج التي تم الحصول عليها من هذه الدراسة أمكن استخلاص الاستنتاجات التالية:
1-أوضحت نتائج حركية التادرت أن درجة التادرت تزداد كلما زاد زمن التادرت.
2-عملية التادرت يتم بثلاث مراحل:المرحلة البطيئة، والمرحلة المحفزة ، المرحلة السريعة.

Studies on the Effect of Mono-Alkoxy Pyrophosphato Coupling Agent on the Mechanical Properties of Magnesium Hydroxide Filled HDPE/EVA/Mg(OH)₂/Composites

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ABSTRACT

Effect of treatment of coupling agent (mono-alkoxy pyrophosphato coupling agent) on mechanical properties of composites made from HDPE/EVA/Mg(OH)₂/ Composites is reported here. The coupling agent in the form of solution (1.5%) was used for treatment of the filler .The treatment resulted in enhancement of mechanical properties of composites when compared with composites containing untreated Magnesium hydroxide. The properties under consideration were tensile strength, modulus at (%) elongation at break, elastic modulus, hardness, etc. Although good reinforcement was observed due to treatment of 1.5% coupling agent, observed was very much remarkable by its comparing to untreated once. Comparison of properties of composites filled with treated and untreated Magnesium hydroxide established that the treatment of Magnesium hydroxide imparts better reinforcing properties. The properties under consideration were tensile strength, (%) elongation at break, elastic modulus, hardness, etc. Tensile strength was improved by 19.10%, (%) elongation at break was improved by 9.01%, elastic modulus was improved by 100%, while hardness was improved by 0.5%, at (0.14) volume fraction.

Keywords: Titanate Coupling Agent, Mechanical Properties of Magnesium hydroxide, HDPE/EVA/Mg(OH)₂/TCA-114.

INTRODUCTION

Many engineering polymer materials are multiphase systems. Polymer blends are physical mixtures of structurally different homo- or copolymers. Polymer alloys may be considered as a subclass of polymer blends and is a term that describes multiphase polymer systems with a modified interface [1]. The addition of fillers can significantly change the mechanical characteristics of a material. However the existence of fillers is sometimes essential for



maintaining the mechanical integrity of a material. Some of these additives, such as the coupler/coupler solvent dispersions or the latex, were prepared in solutions and could not be easily extracted to form solid films for evaluation [2]. Coupling agents are additives used in reinforced and filled plastic composites to enhance the plastic–filler–reinforcement interface to meet increasingly demanding performance requirements. In general, there is little affinity between inorganic materials used as reinforcements and fillers and the organic matrices in which they are blended. With silicate reinforcements, silane coupling agents act by changing the interface between the dissimilar phases. This results in improved bonding and upgraded mechanical properties [3].

Magnesium hydroxide as a filler play a key role in modifying and enhancing the mechanical properties of rubber, ceramic, paint, plastic and other industries [4-5].

It is reported [6-8] that titanate coupling agents are considered useful as promoters of adhesion between mineral fillers and organic matrix. These additives provide improved mechanical strength as well as chemical resistance to composites. the Titanate coupling agent with the appropriate functionality provide chemically bonded coupling agent between this mineral filler particles and the plastic network and is responsible for the improved reinforcing action of mineral fillers.

In this work the effect of titanate coupling agent on mechanical properties of composites made from and Magnesium hydroxide (treated and untreated) has been studied. Treated $Mg(OH)_2$ is prepared by mixing 1.5 gram of titanate with 100 g of $Mg(OH)_2$, while untreated does not content coupling agent. Composites of HDPE/EVA with treated and untreated $Mg(OH)_2$ were prepared in various loadings . The magnitudes of properties of composites containing treated and untreated filler are compared.

Tensile strength, (%) elongation at break, elastic modulus, hardness, etc. are used to study the mechanical properties of prepared polymer composites.

EXPERIMENTAL

Materials

High-density polyethylene (HDPE, 5502#, MFR=0.35g/10min) was from Daelim, Korea, Ethylene vinyl-acetate copolymer (EVA, 8450#, VA=15%wt, MFR=1.5g/10min) was from Nippon, Japan. The filler magnesium hydroxide ($Mg(OH)_2$, average particle size=2 μ m) was obtained from Dalian Yatai Science and Technology New Material Co.Ltd. , China, Titanate coupling agent [(TCA-114)] was obtained from Anhui Tianchang Organic Chemical Plant is the organotitanium pilot base of Shanghai Institute of Organic Chemistry.

General characteristics Ethylene vinyl-acetate copolymer, general Characteristics of High Density Polyethylene, Physical characterization of Titanate coupling agents (TCA-114), General Properties of Magnesium hydroxide, are reported in table 1, 2 and 3 respectively.

Table (1): General Characteristics of Ethylene vinyl-acetate copolymer.

Trade Name	NUC 8450 , Nippon Unicar Co ,Ltd
Appearance	White
Mooney Viscosity (100 ⁰ C)	40
Specific Gravity (g/cm ³)	0.94
Melt index (g/10 min)	0.1 -10
Ash Content (%)	0.3

Table (2): General Characteristics of High Density Polyethylene.

Trade Name	HDPE, 5502 , Daelim, Korea
Appearance	White
Specific Gravity (g/cm ³)	0.96
Melt index (g/10 min)	0.05-0.8

Table (3): Physical characterization of Titanate coupling agents (TCA - 114).

Chemical Name	mono-alkoxy pyrophosphato coupling agent
Typical purity (%)	99
Physical form	Liquid
Color	light yellow viscous liquid
Density	(GB4472-84) D30 About 1.03g/cm ³
Flash point (°c)	(GB37-77)open 50
Refractive index	(GB6428-86)ND30 About 1.45
Viscosity (cp)	(GB265-70) 30 About 300mm/s
pH	3
Solubility	Isopropyl alcohol, xylene, Toluene, DOP, Mineral oil, MEK .

Table (4): General Properties of Magnesium hydroxide .

Name	Magnesium hydroxide
Molecular formula	Mg(OH) ₂
Molar mass (g/mol)	58.33
Appearance	White solid
Melting point (°C)	Decomposes at 623 K (350 °C)
Density (g/cm ³)	2.4
Solubility	0.0012 g in 100 g water
pH	6.5-7

Treatment on Magnesium hydroxide by Titanate Coupling Agent

The coupling agent (1.5g) was mixed ^[9-13] with isopropyl alcohol (100 ml) to make a solution for applying to filler (100 g). 1.5 grams of coupling agent was used per 100g of Magnesium hydroxide. The filler (Magnesium hydroxide) was mixed with the solution of coupling agent in isopropyl alcohol with stirring to ensure uniform distribution of the coupling agent, mixing continued for 30 minutes. The treated filler (Magnesium hydroxide) was then dried at 100 °C in an oven for about 6 hours to allow complete evaporation of the alcohol.

Preparation of composites

EVA/HDPE/Mg(OH)₂/TCA-114 composites were prepared via melt compounding at 160 °C in ThermoHaake rheomixer with a rotation speed of 60 rpm, and the mixing time is 6 min for each sample. The mixed samples were transferred to a mold and preheated at 180 °C for 15 min, then pressed at 20 MPa and then successively cooled to room temperature while maintaining the pressure to obtain the composites sheets for further measurements. Before mixing, all the components were dried in vacuum oven at 80 °C for at least 12h.

Table (5): Compounding Recipe For HDPE/EVA/Mg(OH)₂ / TCA-114 .

Volume Fraction of Composites	EVA /HDPE (Wt – g)	Mg(OH) ₂ (Wt – g)
0.00	25/25 =50	0.0
0.04	22.5/22.5 =45	5
0.09	20/20 =40	10
0.14	17.5/17.5 =35	15
0.21	15/15 =30	20
0.28	12.5/12.5 =25	25
0.37	10/10 =20	30
0.48	7.5/7.5 =15	35
Filler (Treated &Untreated)	Variable (0.0 – 0.48 Volume fraction)	
Curing Time (min)	15	
Curing Temp (°C)	180	

Scanning electron microscopy (SEM)

The SEM micrographs of samples were observed by JEOL JSM-5510 scanning electron microscope. The samples are chosen after the tensile test. The content of HDPE/EVA/Mg(OH)₂ at 0.48 Volume fraction . The surface of the treated and untreated samples was coated with a thin layer of gold to avoid electrostatic charging during examination. Photographs of representative areas of the sample were taken at 5000X magnifications.

Measurement of Mechanical Properties

Mechanical properties such as tensile strength, elongation at break, elastic modulus were determined by subjecting dumbbell shaped specimens (in confirmation with ASTM D – 638) to a universal testing machine (Shenzhen Reger Instrument Co. Ltd, China). The sheets from which specimens were cut had been conditioned for 24 hours prior to subjecting to universal testing machine (100 kg load cell), at a crosshead speed of 50 mm / min. Hardness was measured on Machine –LX–A ,produced by Shanghai , Liuzhong meterage , factory .

RESULTS AND DISCUSSION

Tensile strength

The dependence of the tensile strength on volume fraction of magnesium hydroxide is represented in figure 1. It is seen that on increasing the volume fraction of (both treated and untreated) magnesium hydroxide , the tensile strength increases up to a certain value and it declines .The peak values of tensile strength of the composites correspond to 12.5 MPa and 10.5 MPa for treated and untreated Magnesium hydroxide composites respectively. It is noteworthy that the tensile strength of composites filled with treated Magnesium hydroxide at 0.14 volume fraction is 1.19 higher than that of untreated Magnesium hydroxide composites.

Modulus at (%) elongations at break

The dependence of modulus at (%) elongation at break with volume fraction of treated and untreated HDPE/EVA/Mg(OH)₂ Composites is depicted in Fig. 2. In the cases modules for treated one increased initially, attained the maximum value for particular value of concentration of fillers and decreased. The modulus of treated magnesium hydroxide at

0.14 volume fraction is about 10.01 times higher than that of untreated magnesium hydroxide .The rate of increment in the property with increasing volume fraction of the filler.

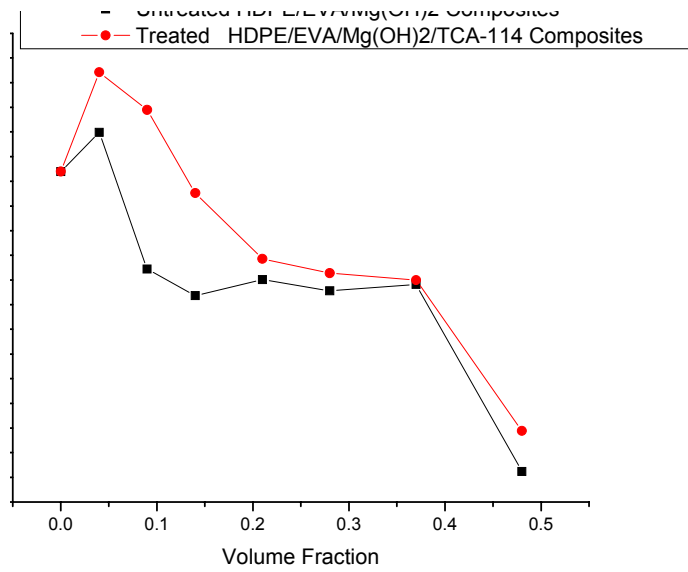


Figure (1): Tensile Strength of the Treated & untreated EVA/HDPE/Mg(OH)₂/TCA-114, Composites.

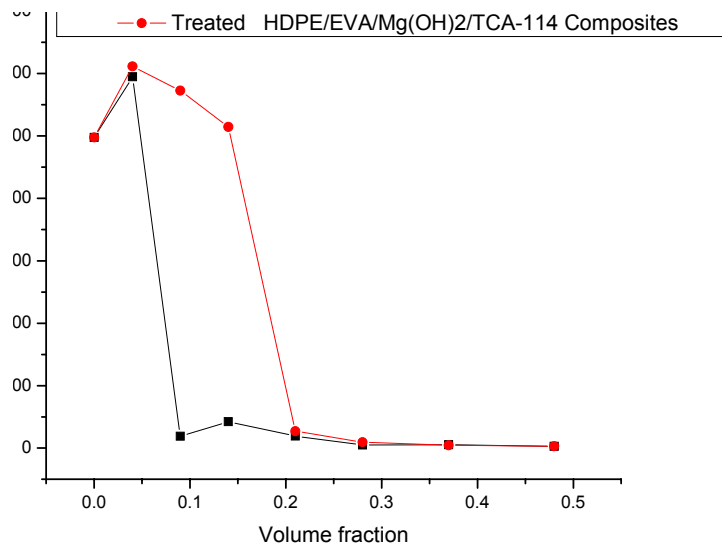


Figure (2): Elongation at break of the Treated & untreated EVA/HDPE/Mg(OH)₂/TCA-114, Composites.

Elastics Modulus

Figure 3 shows the dependence of elastics modulus on concentration of treated and untreated filler in HDPE/EVA. It is seen that, Elastics Modulus of both treated and untreated $Mg(OH)_2$ -EVA composite increased on increasing the concentrations of fillers .

The elastic modulus of treated magnesium hydroxide at 0.14 volume fraction is about 2 times higher than that of untreated magnesium hydroxide .The rate of increment in the property with increasing volume fraction of the filler.

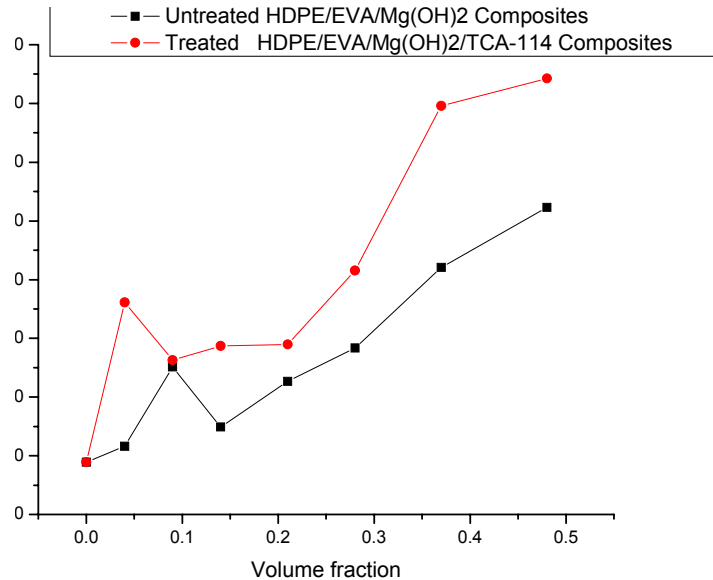


Figure (3): Elastic Modulus of the Treated & untreated EVA/HDPE/ $Mg(OH)_2$ /TCA-114, Composites.

Hardness

Figure 4 shows the dependence of hardness on concentration of treated and untreated filler in HDPE/EVA. It is seen that, hardness of both treated and untreated $Mg(OH)_2$ – HDPE/EVA composite increased on increasing the concentrations of fillers, with a constant rate of increment for composites containing treated and untreated filler (separately) as evidenced by constant and identical slopes of the lines (figure 4). The hardness of treated magnesium hydroxide at 0.14 volume fraction is about 1.01 times higher than that of untreated magnesium hydroxide .The rate of increment in the property with increasing volume fraction of the filler.

SEM of Composites

The SEM photomicrographs of filler magnesium hydroxide and Titanate coupling agent are shown in plate 1&2. It is clear from these photographs that untreated magnesium hydroxide and Titanate coupling agent show tendency to form agglomerates. SEM of HDPE/EVA/ $Mg(OH)_2$ / Composites are shown in plates 3-7 . Untreated composite fracture shows non -adhesive appearance and formation of agglomerates while treated composites

show a very uniform distribution, regular, adhesive appearance indicating further enhancement in polymer–filler attachment.

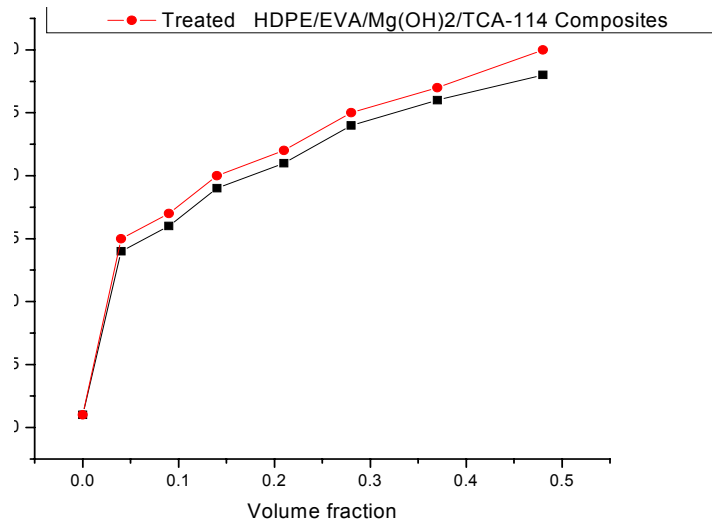


Figure (4): Hardness of the Treated & untreated EVA/HDPE/Mg(OH)₂/TCA-114, Composites.

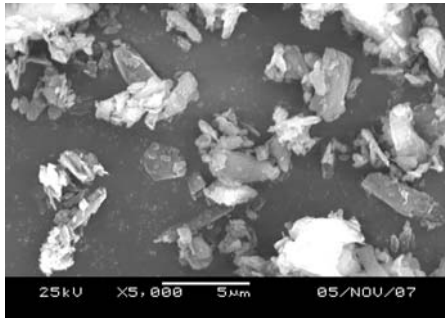


Plate (1): SEM of Mg(OH)₂ Powder (2µm).

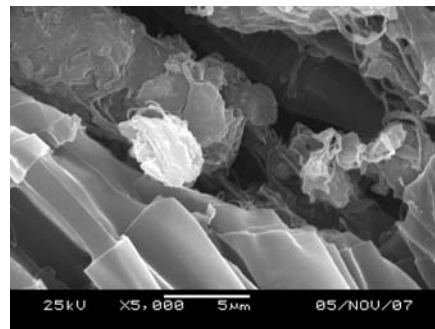


Plate (3): SEM of EVA Composites.

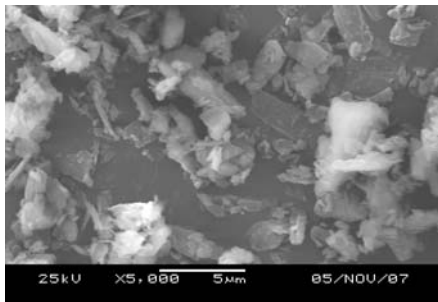


Plate (2): SEM of Mg(OH)₂/TCA-114 Composites.

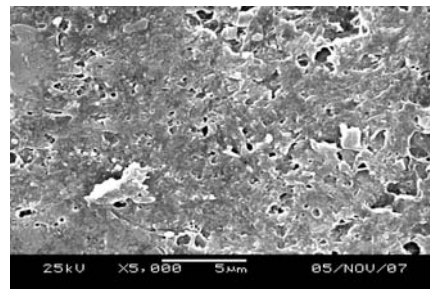


Plate (4): EVA/Mg(OH)₂.

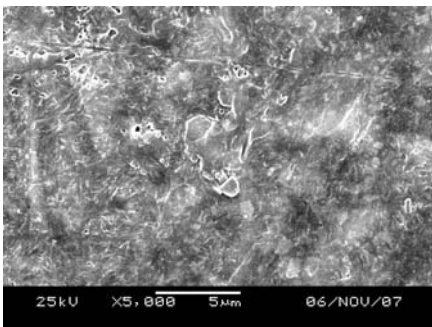


Plate (5): HDPE/Mg(OH)₂.

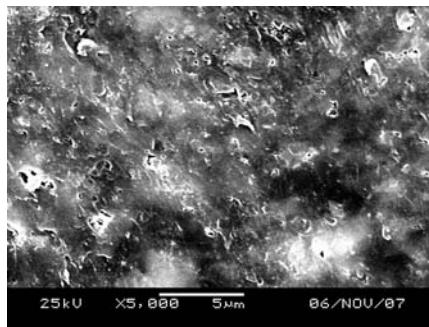


Plate (6): HDPE/EVA/Mg(OH)₂ Composites.

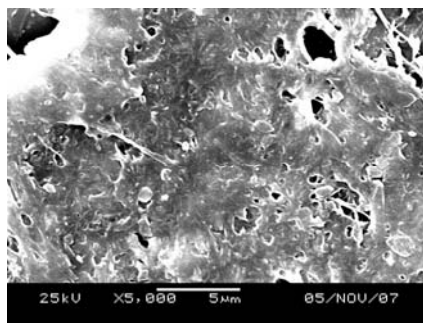


Plate (7): HDPE/EVA/Mg(OH)₂/ TCA-114 Composites.

CONCLUSIONS

Treated Magnesium hydroxide composites showed improvement in mechanical properties and the mechanism of adhesion due to titanate coupling agent is proposed for Magnesium hydroxide as filler.

The treatment of Magnesium hydroxide with titanate coupling agent has effected magnitudes of (%) elongation at break, tensile strength and elastic modulus and hardness of HDPE/EVA/Mg(OH)₂ / Composites. The filler treatment proved to be beneficial by enhancing polymer – filler adhesion as evidenced by SEM study. Considering the cost of the filler and the improvement in properties, the treatment is advisable.

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دراسات في تأثير عوامل الرباط كمواد رابطة في تحسين الخواص الميكانيكية للهيدروكسيد المغنسيوم ، أدخلت كحشوه في متعدد البولي إيثيلين عالي الكثافة ومع كوبوليمر إيثيلين فينيل – اسيتات

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ملخص :

سجلت في هذه الورقة العلمية دراسات في تأثير عوامل رابطة في تحسين الخواص الميكانيكية الميكانيكية للهيدروكسيد المغنسيوم ، أدخلت كحشوه في متعدد البولي إيثيلين عالي الكثافة ومع كوبوليمر إيثيلين فينيل – اسيتات. المواد الرابطة في شكلها سائل بنسبة 1.5% وأضيفت في معالجة مادة الحشوه هيدروكسيد المغنسيوم . المادة المعالجة بعد ذلك أعطيت نتيجة عالية في الخواص الميكانيكية للمكون متى ما قورنت هذه النتيجة مع المكون المحتوى فقط على الهيدروكسيد المغنسيوم الغير معالج . والخواص التي أخذت في الدراسة مثل : استطالة الشد ، معامل القطع ، معامل اللزوجة والقسوه الخ . والدعم كان جيد مع ملاحظة من خلال إضافة المادة المعالجة بنسبة 1.5% من المادة الرابطة والملاحظة كانت النتيجة عالية العلامة بالمقارنة مع الغير معالج كما في الحالة الأولى . ومقارنة الخواص المتكونة من إدخال المعالج والغير معالج من الهيدروكسيد المغنسيوم . وحسنت أن المعالج مع الهيدروكسيد المغنسيوم أعطيت نتيجة أفضل في الخواص الداعمة المتكون (المصنوع) . والخواص المدروسة كانت على النحو التالي : استطالة الشد – معامل القطع 9.01% ، – معامل اللزوجة والقسوه ... الخ . واستطالة الشد أعطت علامة في التحسين هي 19.10% ، معامل القطع 9.01% ، ومعامل اللزوجة أعطت 100% بينما القسوه أعطت علامة 0.5% وبالتحديد عند جزء من الحجم وهي 0.14% .

كلمات رئيسية : عوامل الربط ، الخواص الميكانيكية للمكون ، متعدد البولي إيثيلين عالي الكثافة ، هيدروكسيد المغنسيوم ، كوبوليمر إيثيلين فينيل – اسيتات .

Short Communication

Distribution of Aliphatic Hydrocarbon in Coastal Surface Sediments from the Red Sea of Yemen

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ABSTRACT

During the field work the author has collected samples, but the identification of oil and oil by-products accomplished at the lab. The concentration of Aliphatic from 22.8 $\mu\text{g g}^{-1}$ at Al-Salif Station to 21.72 $\mu\text{g g}^{-1}$ At Ghurirah station. The unresolved complex mixture (UCM) represents components resistant to weathering and bacterial breakdown. This pollution is a consequence of localized oil operation and/or heavy ship traffic. However, some aliphatic hydrocarbons were sourced by natural biogenic processes by marine algae and phytoplankton.

Keywords: Aliphatic Hydrocarbon, Red Sea and Sediments.

INTRODUCTION

Republic of Yemen is one of the seven countries, which have shoreline on the Red Sea; the shoreline of Yemen is an extension of the Red Sea shoreline located in the southeast section of the Red Sea. It extends from Midi in the north to Dhubab near Bab-El-mandab. The season of the monsoon winds drive the upwelling and, in turn, cause a seasonal periodicity throughout the food web. However, there are different types of impacts on the coastal and marine environment of Yemen. These impacts are mainly caused by human and developmental activities, which introduce pollutants to the marine environment and cause the detraction of some special habitats. The most widely recognized issue is that of oil-related pollution, where considerable attention has been focused. However, other areas of concern include the impact of growing industrial and domestic effluents, unplanned coastal development as well as various miscellaneous anthropogenic activities such as fishing, hunting and tourism.

No major spills have yet occurred in Yemen water. However, the potential threat remains strong, either from shipping accidents or through problems at oil transfer points along the coast (EPC, 1996).



Offshore hydrocarbon exploration; As yet offshore production of gas or oil has started. However, plans have been proposed, giving rise to the risk of possible oil spills, discharges of oil-based mud and contaminated water.

The seawater is already polluted by oil and this pollution has, also, affected the beaches in many countries all over the world.

Oil pollution has bad effects on plants; it may interfere with light penetration and photosynthesis. The oil film has also an effect on gas exchange across the water surface.

Description of the Study Area

The Red Sea bibliographic information was extensively cited in Edwards & Head (1987); Morcos & Varley (1990), and Sheppard *et al.* (1992). Moreover, most of the recent information on the Yemen Red Sea coastal environment, flora and fauna was detailed or quoted in IUCN (1987), Rushdi *et al.* (1991), and Dekker & Capelle (1994). The Red Sea is a long narrow basin separating Africa from Asia, and extending from NNW to SSE between latitudes of 30 °N to 12° 30'N almost in a straight line. Its total length is 1932 km and average breadth is 280 km. The maximum breadth is only 306 km in the southern sector near Massawa. It attains its minimum breadth of 26 km at the southern end in the Straits of Bab El-mandab. The area of the Red Sea is $4.6 \times 10^5 \text{ km}^2$ and mean depth is about 500 m. The maximum recorded depth is 3039 m in the axial trough at 19° 35' N, 38 ° 40' E. The real separation of the Red Sea from the Gulf of Aden lies to the north of Bab el Mandab near the Great Hanish Island. The bottom relief of the Red Sea can be divided into the following regions; the coral reef zone, coastal shelves, the main trough, the axial trough, the hot brines region of the Red Sea and the Straits of Bab el Mandab.

Mangroves are salt-tolerant trees usually found in association with mudflats. Globally, mangrove ecosystems contain more than 60 species of trees and provide living space for more than 2000 species of fish, invertebrates and epiphytic plants (Clough, 1993). There are two types of mangroves in Yemen (*Avicennia marina* and *Rhizophora macronata*)

Yemen's mangrove communities include faunal assemblages of fish, crab, shrimp shells, clams, birds, and green turtles and it is important to productive organic carbon

The distribution of coral reefs along both sides of the Red Sea coast shows north-south variations in both coral diversity and community structure. In the southern Red Sea, the steady increase in muddy substrate and mangroves causes significant reef development to be pushed out further from shore. Four factors are thought to be important in limiting coral development in Yemen: 1) the rarity of hard substrates suitable for coral settlement, because of the great depth of alluvial sediments on Tihama and shallow coastal shelf, 2) the shallow bathymetry of the region combined with strong seasonal south-south-westerly winds leads to rough weather that lead to destabilization of soft substrates, high turbidity and sediment stress, 3) there may be localized salinity and sediment stresses resulting from flash floods (IUCN 1987), and 4) the relatively high nutrient levels which promote algal competition (Sheppard *et al.* 1992). Of these, the first is considered to be the most significant limiting factor (IUCN 1987). Three types of coral formation along the coastline were found during the IUCN survey (1987): fringing reefs, patch reefs and bottom reefs. Reefs north of Al Hodiedah differ in their structure from those to the south. In general, reefs in the north of Al Hudaydah were dominated by massive and encrusting types of corals combined with some forest and foliose forms, except in few locations where soft corals were dominant. South of Al Hodiedah, forest and foliose species become more dominant. Percentage of coral cover ranges from less than 10% north of Hodiedah to over

50% in the southernmost reefs near Al Mukha. Coral diversity is higher in the southern region (more than 25 species) than in the northern part (about 10 species) of the Yemeni Red Sea coast. A section of 100% cover of *Galaxea* spp, 200 m wide extending for several kilometers were identified in the region between Al Mukha and Dhubab. Further, the shallow reef rock substrate north of Al Hodeidah is dominated by the brown algae *Sargassum* spp. and red calcareous algae, while *Sargassum* were rare or absent further south.

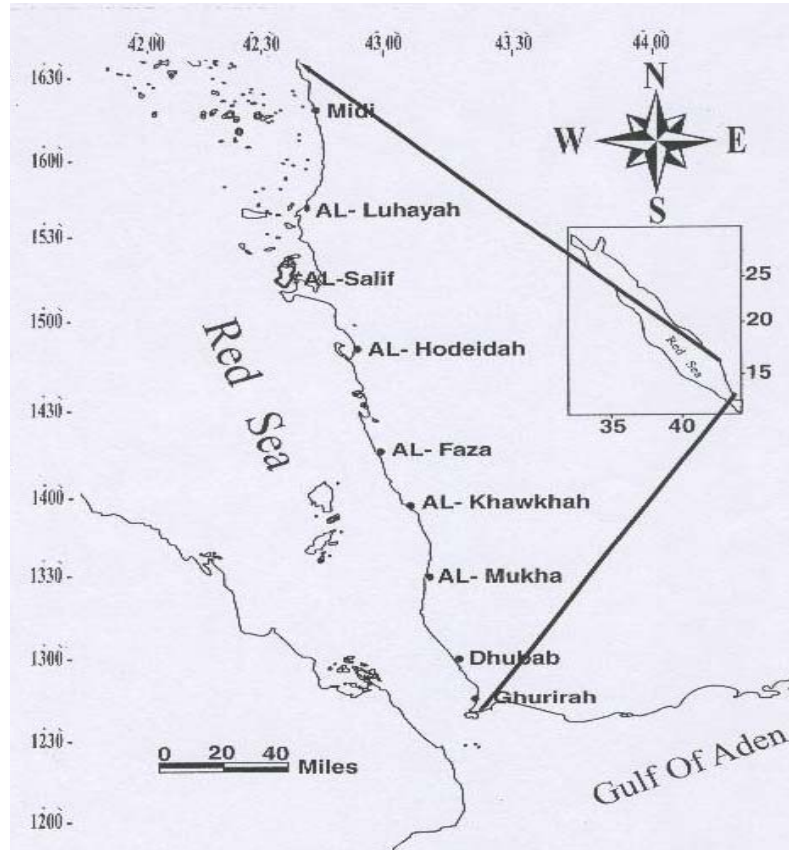


Figure (1): Location of selected sampling sites.

MATERIAL AND METHODS

Samples of surface sediment were collected between February 1999 and January 2000 from Nine stations by Van Van grab samples (Fig, 1). As soon as the samples are acquired, they were placed in glass jars and kept frozen until the time of analysis. A reference station (RF) was selected at Al-Fazaa, being far from oil contamination. The procedure used for extraction and analysis of aliphatic hydrocarbon in the surficial sediment samples were based upon that of IOC (total organic carbon) (1976,1982). For the present work a Perkin-Elmer Spectrofluorometer was used. Blank determinations were carried out by repeating

the procedure with pre-extracted samples. Lotus 1-2-3- we used to subtract the blank spectra from the spectra of the samples. Using a calibration with Kuwait export blend crude oil and the detector suspense was 45.8 mV at 360nm emission wavelength.

Percentage organic carbon (%TOC) was determined by the procedure proposed by El-Wakeel and Riley (1957).

RESULT AND DISCUSSION

The distribution of aliphatic hydrocarbons in the sediment samples from the Red Sea coast of Yemen are summarized in table 1. The range of carbon chain length of n-alkanes for the sediment samples are C10 –C31. The bimodal distribution with two maxima around C17 and C27 suggest two different sources of hydrocarbons both biogenic and anthropogenic (Fig. 2). Biogenic sources for hydrocarbons are indicated by the dominate of the odd carbon n-alkanes (C15, C17, C25 and C29) which are synthesized by marine algae (Blumer, et al., 1971; Hwang, et al, 2002), and higher plants (Matsumoto and Hanya, 1981; Lowe, et al, 2006). The presence of pristane and phytane in significant concentrations supports the biogenic origin of hydrocarbon in these samples; it has been reported to be synthesized by both zooplankton and fish (Burns,et al., 1982).

On the other hand, the anthropogenic contribution of hydrocarbons is evident from the presence of the unresolved complex mixture (UCM) in all of the samples analysed. The UCM represent components resistant to weathering and bacterial breakdown and its presence in chromatograms has frequently been taken as an evidence of petroleum contamination (Farrington, et al., 1977).

This study also shows the presence of even- carbon numbered n-alkanes, which may be related to a contribution from artificial sources (Matsumoto and Hanya, 1981). The carbon preference index (CPI), which is an important parameter in relation to hydrocarbon sources (Mazurek and Simoneit, 1984) has a ratio close to unity and is assigned to a polluted environment. CPI for the sediment samples ranged from ND in Al-Fazaa to 2.0 in Al-Salif, which may indicate biogenic in these sediment samples.

The presence of squalane, a major organic constituent in polluted water, was intimately correlated with anthropogenic sources of hydrocarbons (Matsumoto and Hanya, 1981). This compound was encountered in all sediment samples of the Red Sea coast of Yemen. (Table 2) and may serve to indicate the polluted nature of the region. Burns et al., (1982) reported elevated values of squalane in some sediment samples, which are constantly subjected to oil pollution.

The sources of these hydrocarbons include disposal of automobile and industrial lubricants, spillage from oil- storage facilities and leakage from motor vehicles (Al-Shwafi, 2000; Hamid, 1990).

From (Table 2), the % TOC ranges from 0.03 at Ghurirah station to 0.07 at Al-Hodiedah station. The concentration of Aliphatic hydrocarbon in surficial sediment of the Red Sea coast of Yemen do not relate to % TOC.

CONCLUSION

In conclusion, the distribution of n-alkane (Aliphatic hydrocarbon) in surficial sediments sample from the Red Sea Coast of Yemen were found to contain measurable amounts of hydrocarbons. The components seem to be derived from both biogenic as well as

anthropogenic source, The concentration of Aliphatic hydrocarbon in surficial sediment of the Red Sea coast of Yemen do not relate to % TOC (Table 2).

A major fraction of petroleum consists of aliphatic hydrocarbons which may be used to detect its presence in the environment. The local marine environment of Yemen is exposed to a relatively high chronic input of petroleum hydrocarbons from industrial effluent, sewage and oil spills.

Table (1): n-Alkane concentrations in surface sediments ($\mu\text{g/g}^{-1}$ dry weight).

Location	C ₁₀	C ₁₁	C ₁₂	C ₁₃	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C ₂₀
Midi	0.07	0.04	0.06	0.06	0.05	0.09	0.08	0.05	0.06	0.07	0.05
Al-Luhayah	0.06	0.04	0.05	0.4	0.04	0.08	0.06	0.04	0.06	0.06	0.05
Al-Salif	0.09	0.08	0.8	0.7	0.9	1.00	1.1	1.02	1.3	1.6	1.07
Al-Hodiedah	0.08	0.09	0.07	0.6	0.7	1.00	1.00	1.02	1.2	1.3	1.05
Al-Fazaa	0.02	0.01	0.01	0.03	0.01	0.04	0.01	0.02	0.03	0.05	0.04
Al-Khawkhah	0.03	0.07	0.07	0.09	0.4	0.3	0.09	0.3	0.07	0.1	0.8
Al-mukha	0.07	0.08	0.08	0.5	0.8	0.9	1.0	1.01	1.1	1.01	1.1
Dhubab	0.09	0.08	0.09	0.7	1.0	0.9	1.1	1.09	1.07	1.1	1.2
Ghurirah	1.0	0.9	0.09	0.8	1.1	1.0	1.2	1.1	1.1	1.2	1.3
Location	C ₂₁	C ₂₂	C ₂₃	C ₂₄	C ₂₅	C ₂₆	C ₂₇	C ₂₈	C ₂₉	C ₃₀	C ₃₁
Midi	0.06	0.09	0.04	0.02	0.08	0.09	1.1	0.9	0.07	0.8	0.4
Al-Luhayah	0.04	0.08	0.04	0.03	0.05	0.08	0.09	0.07	0.04	0.7	0.3
Al-Salif	1.05	1.3	1.8	1.7	1.3	0.9	0.7	1.4	0.6	1.3	1.09
Al-Hodiedah	1.03	1.2	1.6	1.2	0.6	0.4	0.6	1.2	0.5	1.2	0.8
Al-Fazaa	ND	0.01	0.01	ND	0.03	0.01	0.01	ND	0.01	ND	ND
Al-Khawkhah	0.09	0.08	0.4	0.1	0.09	0.7	0.04	0.1	0.6	0.07	0.5
Al-Mukha	1.02	1.1	1.3	1.2	0.9	0.7	0.3	1.1	0.2	1.0	0.09
Dhubab	1.03	1.09	1.3	1.07	1.02	0.9	0.7	1.2	0.4	1.3	1.1
Ghurirah	1.0	1.1	1.4	1.04	1.0	1.0	0.9	1.3	0.5	1.3	1.2

Table (2): Pristane, Phytane, Squalane and Total n-Alkanes ($\mu\text{g/g}$ -dry weight) in Sediments, CPI, UCM values and % TOC

Location	Pristane	Phytane	Squalane	Total n-Alkanes	CPI	UCM	% TOC
Midi	0.02	0.06	0.04	4.72	1.1	0.8	0.05
Al-Luhayah	0.02	0.01	0.03	2.46	0.9	0.5	0.04
Al-Salif	1.01	0.9	1.2	22.8	2.0	2.1	0.05
Al-Hodiedah	0.5	0.4	0.6	18.44	1.3	1.6	0.07
Al-Fazaa	ND	ND	ND	0.35	ND	0.4	0.05
Al-Khawkhah	0.03	0.01	0.09	4.91	0.5	0.5	0.04
Al-Mukha	0.5	0.3	0.4	16.56	1.2	0.9	0.06
Dhubab	0.09	0.6	1.02	19.53	1.5	1.6	0.04
Ghurirah	1.0	0.9	1.1	21.72	1.8	1.9	0.03

ND- Non Detection

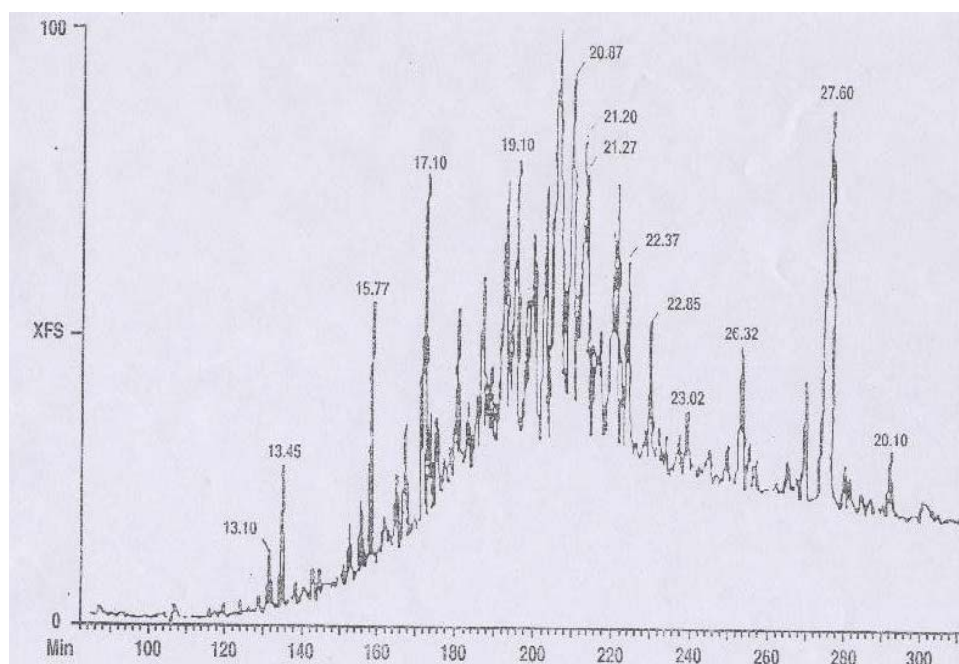


Figure (2): Chromatograms of aliphatic hydrocarbon in Red Sea sediment cost of Yemen.

RECOMMENDATIONS

It is recommended that a continuous monitoring program for the Red Sea coast of Yemen should be formulated and conducted to ensure that the concentrations of Aliphatic hydrocarbons remain within the baseline levels established during the present survey

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توزيع المركبات الهيدروكربونية الأليفاتية في رسوبيات الساحل اليمني على البحر الأحمر

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ملخص

أنجز هذا العمل لغرض التعرف على بقايا التراكيز النفطية الأليفاتية المتواجدة على الساحل اليمني للبحر الأحمر وكانت هذه التراكيز من 22.8 ميكروجرام في محطة الصليف إلى 21.72 ميكروجرام في محطة الغردقة وأظهر التحليل بان الخليط المعقد غير المنحل بواسطة التعرية والبكتيريا يعود إلى إن هذا المتبقي من النفط مصدره غير طبيعي وهو العمليات النفطية المحلية وكذلك من مرور السفن والبواخر، أما وجود بعض المركبات الأليفاتية فان مصدرها طبيعي من الطحالب والكائنات النباتية الخضراء الموجودة في عمود مياه البحر.

المصطلحات الرئيسية: الهيدروكربونات الأليفاتية ، البحر الأحمر ، الرسوبيات .

Development of Advanced Monitoring System in Real Time Environment

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ABSTRACT

In this paper, the problem of providing a good computerized monitoring system is discussed. As an application, Advanced Monitoring System (AMS) is designed and built for this purpose. AMS is an image processing, a soft real time system, which is concerned with enhancement of the monitoring services with lower cost and a high performance. It seems to be very broad area of research as it relates to many important subjects of computer science. The produced system has accomplished its intended functions and services in trusted fashion.

1. INTRODUCTION

Since computer invention, there have been many persistent attempts to use it in a range of areas. The common main goal is to achieve easier life by using the modern technology. Day by day, computer has become the important element in most of daily life activities such as education, medicine, engineering, management...etc.

Today, digital image processing represents one of the most important fields of the computer science. Here, the applications and needs of digital image processing include very long list that contains: Games, Motion Detection, Pattern Recognition...etc. The main issue of digital image processing is the huge computation which might be required [1,5].

"Real time system is any system where a timely response by the computer to external stimuli is vital". The term timely means that the real time system runs tasks that have dead lines [2].

This work is an application of digital image processing in real time environment that aims at developing an automatic monitoring software system which can help in security purposes. The intended system should process a stream of image frames. For the purpose of monitoring, an on-line camera which is connected to the computer must focus on the monitored place.



Practically, the intended system is indeed a real time software system. Here, the task of developing digital image processing software to work in real time mode takes its attraction. This is actually because of time sensitivity and required huge computation.

AMS takes its importance because of the following reasons:

Firstly, monitoring rigid places is a hard human job, considering the time those humans remain to monitor a needed place.

Secondly, there are some noticed cases at many places in which the computer is used. However, no alert actions can be considered. It is just to use the computer screen in human monitoring. Consequently, it is a computer abuse manner without high benefits.

Finally, full automated monitoring systems may achieve the monitoring targets but with a huge costs.

AMS is a trial to solve the above problems with simple solutions.

It starts working when the user requests the monitoring via connected camera for creating the base frame. Then, it starts monitoring services.

During the monitoring, the AMS senses any change in the monitored place, as a result of this, it informs the user by running a suitable alert response. The other system response is done by recording the occurred actions.

Digital image processing remains a challenging domain of programming for several reasons. First, the issue of digital image processing appeared relatively late in computer history, it had to wait for the arrival of the first graphical operating systems to become a true matter. Secondly, digital image processing requires the most careful optimizations and especially for real time applications. Comparing image processing and audio processing is a good way to fix ideas. Let us consider the necessary memory bandwidth for examining the pixels of a 320x240, 32 bits bitmap, 30 times a second: 10 Mo/sec. Now with the same quality standard, an audio stereo wave real time processing needs 44100 (samples per second) x 2 (bytes per sample per channel) x 2 (channels) = 176Ko/sec, which is 50 time less [1,5].

Obviously, it could not use the same signal processing techniques in both audio and image. Finally, digital image processing depends almost on definition of two dimensional domain; this somehow complicates things when elaborating digital filters.

2. PROBLEM DETERMINATION AND OBJECTIVES DEFINITION

There are many problems associated with monitoring systems, which can be listed as follows:

- The monitoring using human capabilities without computerized system is a hard job.
- The computerized monitoring systems without any advanced services such as (change detection, user alarm) are also not efficient systems, because the users of those systems also need continuous watching of the monitoring screen(s).
- The cost needed to develop an efficient monitoring system is very high, because the hardware components will cost a lot of money.

Consequently, the main objectives of the AMS system are to:

- minimize human effort during the monitoring job;
- enhance the monitoring services by using the changes detection, recording the changes and user alert services;

- reduce the cost of the computerized monitoring system, by using the normal hardware attributes required for system work;
- make the monitoring more easily and more powerful; and
- allow the users of the monitoring systems to do another works during monitoring such as reading.

3. SYSTEM DESIGN AND IMPLEMENTATION

The intended AMS has to perform the following basic functions:

- monitor the considered places;
- alert the user when any change has occurred during monitoring; **and**
- record the happened changes.

In addition, to provide system integration the following functions are included:

- user-friendliness;
- reliability; and
- efficiency

As any real time system, AMS Design Phase is very crucial, therefore the designer has to make the desired design simple but highly efficient [2]. The design phase starts from the general design specification toward more detailed design specification i.e., from top to bottom.

3.1 General Design Specifications

The system picks up the input from a user and a connected online camera. Then, it processes the input to produce some results that represent the output. Consequently, the input falls into two categories. The first category is user's input (user name, user password and system setting). The camera's input represents the second category of the intended system input (captured frames). Here, the major component of the AMS system is said to be Core Processing Unit (AMSCPU). The AMSCPU is responsible for most of AMS work functions. Briefly, it gets the input from a user and camera. Then it applies the input to the processing operations. Specifically, the AMSCPU is responsible for accomplishing four main successive tasks. In those tasks, AMSCPU should firstly get user settings and check if a base frame is captured. In case of no base frame, the user must acquire the base frame to continue the work. Secondly, acquired frames via the connected online camera are compared with the base frame. Thirdly, during the monitoring phase, if there is any change detected in the acquired frame, then it alerts the user, and records a stream of frames that have the detected change. All the previous steps are presented in the following points:

- a. store each action in the log files; and
- b. control the possible errors.

Side by side, the output could include:

- a. informing the user with any performed action;
- b. displaying the stream of captured frames;
- c. storing to log files; and
- d. Alerting actions.

Figure (1) summarizes the general view of the AMS system.

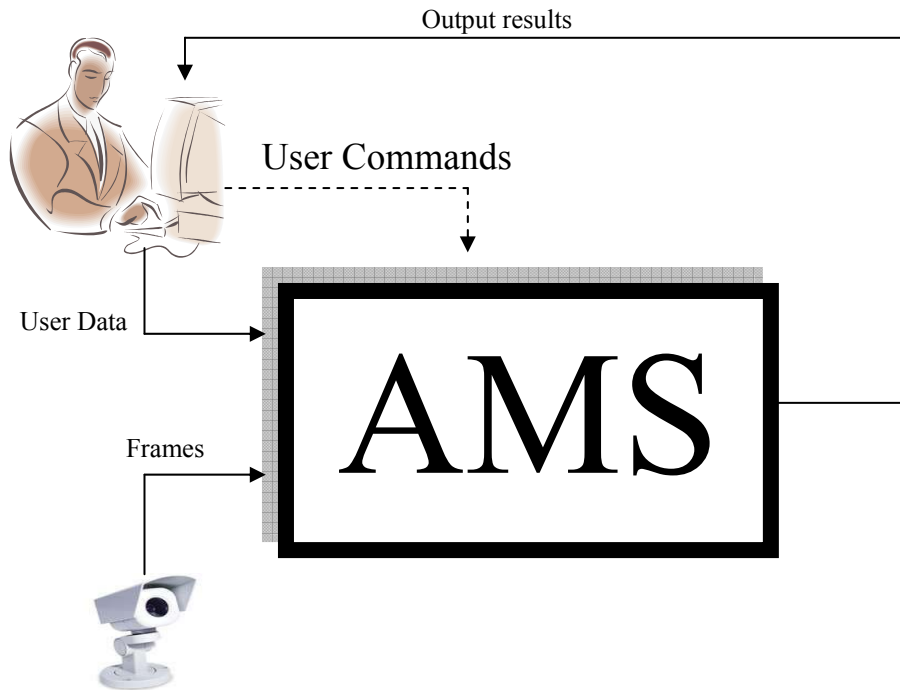


Figure (1): AMS general view.

3.2 Detailed Design Specification

According to figure (2), it is easy to note that the AMS design is constructed by using layering with modularity approach. Clearly, three main layers can be distinguished as in the following:

- 1- AMS Input Unit Layer (Upper layer);
- 2- AMS Core Processing Layer (Middle layer); and
- 3- AMS Output Unit Layer (Lower layer).

3.2.1 AMS Input Unit Layer (AMSIU)

The AMS Input Unit Layer is built of AMS Input Interaction Unit (AMSIIU). The AMSIIU is one of the AMS system main components, which is responsible for acquiring and processing user and camera input. In other words, AMSIIU should:

1. identify system input;
2. control possible input error;
3. deliver the received input to the lower layer; and
4. store the input actions to the log file.

According to the two input categories, the AMSIIU is divided into two modules including User Input Manipulation Unit (UIMU) and Camera Input Manipulation Unit (CIMU).

The UIMU module is responsible for:

1. getting the configuration setting of the user;
2. getting the configuration setting of the system depending on the user's needs;
3. sending user commands to Camera Input Manipulation Unit;
4. sending user setting to Camera Input Manipulation Unit;
5. sending User setting and/or system configuration setting to AMS Core Processing Unit (AMSCPU);
6. informing user against the system errors; and
7. receiving commands come from AMSCPU.

The CIMU module is responsible for:

1. interfacing with camera device;
2. delivering acquired frames to the AMS Core Processing Unit (AMSCPU);
3. receiving and analyzing user commands;
4. sending connection states to the user;
5. sending connection commands to the AMS Core Processing Unit (AMSCPU); and
6. informing the user with the error(s) in the system.
- 7.

3.2.2 AMS Core Processing Unit Layer (AMSCPU)

The AMSCPU layer can be considered as the heart of the AMS system. This layer is responsible for providing the most important AMS functions. The AMSCPU consists of three separated and related modules including Input Interface Unit (IIU), Processing unit (PU) and Output Interface Unit (OIU).

The Input Interface Unit (IIU) represents an interface to the AMSIU layer (Most Upper layer). It performs the initialization of the PU services. Logically, IIU module is divided into Camera Interface Unit (CIU) and User Interface Unit (UIU). The CIU is responsible for manipulating the Camera Input Manipulation Unit output.

CIU consists of two parts:

- Commands unit (Camera interface command unit. CICU) which is responsible for receiving the CIMU unit and Processing unit (PU) commands, and sending the command(s) to the appropriate unit(s): CIMU, Commander and Frame Receiver; and
- Data manipulation unit (Frames Receiver) which is responsible for receiving the commands from CICU and the data from CIMU, and sending the data to the (Transformer in PU).

UIU is responsible for manipulating the User Manipulation Unit intended output. UIU consists of two parts:

- Command unit (User Interface Command Unit. UICU) which is responsible for receiving the commands from the User Input Manipulation Unit (UIMU), and Processing unit (PU), and sending the command(s) to appropriate unit(s) (Recording Checker, UIMU and Commander in PU).

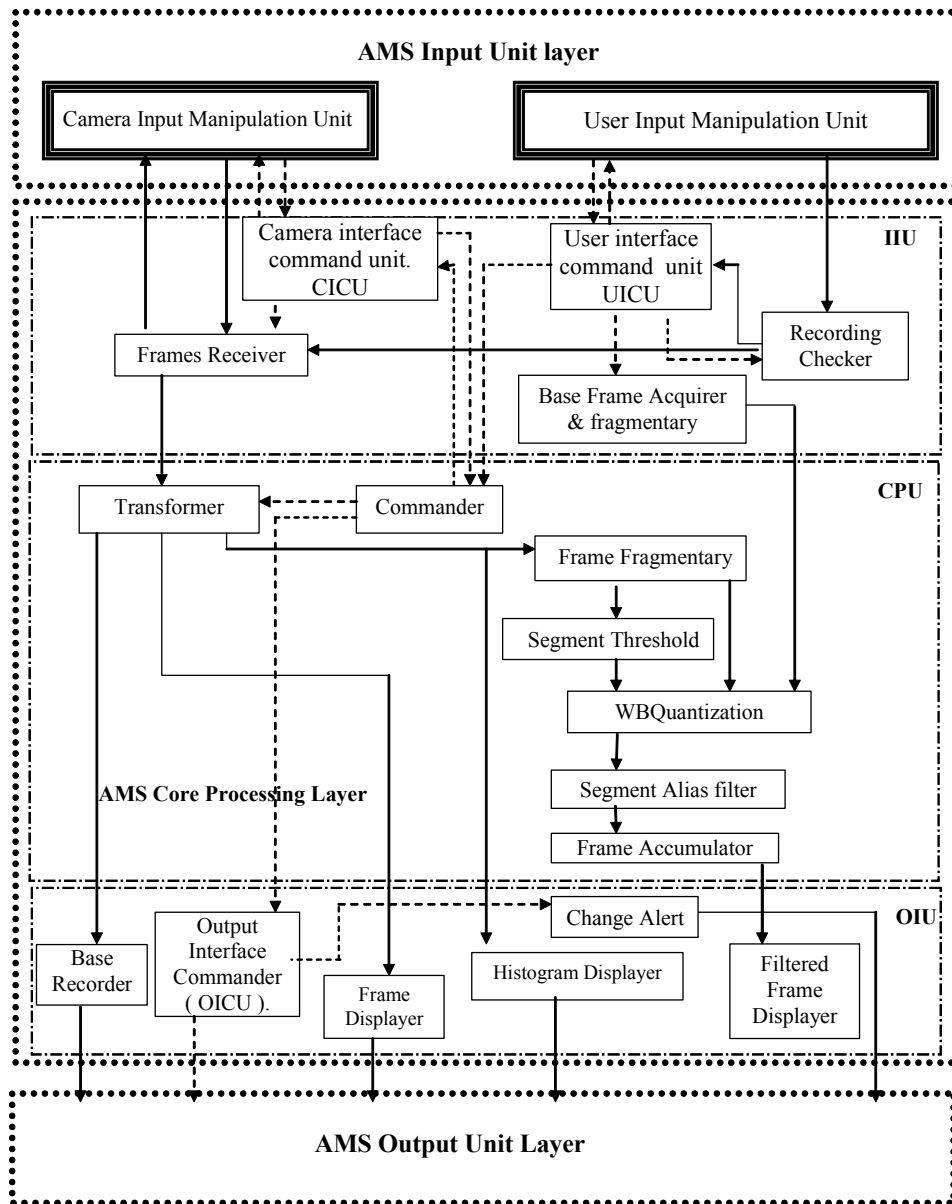


Figure (2): AMS layered view.

- Data manipulation subunits which consist of:
 - Base Frame Acquirer& fragmentary which is responsible for loading and fragmenting the base frame.
 - Recording Checker which is responsible for checking the recoding space and determining the best path of the stream file.

PU is the engine of the AMS monitoring services. Logically, PU consists of two main subunits:

1. Command processing unit (Commander) which is responsible for routing the commands inside the AMSCPU.

2. Data processing unit which is responsible for managing received data.

Data processing unit is in turn divided into six modules:

(1) Transformer which is responsible for filtering, transforming and transferring the frames to the appropriate unit(s).

(2) Frame Fragmentary. The main role of Frame Fragmentary is to fragment the frames.

(3) Segment Threshold. The Segment Threshold is an important module, and its importance dwells in finding the threshold value, which is the backbone of matching process. Here, threshold value is computed via the *Adaptive thresholding algorithm*. During its work, it has only one case, which receives the segment from Frame Fragmentary.

(4) WBQuantization. WBQuantization module is responsible for quantizing the (white black) frame segments from the filtered input frame. During its work, it has one case which receives the segment from the Frame Fragmentary and then quantizes it.

(5) Segment Alias filter. Segment alias filter module is responsible for the linear matching process. It has a case which receives segment from Frame Fragmentary, then matches it by comparing the number of white pixels in it with the threshold value.

(6) Frame Accumulator. Frame Accumulator is responsible for accumulating the filtered and fragmented frame. During its work, it has a case which receives the segment from the Segment Alias filter, and then reassemble the fragmented frame.

The next section demonstrates some algorithms that are used to implement the above modules.

The OIU unit is the interface between the AMSCPU layer and the AMSOU layer. It manages the output coming from PU unit.

Briefly, OIU reforms the output to appropriate a suitable form for the outputting. It consists of two main subunits:

1- Command manipulation unit is implemented by:

- Output Interface Commander (OICU) which is responsible for routing the commands between OIU and PU.

2- Data manipulation units are implemented by:

- Change Alert;

- Histogram Displayer;

- Base Recorder (Gateway);

- Frame Displayer (Gateway); and

- Filtered Frame Displayer (Gateway)

3.2.3 AMS Output Unit (AMSOU) Layer

AMSOU is responsible for manipulating the system's output. Normally, it receives the output from (OIU in AMSCPU) and manipulates them.

3.3 AMS Implementation

AMS is an image processing and soft real time system. Therefore the computation speed is more valuable than other processing constraints (space ...). Here a programming

language is a very sensitive factor. It affects the implementation of similar systems. The decision is based on the used programming language facilitates. These facilitates can support the implementation of the presented design. The needed facilitates should include:

1. the smallest size of the executive program image (.exe file);
2. high speed run time code;
3. abilities of good memory management; and
4. the power of interaction with the operating system

Regarding the above facilitates, (C++) programming language is the best choice .Due to including graphical user interface MS Visual C++ 6.0 is used to the AMS implementation [3,4]. The most important implementation constraints can be summarized as the following:

1. The monitored object must have a different color than the background color in acceptable distance between these colors.
2. The monitored object must have an acceptable size, regarding the monitoring camera qualities.

Figure (3) shows one of the ASM interfaces.

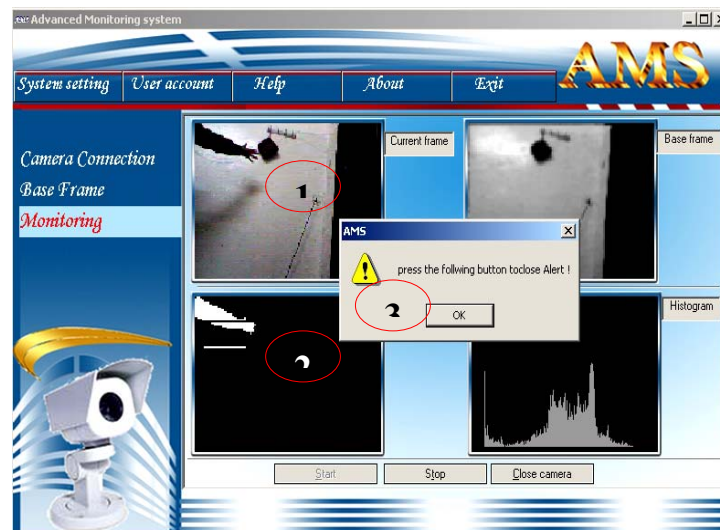


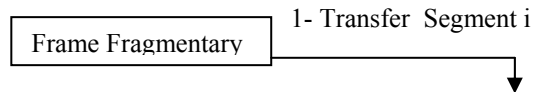
Figure (3): Monitoring with alert.

As seen in Figure(3) above, when any change is detected (1), you will see the change in the filtered frame (2) with the white color and the alert will be run. With this action, a message box will appear with this message ("press the following button to close Alert") (3), which allows you to stop the alert.

4. AMS IMAGE PROCESSING ALGORITHMS

Here, the researcher demonstrates some algorithms [1,5] which are used to implement the data processing unit. These algorithms affect directly the performance of the AMS system. In fact, the used image processing algorithms affect the AMS computation speed and accuracy. That is why the considered algorithms have to be stated. On the other hand, many improvements could be done in this area by enhancing or replacing a distinct algorithm.

4.1 Adaptive Thresholding Algorithm



For each received segment, do the following algorithm.

Adaptive thresholding algorithm.

Input: the segment

Output: adaptive threshold value of that segment

begin

- 1- compute the segment i histogram.
- 2- Store the result histogram into matrix (**Histo[256]**)
- 3- Y axis is the value in the Histo matrix ,and X axis is the index of the Histo matrix..
- 4- Find Maximum Y value (**MaxY**) and it is index (**MaxX**).
- 5- Find Minimum Y value (**MinY**) and it is index (**MinX**).
- 6- The distance (**d**) equation is : (see specification).

$$d = \text{root} (\text{Square} (\text{MaxX} - \text{MinX}) + \text{Square} (\text{MaxY} - \text{MinY}))$$

- 7- initialize the threshold value .

$$\text{MinX} - \text{MaxX} \quad r = (\text{MinY} - \text{MaxY}) /$$

$$\text{Threshold} = \text{MaxX} - 1$$

$$\text{MaxY} = r * (\text{Threshold} - \text{MinX}) + (\text{MinY})$$

$$\text{newX} = (y - \text{MinY} + (\text{MinX} * r)) / r$$

$$\text{MinY} = \text{Histo} [\text{Threshold}]$$

/* Compute d_threshold */

$$d_threshold = \text{root} (\text{Square} (\text{Threshold} - \text{newX}) + \text{Square} (\text{newY} - \text{MinY}))$$

- 8- search for the threshold value

For i =MaxX-2 **down to** i>MinX

begin

$$y = r (i - \text{MinX}) + (\text{MinY})$$

$$\text{newX} = (y - \text{MinY} + (\text{MinX} * r)) / r$$

$$\text{newY} = \text{Histo} [i]$$

/* Compute di */

$$d_i = \text{root} (\text{Square} (i - \text{newX}) + \text{Square} (\text{newY} - \text{MinY}))$$

if $d_i > d_threshold$ **then**

begin

$$\text{Threshold} = i$$

$$d_threshold = d_i$$

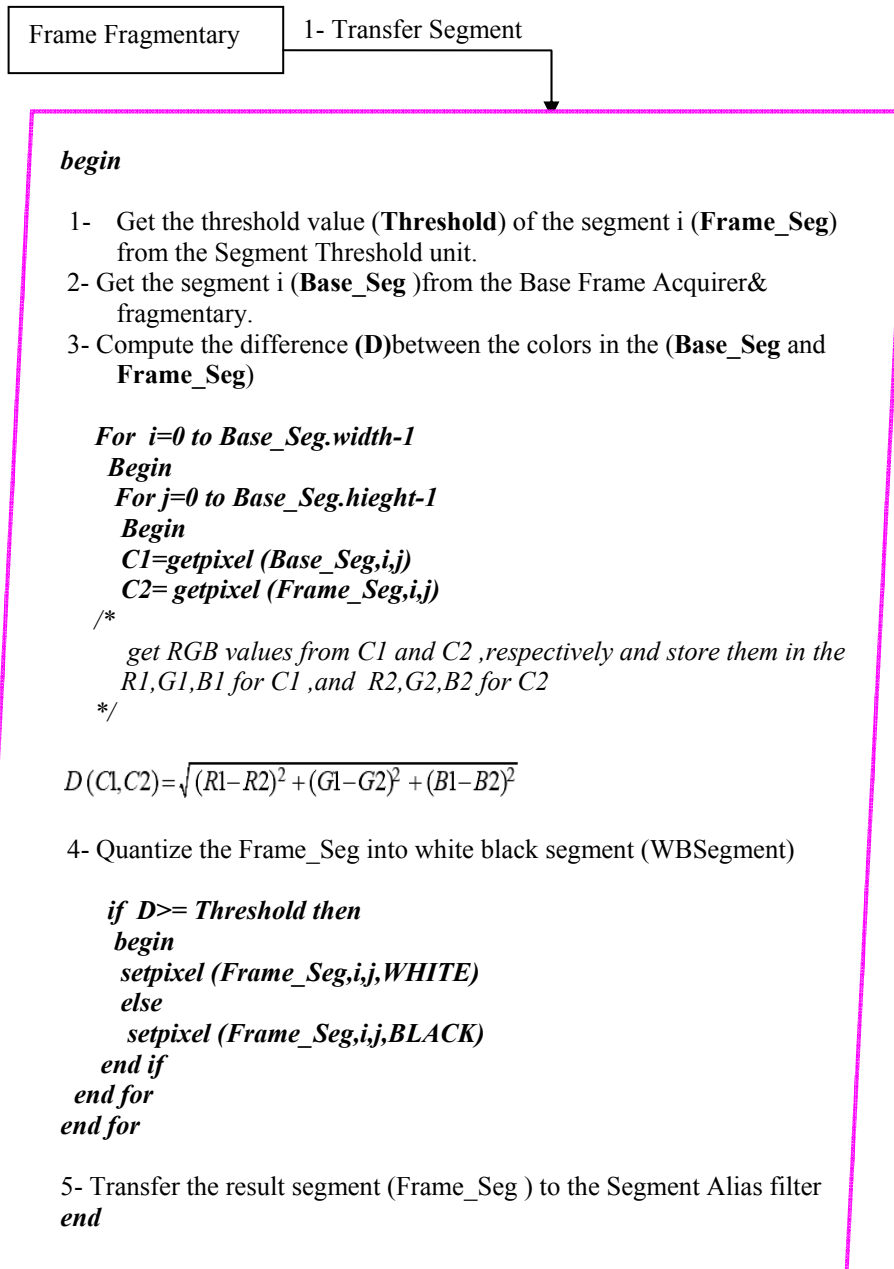
end if

end for

- 9- Send the threshold value to the WBQuantization unit.

end

4.2 WBQuantization Algorithm



4.3 Segment Alias Filter Algorithm

Frame Fragmentary

Transfer Segment i

Begin

1- Get the threshold value (**Threshold**) of the segment i (**Frame_Seg**) from the (Segment Threshold unit).

2- *For each pixels in the segment*
Begin

If the number of the white pixels \geq threshold then
Begin

Make all pixels in the segment white

WBsegment_state=1

Else

Make all pixels in the segment black

WBsegment_state=0

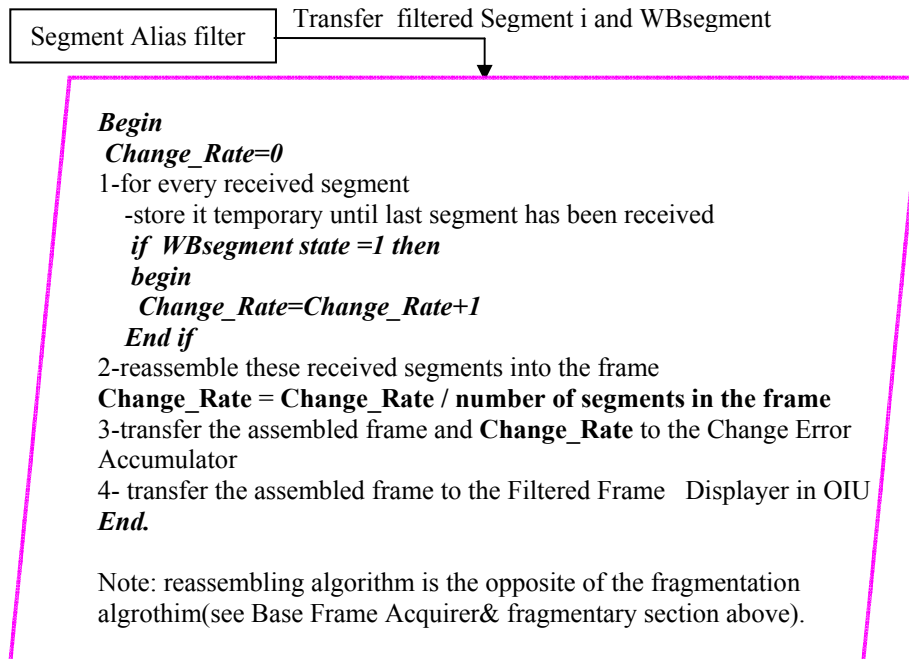
End if

End for

3- transfer the result segment and it's *WBsegment_state* to the Frame Accumulator unit

end.

4.4 Frame Accumulator Algorithm



5. DISCUSSION, CONCLUSION AND FURTHER WORKS

In this section, some important discussion is given to ensure the AMS assessment. Then, the conclusion is provided. After that, some ideas for further works are derived.

5.1 Discussion and Conclusion

There are many challenges in image processing applications, especially the applications that work in a noisy environment, but they also have the motivation and good research areas to carry on. AMS has been considered the situations of Noise Analysis and Cancellation, Image Thresholding and Computation Speedup.

The noise that AMS system processes is an *Amplified* type. This type of noise can be canceled by smoothing and blurring the image to balanced form, i.e. saving image form and removing the noise. However, some colored noise may still be in the smoothed image. So gray scaling is used to remove that noise.

Finding the image threshold value can be simple, but the computed value may not be precise depending on the image thresholding technique. There are many techniques that could be used to find image threshold. The AMS system uses *Triangle algorithm* (due to Zack). This algorithm is very efficient in finding threshold value, but when applying this algorithm on the image as a whole block, the computed threshold value may not be efficient in matching process. However, by applying it in region by region of the image then

calculating the regions threshold values will improve the matching process. This is done by using region by region matching.

One key element of the real time systems succession is the implementation algorithm that is used. The major challenge in designing efficient implementation algorithm is the application nature. In image processing applications, many processes are usually performed on the same image. In other words, the processes are performed on the same array as serious procedures which may increase the computation time. So, AMS system uses the parallelism technique when loading the image from camera to memory.

Here, it is important to clarify that the system in question has been checked for a suitable time at the laboratories of Faculty of Computer Science and Information System, Tamar University. As a result of this, the researcher can conclude clearly that the built AMS system can indeed do its function and provide the intended services of monitoring in real time environment.

5.2 Further works

As any security system, AMS system may have some imprecise issues. For example, finding threshold value may not be very effective regarding some mathematical approaches. So there are more powerful development approaches that can be applied to AMS system to increase its efficiency on achieving monitoring functions.

Suggested further development approaches include Thresholding by minimizing fuzziness.

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تطوير نظام مراقبة متقدم في بيئة زمن حقيقي

خليل الوجيه

كلية الحاسوب و تقنية المعلومات – جامعة ذمار

ملخص

تتناقش هذه الورقة مسألة توفير نظام جيد للمراقبة بواسطة الحاسوب. وكتطبيق لذلك فقد تم تصميم وبناء نظام المراقبة المتقدم (AMS). يعد النظام المطور نظام زمن حقيقي للمعالجة الصورية لغرض تعزيز خدمات المراقبة بكلفة منخفضة وأداء عالٍ. يأتي هذا التطبيق ضمن مساحة بحثية واسعة لعلاقته بعدة مواضيع مهمة في علم الحاسوب وقد جرى التحقق من إنجاز الوظائف والخدمات المستهدفة للنظام بشكل موثوق.

A Study of $\alpha \beta$ Tracker with Some New Algorithms for Selecting α and β Parameters

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ABSTRACT

This paper deals with target tracking using $\alpha \beta$ Tracker. Some of the theoretical properties of this classical tracker are discussed and the problem of selecting the suitable α and β parameters is studied. Two well known classical methods of estimating α and β parameters are veiwed and another three new algorithms of estimating these parameters are suggested. Some simulation experiements are performed in the cases of white noise and color noise to check the accuracy of the classical as well as the new ones. The performance of our new suggested algorithms seems to be very well.

1. INTRODUCTION

The $\alpha \beta$ tracker is a very simple filter still used in many tactical military systems although it has been used firstly in tracking radar at the early of 1960's. This tracker has an excellent performance for tracking non-maneuvering targets. Because of its simplicity, it is often considered as a candidate filter (Bhagavan and Polge [1974] and West and Blair [2001]).

$\alpha \beta$ tracker is one of the type fading memory filters with fixed gain and it can be implemented recursively. i.e., data received in the past are included in the present estimates (Hanna [1989]). This tracker is one step ahead predictor of position that uses the current error in order to predict the next position.

Sklansky, in his seminal paper, analyzed the behavior of an $\alpha \beta$ tracker (Sklansky [1957]). His analysis of the range of values of the smoothing parameters α and β which resulted in a stable filter constrained the parameters to lie within a stability triangle. He also derived closed form equations to relate the smoothing parameters for critically damped transient response and the ability of the filter to smooth white noise. Following his work, Benedict and Brodner [1962] used calculus of variation to solve for an optimal filter which minimizes a cost function which is a weighted function of the noise smoothing and the transient (maneuver following) response bringing a constraint to the optimal filter. Schooler [1975], discussed the inaccuracies of $\alpha \beta$ tracker and modeled them; then he provided an optimal $\alpha \beta$ tracker for the systems with modeled inaccuracies. Lefferts [1981] studied



the correlation regions assumed of independent and Gaussian distributed error. He used a dynamically varying correlation region to yield improved tracking performance.

In 1990's there were many studies and researches related to α β tracker and further improvement was obtained, (see for example Yosko and Kalata [1992], Aubree et al. [1995], Llinas et al. [1998] and West and Blair [2001]). Anyway, tracking through α β tracker still is an attractive area, which needs rich analysis and improvement.

The usefulness of α β tracker as compared to others with superior performance lies mainly in the ease of implementation and limited computational requirements. This means that it may be needed as a result of computational limitations if the sampling interval is short, or if many targets must be engaged (Leffertds [1981] and Hanna [1989]). α β tracker provides a good performance for non-maneuvering, constant velocity targets. It has the ability to deal with a maneuvering target if it is modeled as a constant – velocity system with random maneuvering.

However, α β tracker is just one step ahead position predictor; this restricts the ability to predict the target path through next n steps of times. It has fixed coefficient parameters, so its gain is not adaptively hanged it has little capability to track severely maneuvering targets (Bhagvan and Ploge [1974] and Lefferts [1998]).

It is well known that it is not possible to select smoothing parameters on line which are optimal in all cases, so it is frequently necessary to use several sets of smoothing parameters to achieve a practical system. The α β tracker however, is obtained by neglecting the acceleration term in the equation of motion, the manner that affects dealing with maneuvering targets. This work therefor, is trying to minimize the problem by selecting suitable values of α and β parameters, on line with minimum error.

2. α β TRACKER

The form α β tracker equations can be derived from Newton's laws of motion. Consider the motion of point mass with constant acceleration. It is well known that this motion is described by integrating the Newton's First Law.

Let $x(t)$ denotes the position of a point mass at time t , then the equation of motion can be reduced to (Llinas et al. [1998]) :

$$x(t) = x(0) + v(0)t + \frac{1}{2}at^2 \quad , \quad (1)$$

where $x(0)$ is the initial position, $v(0)$ is the initial velocity and a is the acceleration which is assumed here to be constant independent of time. Now, if the acceleration is negligible then the equation (1) can be written as:

$$x(t) = x(0) + v(0)t \quad . \quad (2)$$

Assuming that we have measurements at discrete time points; say $t = 1, 2, \dots$. Substituting the initial conditions $x(0)$ and $v(0)$ by the smoothed position x_s and the smoothed velocity v_s , respectively, then the following equation of one-step-ahead prediction is obtained (Llinas et al. [1998]) :

$$x_p(t+1) = x_s(t) + Tv_s(t) \quad ; t = 1, 2, \dots \quad (3)$$

where $x_p(t+1)$ is the 1st-step ahead predicted position at time t ,

$x_s(t)$ is the smoothed position at time t ,

$v_s(t)$ is the smoothed velocity at time t ,

T is the sampling time interval.

The innovation, or prediction error, at time t is denoted by $e(t)$ and defined as the difference between the measured position $x_m(t)$ and the predicted position $x_p(t)$.

I.e.:

$$e(t) = x_m(t) - x_p(t) \quad ; t = 1, 2, \dots \quad (4)$$

Assuming the ratio of the difference between the smoothed position and the predicted position to the innovation is a constant, say α acting as a smoothing parameter of the position and computed as:

Hence, the smoothed position can be obtained from the following equation:

$$\alpha = \frac{x_s(t) - x_p(t)}{x_m(t) - x_p(t)} \quad (5)$$

$$x_s(t) = x_p(t) + \alpha [x_m(t) - x_p(t)] \quad ; t = 1, 2, \dots \quad (6)$$

Also similarly, the smoothed velocity can be obtained by using the well known physical law : velocity = distance / time , and letting :

$$\beta = \frac{v_s(t) - v_s(t-1)}{[x_m(t) - x_p(t)]/T} \quad (7)$$

Then the smoothed velocity equation is given by:

$$v_s(t) = v_s(t-1) + \beta/T [x_m(t) - x_p(t)] \quad ; t = 1, 2, \dots \quad (8)$$

3. INITIALIZING THE $\alpha \beta$ TRACKER

$\alpha \beta$ tracker is a recursive filter as the prediction equation (3) is in recursive form, this means that it needs to be initialized. Two measured target positions are required to determine the initial smoothed velocity, causing the target position prediction begins at the third time step. The measured position is considered to be the initial predicted target position till the second time step. The initial smoothed velocity is calculated as (Llinas et al. [1998]):

$$v_s(2) = \frac{x_m(2) - x_m(1)}{T} \quad (9)$$

The first predicted position is then calculated as:

$$x_p(3) = x_m(2) + Tv_s(2) \quad (10)$$

Figure (1) illustrates the track initialization. It is clear that the initial innovation is zero and the smoothing parameters have no influence of the initial prediction.

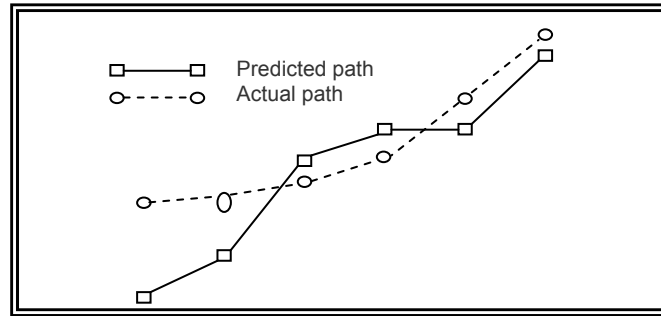


Figure (1): Track Initialization.

4. STABILITY ANALYSIS

The regions of stability at different transient response characteristics of $\alpha \beta$ tracker can be specified in the $\alpha \beta$ space. Writing equations (3), (6) and (8) in the z -domain and substituting x_s and v_s into the prediction equation (3) yielding the transfer function of the $\alpha \beta$ tracker in the z -domain $G(z)$ as follow (Llinas et al. [1998]) :

$$G(z) = \frac{\alpha(z-1) + \beta z}{z^2 + (\alpha + \beta - 2)z + (1 - \alpha)} \quad (11)$$

which can be used to determine the region of stability of the $\alpha \beta$ tracker. Stability requires that roots of the characteristic polynomial lie within the unit circle in the z -domain. The characteristic polynomial is given by the denominator of equation (11). To prove that the roots lie within the unit circle, one can transform equation (11) into the w -domain, mapping the unit circle of the z -domain to the left half plane of the w -domain and applying one of the known stability criteria in continuous domain. Another approach is to check the stability directly in the z -domain using Jury's Stability Test.

The Jury's Stability Test can be used to analyze the stability of the system without explicitly solving for the poles of the system. Therefore, it is used to determine the bounds on the parameters which result a stable transfer function in the z -domain.

Llinas et al. [1998] showed the stability region of the $\alpha \beta$ tracker is defined by the following three constraints:

$$(i) \quad 0 < \alpha < 2 \quad , \quad (12)$$

$$(ii) \quad \beta > 0 \quad \text{and} \quad (13)$$

$$(iii) \quad 2\alpha + \beta < 4 \quad . \quad (14)$$

The characteristic polynomial is:

$$z^2 + (\alpha + \beta - 2)z + (1 - \alpha) = 0 \quad (15)$$

and the roots of this characteristic equation are:

$$z_1 = \frac{-(\alpha + \beta - 2) + \sqrt{(\alpha + \beta - 2)^2 - 4(1 - \alpha)}}{2} \quad , \quad (16)$$

$$z_2 = \frac{-(\alpha + \beta - 2) - \sqrt{(\alpha + \beta - 2)^2 - 4(1 - \alpha)}}{2} \quad . \quad (17)$$

Critical damping is obtained when $z_1 = z_2$ i.e. when

$$(\alpha + \beta - 2)^2 - 4(1 - \alpha) = 0 \quad (18)$$

i.e. when

$$\alpha + \beta - 2 = \pm 2\sqrt{1 - \alpha} \quad (19)$$

i.e. when

$$\beta = 2 - \alpha \pm 2\sqrt{1 - \alpha} \quad . \quad (20)$$

Equation (20) is valid for all $\alpha \leq 1$ and the system is oscillating if the poles in equation (11) contains a non-zero imaginary part.

Llinas et al. [1998] have shown that when $\alpha > 1$, then the roots of the equation (15) are never negative so the above approach can not be applied. Hence, the final stability boundaries are:

$$0 < \alpha \leq 1 \quad \text{and} \quad (21)$$

$$\beta = 4 - 2\alpha \quad . \quad (22)$$

Figure (2) shows the stability region of $\alpha \beta$ tracker.

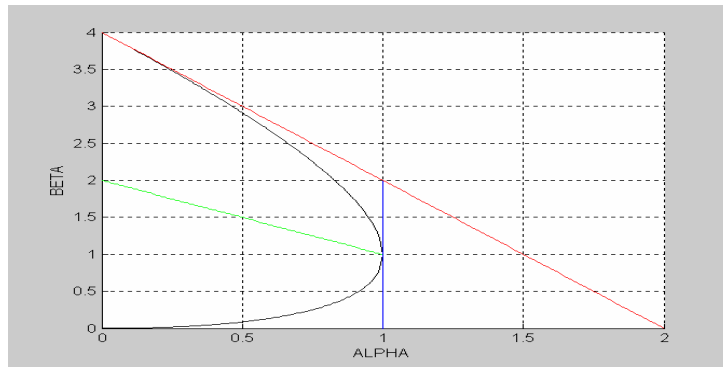


Figure (2): Stability Region of α β Tracker.

5. CHOICE OF α AND β PARAMETERS

In this section we describe the standard methods of selecting α and β parameters. Also, three new methods are suggested.

5.1 CLASSICAL METHODS

The classical α β tracker is designed originally to minimize the mean square error in the filtered position and velocity. The problem with α β tracker is that its design implies a compromise between good noise smoothing, i.e. required small α and β , and good maneuver following capability, i.e. required large α and β values (Hanna [1989]). One of the well known estimates of α and β parameters are (Llinas et al. [1998]) :

$$0 < \tilde{\alpha} \leq 1 \quad (23a)$$

$$\tilde{\beta} = \tilde{\alpha}^2 / (2 - \tilde{\alpha}) \quad (23b)$$

Now, the main objective here is to use the possibility to change α and β parameters during confirmed tracking. Thus, the unknown target maneuvers must influence the α and β parameters by increasing swiftness or stability according as the target is accelerating or not (West and Blair [2001]). Hence, the other criterion for selecting the α and β parameters is based on the best linear track fitted to the radar data in a least squares sense. This is leading to use the evolutive parameters which are given as (Skolink [1981] and West and Blair [2001]):

$$\hat{\alpha} = \frac{2(2n - 1)}{n(n + 1)} \quad , \quad (24a)$$

$$\hat{\beta} = \frac{6}{n(n + 1)} \quad , \quad (24b)$$

where n is the sequence number of the target measurements and $n > 2$.

5.2 NEW SUGGESTED METHODS

In the last subsection, we have considered two classical methods for estimating α and β parameters. The first method (M1), based on selecting a given value of the parameter α from the interval $(0, 1)$, usually near zero; say $\alpha=0.05$ or $\alpha=0.01$, and then the corresponding value of β is obtained from the equation (23b). The second method, (M2) based on calculating the estimated values of α and β as functions of the available number of measurements n .

We describe now three suggested methods for estimating α and β . The first suggested method, method 3 (M3) is based on the two estimates of β obtained by the previous two methods. A linear combination of two estimated β from the equations (23b) and (24b) can be considered as alternative estimate and denoted by β_{LC} . This suggested estimate is defined as :

$$\beta_{LC} = w\tilde{\beta} + (1 - w)\hat{\beta} \quad , \quad (25)$$

where w is a given weight such that $0 \leq w \leq 1$. The choice of w can be based on optimization strategies such as the minimization of the mean square error or the minimization of the mean absolute error.

The statistical properties of β_{LC} , like unbiasedness and consistency, can be studied if the statistical properties of $\tilde{\beta}$ and $\hat{\beta}$ are known. If both $\tilde{\beta}$ and $\hat{\beta}$ are unbiased estimates of β , such that

$$E(\tilde{\beta}) = E(\hat{\beta}) = E(\beta)$$

and $E(\cdot)$ is the expectation operator. Then, it is easy shown that β_{LC} is also unbiased estimate of β , i.e.,

$$\begin{aligned} E(\beta_{LC}) &= E[w\tilde{\beta} + (1 - w)\hat{\beta}] \\ &= wE(\tilde{\beta}) + (1 - w)E(\hat{\beta}) \\ &= w\beta + (1 - w)\beta \\ &= \beta \quad . \end{aligned}$$

On the other hand, when we take the variance operator of both sides of (25), and assuming that $\tilde{\beta}$ and $\hat{\beta}$ are independent, then

$$\text{var}(\beta_{LC}) = w^2 \text{var}(\tilde{\beta}) + (1 - w)^2 \text{var}(\hat{\beta})$$

Hence, if $\tilde{\beta}$ and $\hat{\beta}$ are consistent estimates of β , then

$$\text{var}(\tilde{\beta}), \text{var}(\hat{\beta}) \rightarrow 0 \quad \text{as} \quad n \rightarrow \infty$$

Therefore,

$$\text{var}(\beta_{LC}) \rightarrow 0 \quad \text{as} \quad n \rightarrow \infty$$

and β_{LC} will be also consistent estimate of β .

To avoid the arbitrary choice of α , and also to obtain good maneuver following capability, we can use the estimate (24a) for α , which is denoted by $\hat{\alpha}$.

The summary of the above discussion can be observed in the following algorithm.

ALGORITHM (1) : α β Tracking by Linear Combination Method M3.

Step 1: Fix the value of α at α_0 .

Step 2: Calculate the value of β^- and β^+ from equations (23b) and (24b), respectively.

Step 3: Search for optimal weight w , to obtain the optimal value of β_{LC} .

The second suggested method (M4) is called the adjusted α β tracker. In this method, we suppose that there is a moving window which moves through the measurements during α β tracker computations. Through the moving of the window, the optimal values of α parameter is found for the measurements inside the window with respect to the window innovation. The diagram in Figure (3) describes the adjusted α β tracker.

Again, the value of corresponding β is obtained from equation (23b). The adjusted α and β parameters are then employed for the next stage of tracking. To decrease the computation time, the parallel approach maybe used in manner of calculating optimal α and β parameters for a given window in parallel way during α β tracker computations. However, the parallelism will be used clearly in the next suggested method.

The summary of M4 can be observed in the following algorithm.

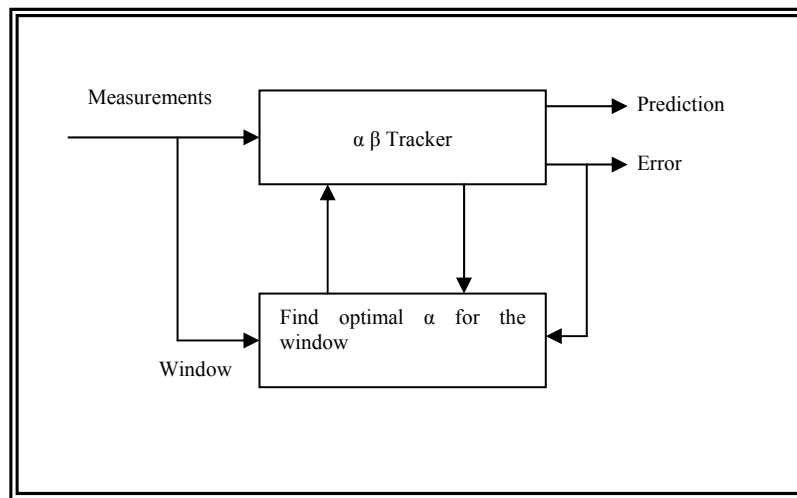


Figure (3): α β Tracking with Adjusting through Window.

ALGORITHM (2) : α β Tracking with adjusting through a window Method M4

Step 1: Fix the values of α and β at α_0 and β_0 respectively.

Step 2: Track by α β tracker.

Step 3: While tracking, search for optimal α and β for a given window.

Step 4: Adjust α and β parameters by those in step 3.

Step 5: Go to step 2.

The third suggested method (M5) is based on Parallel Processing principles. It is well known that Parallel Processing is a computer trend for improving processing speed by

doing more than one function at the same time. This is depending on Parallel Computers or Parallel Processors (Wagih [1999]). The method M5 is called Parallel $\alpha \beta$ tracking, and it supposes that there are K $\alpha \beta$ trackers each one is a fixed gain tracker but with different α and β parameters. Tracking will be done through all those trackers in parallel manner and the prediction with lowest innovation will be considered as the best prediction in the mean square (or mean absolute) error sense (see Figure (4)). The number of the trackers is constrained by hardware availability. Hence, as the number of the trackers increases, the prediction will be more accurate and vice versa. On the other hand, if the tracking lies between two neighbor trackers for long time, we can increase the trackers between them. However, if the time interval between two measurements (sampling rate) is not too small, M5 can be simulated in the sequential mode easily. The summary of M5 can be observed in the following algorithm.

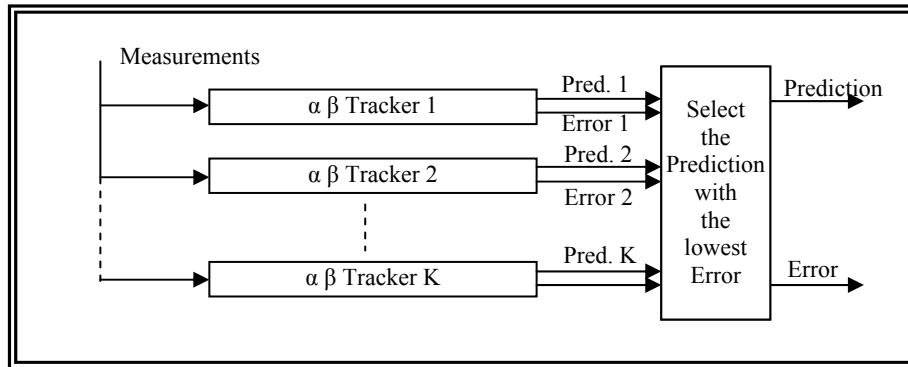


Figure (4): Parallel $\alpha \beta$ Tracking.

ALGORITHM (3) : Parallel $\alpha \beta$ Tracking Method M5.

Step 1: Prepare the K $\alpha \beta$ trackers with different α 's and β 's parameters.

Step 2: Track with all K $\alpha \beta$ trackers.

Step 3: At each time step, consider the prediction value with the lowest absolute error as optimal one.

6. SIMULATION EXPERIMENTS

In this section we try to check the performance of the trackers discussed in the last section through simulation approach. Ten sets of simulated radar data were generated, half of them were corrupted by Gaussian white noise and the others were corrupted by Gaussian colored noise. Each set of the data was treated by each five methods M1, M2, M3, M4 and M5.

To specify the major of optimality, we need to measure the distance between the true position and the predicted position by each method. Usually, the Root Mean Square Error (RMSE) is used in this context which is obtained as:

$$RMSE = \sqrt{\frac{\text{sum}(\text{actual position} - \text{predicted position})^2}{\text{no. of measured data}}} \quad (26)$$

Figures (5) and (6) show comparisons between actual, measured and predicted track using α β tracker by the five methods of selecting α and β parameters and for white and colored noised corrupting; respectively. Figures (7) and (8) as Tables (1) and (2) show the $RMSE$ of these results in each case, again for white and colored noise corrupting; respectively. A quick look at these two tables indicates the efficiency of the suggested methods. It is clear that method M5 gives very lower $RMSE$ than other methods.

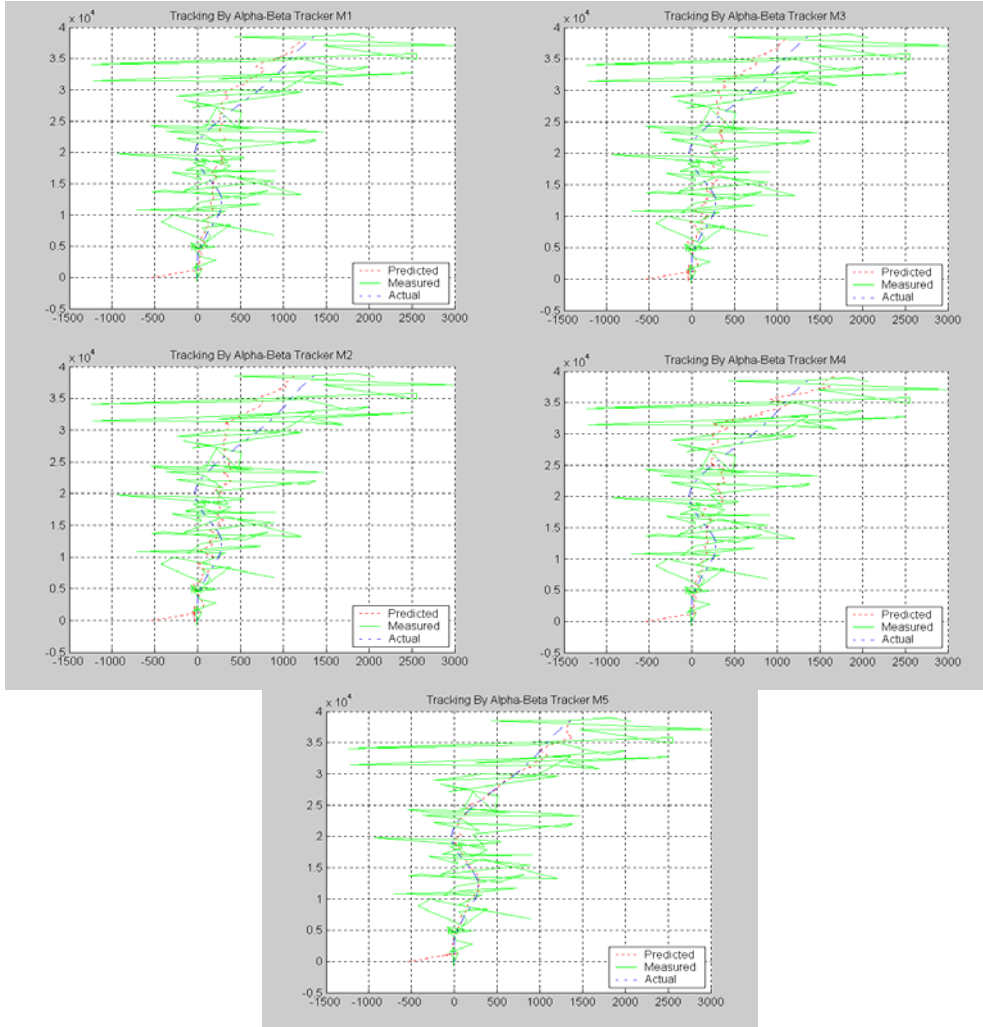


Figure (5): Tracking simulated measurements corrupted by white noise.

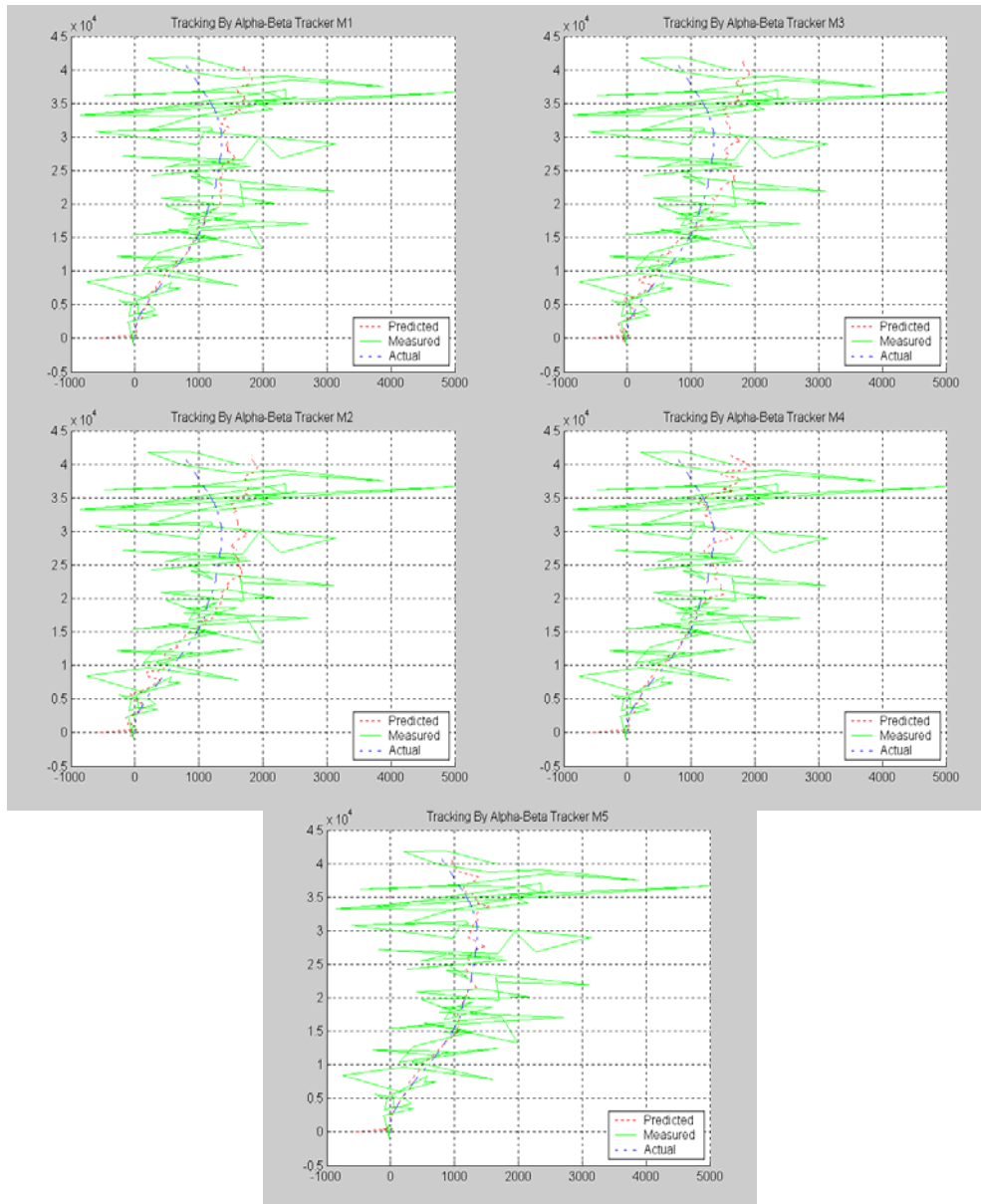


Figure (6): Tracking simulated measurements corrupted by color noise.

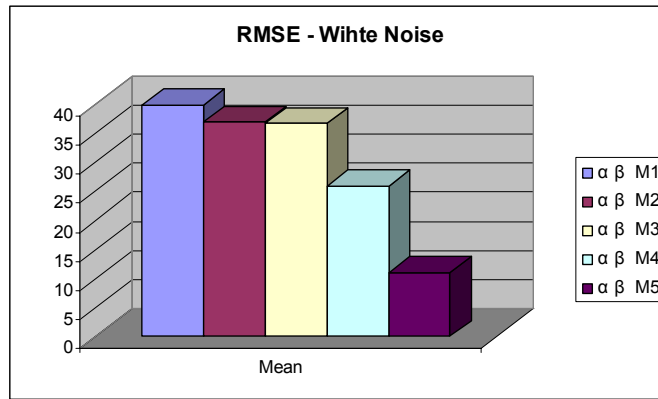


Figure (7): RMSE of the 5 methods – White Noise

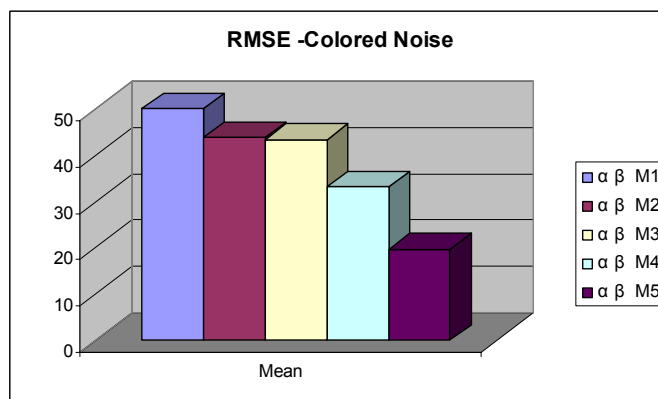


Figure (8): RMSE of the 5 methods – Colored Noise

Table (1): RMSE of tracking white noised simulated data by 5 methods of α β trackers.

Experiment	Coordinates	M1	M2	M3	M4	M5
1	X	28.4589	26.6355	26.6802	25.3460	10.4094
	Y	291.7924	63.6673	66.8815	265.2983	17.7988
2	X	45.8634	47.2913	46.9485	26.1398	11.0342
	Y	299.7238	55.5100	59.1243	268.2146	18.2989
3	X	34.2435	29.6414	29.7098	31.4221	10.1660
	Y	296.4985	60.3253	63.7537	268.3542	17.0756
4	X	40.6228	27.5558	26.8202	23.5749	11.5605
	Y	299.8339	75.4845	78.4928	278.6082	19.1451
5	X	50.5104	53.6469	53.1669	22.7810	11.9972
	Y	307.2010	61.0965	64.9569	278.5191	19.2215

Table (2): RMSE of tracking colored noised simulated data by 5 methods of $\alpha \beta$ trackers.

Experiment	Coordinates	M1	M2	M3	M4	M5
1	X	43.7560	44.4713	44.0580	25.6035	17.0891
	Y	292.9631	78.7142	79.2807	273.4808	21.6512
2	X	77.0170	51.1982	50.0406	44.6987	17.2379
	Y	277.4844	84.7149	84.5255	259.9784	22.3588
3	X	43.2933	30.5868	29.9748	33.5310	17.4046
	Y	295.8656	102.6156	102.3371	284.3615	21.0918
4	X	36.7597	39.6073	39.6211	36.5744	18.2052
	Y	306.6647	79.9087	80.7844	285.2135	23.2990
5	X	49.1139	53.1412	52.7067	25.1383	16.5149
	Y	300.8425	85.0407	85.7739	283.3336	21.0630

7. DISCUSSIONS AND CONCLUSIONS

In this paper we studied the classical $\alpha \beta$ tracker and focused our attention on the problem of selecting the α and β parameters. Five methods of selecting these two parameters were considered, two of them are classical, and the others are suggested by the authors of this paper. These five methods are tested through simulation technique and based on realizations of white and colored noise. The simulation exercise is applied on five different experiments.

We start our discussion by considering the two classical methods M1 and M2, as the base for the purpose of comparison. Table (3) shows the averages of the differences between the *RMSE* obtained from each of the classical methods and each of the suggested new methods and when the noise is white. Table (4) shows these averages but when the noise is colored. It is quite obvious that these averages are positive in all cases except when we compare M4 with M2 in the Y-coordinate. In fact, a statistical paired t-test is applied on these differences and indicated a very highly significance difference in *RMSE* obtained for these comparisons. Hence, we may conclude that our suggested algorithms are significantly differing than the classical ones in the positive direction.

In order to see the respective efficiency of the suggested algorithms with respect to the classical ones, we fix method M1 as the base. Then, we compute the percentage of change of *RMSE* of each of M3, M4, and M5 ($RMSE_{Mi} ; i = 3,4,5$) with respect to that of M1 ($RMSE_{M1}$), which is defined as

$$PC_{i,1} = \frac{(RMSE_{M1} - RMSE_{Mi})}{RMSE_{M1}} * 100\% \quad ; \quad i = 3,4,5 \quad . \quad (27)$$

Table (3): The averages of the difference RMSE of Table (1).

Relative to	Coordinates	M3	M4	M5
M1	X	4.7713	14.0870	28.9063
	Y	232.2810	27.3819	280.7019
M2	X	0.3343	11.8137	25.9207
	Y	3.5122	-208.4113	44.9087

Table (4): The averages of the difference RMSE of Table (2).

Relative to	Coordinates	M3	M4	M5
M1	X	9.4102	16.8788	32.6976
	Y	208.2237	17.4905	272.8713
M2	X	0.5262	11.8695	26.5106
	Y	0.5287	-191.0747	64.3061

Tables (5) and (6) show the obtained values of $PC_{i,l}$ and when the noise is white and colored; respectively. Obviously, algorithms M4 and M5 give high and positive $PC_{i,l}$ values, e.g., 10.9382 means that $RMSE$ of M4 is 10.9382 % lower with respect to M1.

From the previous tables we may draw a main conclusion that algorithm M5 is the best, then M4, and then M3.

Table (5): The percentage (%) of change of RMSE with respect to M1 - white noise.

Experiment	Coordinates	M3	M4	M5
1	X	6.2501	10.9382	63.4230
	Y	77.0790	9.0798	93.9002
2	X	-2.3659	43.0050	75.9412
	Y	80.2737	10.5127	93.8947
3	X	13.2396	8.2392	70.3126
	Y	78.4978	9.4922	94.2409
4	X	33.9775	41.9663	71.5418
	Y	73.8212	99.0708	93.6148
5	X	-5.2593	54.8984	76.2481
	Y	78.8552	9.4433	93.7430

Table (6): The percentage (%) of change of RMSE with respect to M1 - Colored noise.

Experiment	Coordinates	M3	M4	M5
1	X	-0.6902	41.4857	60.9446
	Y	72.9383	6.6500	92.6098
2	X	35.0266	41.9626	77.6181
	Y	69.5368	6.3088	91.9423
3	X	30.7634	22.5492	59.7984
	Y	65.4109	3.8882	92.8712
4	X	-7.7841	0.5041	50.4751
	Y	73.6571	6.9950	92.4025
5	X	-7.3152	48.8163	66.3743
	Y	71.4888	5.8199	92.9987

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Appendix: List of Symbols.

x	Target position coordinate.	V	Target velocity.
\mathbf{a}	Target acceleration.	T	Time instant.
x_p	Target predicted position.	x_s	Target smoothed position.
v_s	Target smoothed velocity.	T	Time period between two scans.
e	Prediction error.	x_m	Target measured position.
α	Position smooth parameter.	B	Velocity smooth parameter.
z	Z-transform coefficient .	G	Transfer function.
w	Weight parameter in method 3.	N	Number of measurements.
K	Number of $\alpha\beta$ trackers in method 5.	$RMSE$	Root Mean Square Error.
$\tilde{\alpha}$	Estimated α by method 1.	$\tilde{\beta}$	Estimated β by method 1.
$\hat{\alpha}$	Estimated α by method 2.	$\hat{\beta}$	Estimated β by method 2.
β_{LC}	Estimated β by method linear combination of methods 1 and 2.		

دراسة المعقب ألفا بيتا باستخدام بعض الخوارزميات الجديدة لاختيار المعلمتين ألف وبيتا

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ملخص

تدرس هذه المقالة مسألة تعقب الهدف و ذلك بواسطة معقب ألفا بيتا، وناقش بعض الخواص النظرية لهذا المعقب مع التركيز على طريقة اختيار المعلمتين ألفا و بيتا. تستعرض أولاً طريقتين تقليديتين في اختيار هاتين المعلمتين ومن ثم تقترح ثلاثة خوارزميات جديدة لهذا الغرض. تجرى تجارب بالمحاكاة على مشاهدات مولدة في حالتها التشويش الأبيض و الملون، وتظهر الخوارزميات المقترحة كفاءة جيدة مقارنة بالطريقتين التقليديتين.

A Study on the Prevalence of Mange Among Arabian Camels in Najaf Province / Iraq

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ABSTRACT

The study was carried out at Najaf abattoir and some nomadic areas, for a period of five months , between January and May 2000 . A total of (434) camels were examined clinically, and skin scrapings collected from suspected animals for laboratory investigation. Sarcoptic mange was diagnosed in (114) camels with an infestation rate of (25, 9%).The highest prevalence rate was recorded in January (38%), and the lowest was in May (14.8%).

The main clinical findings observed included easlessness, intensive itching, and the affected areas of the skin became hairless, thickened, corrugated and grey in color. Regarding affected regions of the body the highest incidence of mange lesions were recorded on the neck (60.5%) and the lowest incidence were on the tail (5.2%).There were no lesions observed on the hump.

Sex of animals showed no effect on the prevalence as well as the severity of the disease. Regarding age, the higher prevalence rate of the disease occurred in animals under 4 years of age.

INTRODUCTION

Mange is a very common and widely spreading disease of camels in most camel rearing countries⁽¹⁾, including Iraq⁽²⁾. It is a highly contagious obstinate and debilitating disease⁽³⁾ and zoonotic one ⁽⁴⁾. The causative mite, Sarcoptes scabiei var camel, is one of many definitive forms of Sarcoptes scabiei ⁽¹⁾. The cross infestation between different hosts occurred due to incomplete host specificity of parasite⁽⁵⁾. The disease transmitted either by direct contact of animals or by fomile like blankets or saddles. Climate and season of a year have great bearing on the occurrence and spreads of mange ⁽³⁾. The disease is more common in wet and colour weather, and spread slowly during summer months ⁽⁶⁾. This study was carried out in Najaf abattoir and some nomadic areas surrounding Najaf for a period of five months (January-May 2000) to determine the prevalence of sarcoptic mange in camel, the effect of weather



(month of the year) , as well as age & sex . The distribution of lesions on various parts of the body was studied too.

MATERIALS AND METHODS

The study was conducted in two places:-

1-Abattoir of Najaf governorate: Through six visits /month, a total of (151) camels were examined.

2-Najaf nomadic surroundings through two visits/month a total of (288) camels were examined.

Collection and examination of Skin scrapings: skin scrapings were collected from all suspected cases. After restraining of camels in recumbency position, the skin scrapings were taken from edges of lesions by scalpel blade until blood oozed. Scrapings were mixed with (10%) potassium hydroxide, heated to dissolve skin debris and after centrifugation, the sediment were microscopically examined for the presence of mites ^(7,8).

RESULTS

During the five months of the study a total of (439) camels of various ages were examined in two places .Of these (114) camels were infested with sarcoptic mites, i.e. an infestation rate of 25.9% . Prevalence of sarcoptic mange according to a month of examination is presented in table (1). The highest prevalence was recorded in the month of January with an infestation rate of (38%) , and then months of February , March and April with an infestation rate of (33.8%), (20.9%) and (20.9) respectively. The lowest prevalence rate recorded, was in May (14.8% only).

The main clinical findings of sarcoptic mange in camels were restlessness, intensive itching, scratching of the skin lesions by hind legs or by biting. The affected areas of the skin became hairless, thickened, corrugated and grey in colour with a progressive loss of body condition.

The distribution of skin lesions on camels body are shown in table (2). The highest incidence of mange lesion occurred on neck region (60.5%) and the lowest occurred on tail region (5.2%). There were no lesions observed on the hump region.

Number and infestation percentage of camels with sarcoptic mange on sex and age bases are shown in table (3) .Both sexes were infested in the same way; and regarding age of the infested camels the prevalence of disease was higher in animals under four years (33.3%) than in animals over four years of age (18.5%).

Table (1): No. and (%) of infested camels during months of the study.

Month	No. of camels examined	No. & % of camels infested
January	92	35 (38%)
February	62	21 (33.8%)
March	43	9 (20.9%)
April	215	45 (20.9%)
May	27	4 (14.8%)
Total	439	114 (25.9%)

Table (2): The distribution of sarcoptic skin lesions on 114 infested camels.

Body region	No. of infected animals	%
Head	40	35
Neck	69	60.5
Thorax	21	18
Forelimbs	25	21.9
Axellae	16	14
Rumps	33	28.9
Inguine	12	10.5
Hind limbs	9	7.8
Tail	6	5.2

Table (3): The effect of sex and age of camel on rate of infestation with sarcoptic mange.

	Sex		Age	
	female	male	Under 4 years	Above 4 years
No. of examined animals	256	183	207	232
No. (& %) of infested animals	68 (26.5%)	46 (25.1%)	69 (33.3%)	45 (18.5%)

DISCUSSION AND CONCLUSION

The high prevalence of sarcoptic mange in camel is in agreement with Higgins ,A.J. (1984) and Hassan, M.A.A.(1986) ^(1,2). The high prevalence in the month of January, and the low prevalence in the month of May is in agreement with the findings of others ^(2,9,10,11). A shorter hair with a cleaner animal skin toward the hot month of May will ensure an active blood circulation and active sweat glands, and hence unsuitable circumstances for the mange mites. The opposite is expected to happen during the cold and wet months of January and February ^(1,3).

The clinical signs observed during the study were recorded by Hassan, M.A.A.(1986) and Lodha ,K.R.(1966) ^(2,3), and the highly infested neck region is in accordance with others ^(1,2). There were no lesions observed on the hump, as recorded by Basu, A.K; Aliyu ,A. and Mohammed ,A. (1995) ⁽¹⁰⁾. Sex of camels had no effect on the prevalence as well as the severity of disease and this finding was noticed by Rathor M.S. and Lodha, K.R. (1973) ⁽⁹⁾ too .The high prevalence of mange in camels under four years of age may be due to skin tenderness ⁽¹²⁾.

Sarcoptic mange in camels has a high prevalence in Najaf province. The rate of infestation with mange was higher in the cold and wet months. The neck was the mostly affected region of the body. The disease is expected to occur in camels under four years of age than in those above 4 years of age.

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دراسة حول انتشار مرض الجرب في الجمال العربية في محافظة النجف في العراق

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2- كلية الزراعة والطب البيطري - جامعة ذي قار / ذي قار / اليمن

ملخص

أجريت الدراسة في مجزرة النجف وبعض مناطق البادية هناك ولفترة خمسة أشهر امتدت من كانون الثاني إلى مارس 2000. تم فحص (434) جملاً فحصاً سريرياً وجمعت الكشطات الجلدية لأعراض الفحص المختبري. شخص مرض الجرب من نوع Sarcoptic في (114) جملاً وبنسبة خمج بلغت (25.9%). كان أعلى حدوث لمرض الجرب في شهر كانون الثاني وبنسبة (38%) وسجلت أقل نسبة في شهر مارس حيث بلغت (14.8%). إن أهم العلامات السريرية التي لوحظت على الجمال المخمجة هي عدم الراحة والحكة الشديدة وكان الجلد المغطي للآفة خالياً من الشعر ومنتخن ومنتجد ورمادي اللون، وفيما يتعلق بمناطق الجسم المصابة فإن أكثر أجزاء الجسم تعرضاً لأفات مرض الجرب هي الرقبة (60.5%) وأقل المناطق تعرضاً هي منطقة الذيل (5.2%) ولم تلاحظ آفات المرض على منطقة السنام. لم يلاحظ تأثير لجنس الحيوان على حدوث المرض أو شدته، وفيما يتعلق بعمر الحيوان فإن أعلى نسب للمرض سجلت في الحيوانات التي لم يتجاوز عمرها (4) سنوات.

The Occurrence of Gallbladder Carcinoma in Yemeni Patients Undergoing Cholecystectomy in Two Hospitals

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ABSTRACT

Background: Primary cancer of the gallbladder has wide geographical, ethnic and cultural variations with poor prognosis. Currently, there is no study about gallbladder carcinoma in Yemen.

The aim is to detect the occurrence of gallbladder carcinoma in Yemeni patients undergoing cholecystectomy.

Patients and Methods: A descriptive retrospective study of data of 940 patients operated for gallstones in two university hospitals in Yemen between 2002 and 2006 was carried out with respect to results of histopathological analysis of gallbladder specimens. There were 872 women and 68 men. Patients who have no histopathological reports in their files were excluded. Histopathological reports of 838 patients were retrospectively investigated for gallbladder malignancy.

Results: Gallbladder carcinoma was detected in 4 female patients of median age 66 years. No male patient was affected. Adenocarcinoma was the variety found in all four cases. All four patients with gallbladder cancer have history of longstanding gallstone disease.

Conclusion: The study supports the hypothesis that gallbladder carcinoma is rare and mostly affects elder women with long-standing gallbladder stones. The occurrence of gallbladder carcinoma in the targeted sample is significantly less than that of the western countries. Cooperation between surgeon, sonographer and histopathologist is strongly advised, particularly when gallbladder malignancy is suspected

Keywords: Cholecystectomy, Gallbladder, Cancer.



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INTRODUCTION

Primary cancer of the gallbladder was first described in 1777 by Maximillian DeStoll on the bases of two autopsies as a relatively uncommon tumor and a highly fatal disease (1). It is the most common malignancy of the biliary tract and the fifth common malignancy of gastrointestinal tract /GIT/(2, 3). Cancer of the gallbladder, despite its rarity, is known to vary greatly in incidence in different parts of the world and has also geographical, cultural and ethnic variations (1, 6).

It accounts for less than 1% of all incident of cancer in the USA, (4,5) and 1–2% of cholecystectomy specimens in the UK (4,5). The highest incidences are found in Native Americans and South Americans as well (7.5 per 100 000 for men and 23 per 100000 for women). However, the exact incidence of the disease in Yemen is still unknown.

Gall bladder (GB) cancer is primarily a disease of older women and has been associated with many risk factors including obesity, female gender, high parity among women, advanced age and ethnicity(2,5,6-14). The most common risk factor to gallbladder carcinoma development is longstanding gallstones, which cause chronic inflammation and chronic mechanical irritation in the wall of the gallbladder. This irritation maybe induced by time mucosal dysplasia, which may progress to cancer in situ eventually invasive carcinoma (2, 3, 7, 10). It is postulated that cancer of the gallbladder is associated with presence of longstanding gallstones in 75% to 90% of patients (1, 2). Since the incidence of gallbladder cancer in Yemen is still unknown and there is no enough information about it in the literature. This study attempts to detect the occurrence of this malignancy in Yemeni patients being operated in two university teaching hospitals between 2002 and 2006.

PATIENTS AND METHOD

Between 2002 and 2006 the researchers have performed 940 cholecystectomy for patients with gallbladder diseases particularly gallbladder stones with or without obstructive jaundice. The procedures were performed in two hospitals, Al-Kuwait University hospital in Sana'a and Al-Wahdah University hospital in Thamar. The group consists of 872 women and 68 men (92.8% and 7.2% respectively) at median age 38 years (ranging from 20 years to 78 years). All patients sent to Department of Surgery for cholecystectomy and were preoperatively diagnosed by sonographer who reported the presence of gallbladder stones with or without thickening of the gallbladder wall, in presence or absence of obstructive jaundice. An open cholecystectomy was performed for 192 patients, while laparoscopic cholecystectomy was performed for 748 patients. Gallbladder specimens were sent for histopathological analysis.

Out of 940 operated patients, 102 cases (96 women and 6 men) were excluded from the study because of absence of histopathological reports in their files.

A retrospective study of 838 patients (62 men and 776 women) whose histopathological reports were present in their files was conducted to detect the occurrence gallbladder cancer. Gall bladder histology is almost a standard postoperative procedure in Al-Kuwait University hospital especially for suspected cases.

RESULTS

Gallbladder carcinoma was detected only in 4 female patients (0.47%) from a series of 838 patients with median age 66 years (57, 60, 70 and 77 years). All four cases were associated

with gallbladder stones. Adenocarcinoma variety was found in all four cases. Diagnosis of the gallbladder cancer was made neither preoperatively by sonographer nor intraoperatively by the surgeon, but incidentally postoperatively by histopathologist. In one of the four cases, the diagnosis of malignancy was already suspected intraoperatively because of a gross thickening of gall bladder wall that enforced us to convert operation from laparoscopic to open. Data of patients with GB carcinoma are shown in (Table 1). The indication for admission to the department of surgery for this case was calculi acute cholecystitis. The average age of patients with a gall bladder carcinoma was significantly higher i.e. 66 years (ranges 57-77 years) than the average age of patients with cholelithiasis without carcinoma i.e. 36 (range: 20-78 years).

Jaundice was observed in 45 patients with gall bladder disease combined with obstruction of extrahepatic biliary tree. In 35 patients the diagnosis was calculi acute cholecystitis.

Acalculi acute cholecystitis was found in two patients despite these two cases were diagnosed preoperatively by ultrasound as calculi acute cholecystitis. Twenty three patients had hydrops of the gallbladder due to impacting stone in the neck of the gallbladder. Intraoperative findings of operated patients are shown in (Table 2).

Table (1): Data of patients with gallbladder carcinoma.

No.	Gender	Age	Weight	District	Clinical diagnosis	Intraoperative finding	Type of procedure
1.	female	57	70kg	Sana'a	Cal. cholecystitis	MGBS. No signs of malignancy	LCHCE
2.	female	60	55	Sana'a	Obstr. Jaund. (cholangitis)	MGBS & CBDS No signs of malig.	OCHCE
3.	female	70	65kg	Taiz	Cal. cholecystitis	SGBS&Thickened GB wall	Converted from LCHCE to OCHCE
4.	female	77kg	65kg	Sana'a	Cal. cholecystitis	MGBS	LCHCE

Cal.= calculi. Obstr. Jaund = obstructive Jaundice. MGBS= multiple gallbladder stones. GB= gallbladder
CBDS= common bile stones. LCHCE= laparoscopic cholecystectomy. OCHCE= open cholecystectomy.

Table (2): Cholecystectomy, Intraoperative finding.

Operative finding	number of Patients	% of patients
Chronic cholecystitis	733	87.4%
Acute cholecystitis - calculi	35	4%
- acalculi	2	0.2%
Hydrops	23	2.7%
Obstructive jaundice	45	5.3%
Total	838	100%

DISCUSSION

Cancer of the gallbladder is a fatal and rare malignant tumor with non-specific presentation and incidence of 1-2% (1, 3, 4). It is the most common malignancy of biliary tract and the fifth most common malignancy of GIT (2, 3). Gallbladder cancer is associated with many risk factors including the obesity, female gender, high parity among women, advanced age and ethnicity (2, 3, 6). Longstanding gallstones remain the most common risk factor to

gallbladder carcinoma development (2, 3, 7, 12).

Our goal is to detect the occurrence of the gallbladder carcinoma among Yemeni patients since this rare malignancy usually has wide geographical, ethnic and cultural variations (2, 11) and there are no reports in the literature or in Medline website about the incidence in Yemen. The histopathological reports of 838 patients operated for gallstones have been retrospectively investigated for primary gallbladder malignancy. Gallbladder carcinoma was detected in 4 female patients with incidence of 0.47%. This ratio remains lower if compared with that reported by Saneejv et al (3). High incidences are seen in Native Americans, South Americans and in North India (7.5 per 100000 for men and 23 per 100000 for women). Rates of up to 5 per 100000 are seen in Japanese and Hispanic American countries. Lower incidence was seen in USA, Nigeria and Singapore (3). However; in Chile, the cancer of the gallbladder is the most frequent cause of cancers related death (2, 3).

In the literature the majority of reports suggests that cancer of the gallbladder affects women two to six times more than men and the incidence peaks in the seventh decade of age (2). In our study, we found that all patients with cancer of the gallbladder were women. The absence of men to have carcinoma of GB in this study might be attributed to the small number of men included in the study. The age of affected women was in 6th and 7th decade of life which is consistent with the literature. The high incidence of GB cancer in elder patients might be attributed to the longstanding mechanical irritation of the mucosa of GB wall by gallstones. This repeated irritation of mucosa may induce mucosal dysplasia, which may progress to cancer in situ eventually invasive carcinoma (2, 3, 9).

Adenocarcinoma represents gallbladder malignancies in more than 87%, and then comes mixed adeno-squamous and squamous cell carcinoma (1, 2, 3). In our cases, the only adenocarcinoma variety was detected. We attributed this finding to the small number of patients with gallbladder carcinoma.

The relationship between gallbladder stones and gallbladder cancer has been well established. Gallstones are found in 75-90% of patients with gallbladder carcinoma, while only 1% of patients with gallstones have cancer of the gallbladder (2, 3, 6, 9). This was confirmed in our series of patients where gallbladder carcinoma cases were associated with gallstones.

Large or multiple gallstones filling the gallbladder lumen may well constitute a marker for malignancy over time by possible repeated mechanical irritation of the gall bladder mucosa that may induce mucosal dysplasia, which may progress to cancer in situ eventually invasive carcinoma (2, 3, 10). This mechanism has been postulated by Solan and Jackson(10), that supports the findings of Lowenfells et al.(11) as well as Diehl et al.(12) who reported that gallstone size increases the risk of gall bladder carcinoma. However Moerman et al.(13) denied these findings and claimed that there is no relationship between stone size and gallbladder carcinoma development (13). In our sample, one patient has had solitary stone with unknown size, two patients have had multiple stones and the fourth case has had multiple stone combined with stones in CBD.

It is postulated that dysplasia is more likely to be found in patients with asymptomatic gallstones due to repeated mechanical irritation of the mucosa of gallbladder over a longer period of time, while symptomatic gallstones do not allow enough time for dysplasia to develop (6).

The occurrence of porcelain gallbladder is rare among biliary diseases but has strong association with gallbladder carcinoma (2, 6, 14). However; we did not detect porcelain

gallbladder in this study.

Despite the advances in hepatobiliary imaging techniques, the preoperative diagnosis of gallbladder cancer remains a challenging task because of the disease's non-specific presentation on one hand and lack of experienced sonographer, who usually seeks for stones rather than cancer of the gallbladder, on the other hand. Although the diagnostic accuracy of ultrasonography and computer tomography (CT) is more than 80% (3), the most common comment of findings described for gallbladder cancer is "diffuse thickening of the wall of the gallbladder". However, this is commonly reported for inflammatory conditions of the gallbladder and therefore does not aid in the cancer diagnosis (1). In our series, the preoperative sonographic comment for finding was either "normal wall thickening or mildly thickened gallbladder wall". It is a rarity that Sonographer identifies a polyp or adenomyomatosis of the gallbladder preoperatively. Therefore the diagnosis of gallbladder cancer in our patients was made neither preoperatively nor intraoperatively but postoperatively by a histopathologist. This defect in experience of our sonographers is apparent in two cases with acalculous cholecystitis that were wrongly diagnosed preoperatively by sonographer as calculous cholecystitis.

The small number of patients with positive histopathological reports for GB malignancy (4 cases of 838) does not necessarily reflect the exact number of patients with cancer of gallbladder among Yemeni patients, because of two reasons:

1. Sometimes, pathologists do not actively look for dysplasia, which may be present in some cases.
2. The histopathological analysis of GB specimens is not a standard measure in all Yemeni hospitals. Therefore, further a larger scale study in other hospitals with use of histopathological analysis of gallbladder specimens as standard investigation, is advisable.

CONCLUSION

The present study supports previous reports that postulated that carcinoma of the gallbladder is rare and age-dependent malignancy and in most affects older women with long-standing gallstone disease. It appears that the occurrence of gallbladder malignancy in Yemeni patients is significantly less than that found among western people (0.47%, 1-2% respectively).

The cooperation between surgeon, sonographer and pathologist is recommended in order to diagnose gallbladder malignancy before surgical intervention. Further study in larger scale in other hospitals, where the use of histopathological analysis of gallbladder specimens is standard investigation, is advised.

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ظهور سرطان المرارة بين المرضى اليمنيين الذين خضعوا لعملية إزالة المرارة في مستشفيات

سعيد هادي البهلولي* ، سلطان أحمد سيف** ، أحمد الملاحي*

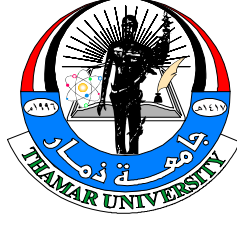
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ملخص

يوصف سرطان المرارة بأنه خبيث جدا وأن ظهوره يتنوع طبقا للمتغيرات الجغرافية والعرقية والثقافية. ولأننا لم نعثر على أي دراسات تعنى بسرطان المرارة عند المرضى اليمنيين فإن الهدف من خلال هذه الدراسة هو تتبع ظهور هذا السرطان عند المرضى اليمنيين الذين خضعوا لعملية إزالة المرارة المرضي وطريقة إجراء الدراسة: أجرينا دراسة وصفية لملفات 940 حالة مرضية (872 إناث و 68 ذكور) كلهم خضعوا لعملية إزالة المرارة في مستشفيات جامعيين في الفترة من 2002 وحتى 2006 ، مركزين على نتائج تحاليل فحص الأنسجة للمرارات. من هذا العدد تم استبعاد الملفات التي لا تحتوي على نتائج للفحص النسيجي للمرارة (102ملفا). إذن 838 حالة هي التي تم دراستها بهدف التعرف على ظهور سرطان المرارة. النتائج: ظهر سرطان المرارة عند 4 حالات كلها إناث وبمعدل عمر عند 66 عاما، جميعها كانت تعاني من آلام حصوات المرارة لفترات طويلة. الموجز: لقد أكدت هذه الدراسة على الفرضية السائدة والتي ترى في سرطان المرارة بأنه مرض نادر يصيب كبار السن من الإناث وبالذات اللاتي يعانين من آلام حصوات المرارة لفترات طويلة ومزمنة. كما إن ظهور سرطان المرارة في العينة المستهدفة بالدراسة يعتبر أقل بكثير مقارنة بالبلدان الغربية. ومن أجل تشخيص سرطان المرارة قبل إجراء العملية فإننا ننصح بالتعاون الوثيق بين الجراح وأخصائي الأشعة واختصاصي فحص الأنسجة خصوصا في الحالات المحتمل إصابتها بسرطان المرارة.

الكلمات الرئيسية: المرارة - عملية إزالة المرارة – سرطان المرارة.

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كلمة رئيس التحرير

بسم الله الرحمن الرحيم

إنه لمن دواعي الفخر أن تصدر جامعة نمار العدد الأول من مجلة جامعة نمار للعلوم الطبيعية والتطبيقية ، وهذا يعكس اهتمام الجامعة و اعتقادها الراسخ بأن تقدم اليمن يعتمد على مقدار ما تحققه من تقدم في البحوث العلمية والتطور الكيفي و النوعي في دراستها وجامعاتها ومؤسساتها التعليمية ، وإصدار مجلة علمية محكمة متخصصة في الأبحاث العلمية وحاصلة على رقم النشر الدولي (ISSN) يعني تنفيذاً لبرنامج فخامة الأخ / رئيس الجمهورية المتعلق بتطوير المعوقه العلمية والبحوث العلمية .

وفي السابق كانت الأبحاث العلمية المتعلقة بالعلوم الطبيعية والتطبيقية ، تصدر في مجلة جامعة نمار بالتناوب مع الأبحاث المتعلقة بالدراسات الإنسانية ، ونحن من هذه البداية المتواضعة نأمل أن تصل البحوث المنشورة في هذه المجلة إلى المستوى العالمي الذي يحقق الفائدة الوطنية والإقليمية والعالمية.

وهيئة التحرير تدعو الأكاديميين في جامعة نمار والجامعات اليمنية الأخرى والباحثين في الجامعات العربية والعالمية أن يرسلوا أبحاثهم للنشر في المجلة ، حيث ستحکم من قبل خبراء وبعد أن تكون مطابقة للمعايير الأكاديمية للنشر والالتزام بشروط المجلة . والمجلة ترحب بأي آراء أو نقد بناء من شأنه أن يسهم في تحسين المجلة وتطويرها.

وفي الأخير لا يسعني إلا أن أقدم الشكر لراعي الجامعة ولنشاطاتها فخامة الأخ / علي عبد الله صالح / رئيس الجمهورية لما يقدمه من رعاية ومتابعة من أجل تطوير الجامعات وتقديمها.

رئيس الجامعة
رئيس هيئة التحرير

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إنتاج صنف جديد من الذرة الرفيعة

عبد الحميد أحمد اليونس

قسم تربية النبات - كلية الزراعة و الطب البيطري - جامعة ذمار - اليمن

ملخص

تُعد طريقة الانتخاب الكمي Mass selection من طرق تربية النبات الفعالة في تحسين الأصناف المتأقلمة لزيادة الحاصل ، إضافة لكونها سريعة النتائج . وتمتلك اليمن العديد من أصناف الذرة الرفيعة المتأقلمة ذات الصفات الجيدة القابلة للتحسين. استخدم الصنف جراحة الواسع الانتشار في المرتفعات الوسطى وصلاحيته للخلط مع دقيق القمح. طبقت التجربة بموسمين 2006 و 2007 وجرى الانتخاب لثلاث مرات. أظهرت النتائج زيادة معنوية عالية في حاصل القصب بمقدار 11.49% ، وفي حاصل الحبوب كانت الزيادة عالية المعنوية أيضاً حيث أعطى الصنف جراحة المنتخب 3.50طن/هـ بينما الصنف الأصل 2.67 طن/هـ وهذه تمثل زيادة مقدارها 23.71% وكانت الزيادة المعنوية العالية الأكثر وضوحاً في عدد حبوب النبات (السبولة) التي بلغت 25.75%. ومن دراسة معامل الارتباط فقد ظهرت علاقة ارتباط موجبة عالية المعنوية (**0.964) بين كل من الحاصل الحبوب وعدد حبوب السبولة و كذلك كانت العلاقة معنوية عالية (**0.701) بين طول النبات (القصبية) وحاصل القصب طن/هـ. نوصي بتكثير بذور جراحة المنتخب لتوزيع بذوره على المزارعين وكذلك الاستفادة منه في برامج تربية النبات مستقبلاً.

مصطلحات رئيسية: ذرة رفيعة ، الأصول الوراثية ، جراحة ، الانتخاب الكمي ، معامل الارتباط .

المقدمة

يُعد محصول الذرة الرفيعة من أهم محاصيل الحبوب الغذائية في اليمن بوصفها تمثل الغذاء الرئيس لغالبية سكان اليمن خصوصاً في الريف سواء كان استخدامها في التغذية المباشرة أو عن طريق تغذية الحيوانات من الماشية و الخيول و الدواجن مارش(2007).

فالذرة تستخدم لعمل العصيدة و العيش و الججين و اللحوح و الهريش. كما يخلط دقيق الذرة مع دقيق القمح بنسبة 30-40% لعمل الخبز، ويشير المصلي (2005) إلى إمكانية إحلال 25% من دقيق القمح بدقيق الذرة الرفيعة (صنف جراحة) لإنتاج خبز القوالب Pan Bread ، ويمكن زيادة الإحلال لأنواع الخبز الأخرى مثل الفرنسي (الصامولي) والرغيف وفقاً لنوعية الدقيق المستخدم ، كما يمكن إحلال 30-40% من دقيق القمح بدقيق الذرة الرفيعة عند إنتاج الكيك و البسكويت.



أشار (Wall and Ross 1970) أن التحليل الكيماوي للبذور أظهر أنها تحتوي على 9.7% بروتين و 3.2% زيت و 78.0% كاربوهيدرات ، مقارنة بحبوب القمح الذي يحتوي بذوره على 14-17% بروتين و 4% زيت و 74% كاربوهيدرات.

ولذلك فإن الذرة الرفيعة كانت وما تزال تستخدم كغذاء رئيسي للسكان في العديد من المناطق في آسيا كإندونيسيا وباكستان و إفريقيا كإثيوبيا والصومال و نيجيريا و الحبشة ، وهو المحصول الأساس في المناطق ذات الأمطار الصيفية كما هو الحال في الأراضي اليمينية ، وزراعتها تنجح في المناطق التي يقل فيها نجاح زراعة القمح بسبب الظروف الجوية غير الملائمة لزراعة القمح، وقدرة الذرة الرفيعة على تحمل ملوحة التربة بنسبة أعلى وكذلك الجفاف و الحد من فقدان ماء النبات بالنتج (Foale et. al. 1984) .

أما النباتات الخضراء وقصب الذرة الجاف و شرف الأوراق (الأوراق المنزوعة من النباتات قبيل الحصاد) فإنها تستخدم في تغذية الحيوانات خاصة الأبقار ، فالقصب الجاف للذرة الرفيعة يحتوي على 56% كاربوهيدرات ، 28% سليلوز و 11.6% بروتين. أما المادة الخضراء فإنها تحتوي على 46% كاربوهيدرات و 25% سليلوز و 11.2% بروتين (Wall and Ross 1970) .

ويجب عند رعي الماشية للذرة الرفيعة أن تكون النباتات قد وصلت عمر 55 يوماً بعد الإنبات تجنباً لحصول التسمم للماشية لأن النباتات الغضة تحتوي على مادة سامة هي الكلوكوسايد Glucoside التي تسبب التسمم عندما ترعى الماشية النباتات الفتية الغضة في الصباح الباكر (Martin and Leonard 1975).

بلغت المساحة المزروعة بالذرة الرفيعة في اليمن حسب إحصاءات عام 2006م، 453011 هكتار أنتجت 401843 طناً بمعدل غلة 887كجم/هـ (نحو 0.9 طن/هـ) وتشكل هذه المساحة 51% و الإنتاج 54% من مجمل مساحة و إنتاج جميع محاصيل الحبوب في اليمن، ومن هنا تتضح أهمية الذرة الرفيعة كغذاء للإنسان و كعلف للماشية و للدواجن، الإحصاء الزراعي (2006). إن أكثر أقطار العالم زراعة و إنتاجاً للذرة الرفيعة هي الهند ثم الولايات المتحدة الأمريكية فالسودان و نيجيريا ، أما على مستوى الوطن العربي فتأتي السودان بالمرتبة الأولى ثم اليمن و مصر (FAO 2006).

وبناء على ما تقدم يهدف البحث إلى إنتاج صنف جديد من الذرة الرفيعة بطرق تربية النبات متفوق الغلة ينتخب من المصادر الوراثية للذرة المحلية.

الأصول الوراثية للذرة الرفيعة في اليمن :

تزرع اليمن بالعديد من الأنواع Species و الأصناف Varieties النباتية التي تشكل مصادر وراثية غاية في الأهمية و التي ظلت عبر قرون عديدة تقاوم الظروف البيئية القاسية و المتباينة من جفاف وحرارة وكذلك مقاومتها للأمراض و الحشرات ، وقد عمل الانتخاب الطبيعي على الأفضل.

وقد بقي الفلاح اليمني يهتم بهذه الأنواع و الأصناف من المحاصيل ويعمل على انتخاب الأكثر مقاومة و أنتاجاً لأنها مصدر لغذائه وعلفاً لحيواناته.

ففي الأراضي اليمينية تنتشر حالياً العديد من الأصناف و الطرز المحلية من الذرة الرفيعة مما تشكل بحيرة وراثية Gene pool ، فمن الذرة الرفيعة تزرع أصناف Varieties و طرز محلية بيئية ecotypes وهي ذات إنتاجية جيدة ، وبعضها مبكر في النضج و الحصاد، كما أن هناك

الأصناف بيضاء البذور و الحمراء و الصفراء و التي تشكل تنوعاً بيئياً Biodiversity يساعد كثيراً في عمليات تربية النبات و التحسين الوراثي، الحكيمي (2000).

وتتنمي معظم أصناف الذرة الرفيعة المزروعة في اليمن حسب (Murth et al. 1981) إلى أن الذرة الرفيعة الحبية Sorghum bicolor تستخدم لغرض إنتاج الحبوب و إنتاج العلف الأخضر و الجاف ، وتختلف أصناف الذرة الرفيعة في اليمن، فلكل منطقة أصنافها وطرزها البيئية المتأقلمة لتلك المنطقة.

وقد درس (Mehra and Amer 1990) العملية التكيفية للذرة الرفيعة في اليمن ، وتعتبر متعددة الاستخدام (حبوب - علف - حطب - بناء مساكن).

وقد أشار المعلم (1982) إلى أن أصناف الذرة الرفيعة تتباين في صفاتها من حيث طول النبات و لون الحبوب و الكفاءة الإنتاجية.

وذكر المجاهد (1980) أن اليمن يمكن أن يكون من المواطن الأصلية لزراعة ونشوء الذرة الرفيعة ، وقد أشار المعلم و آخرون (1993) إلى أن اليمن تعتبر من أهم مواطن التنوع الوراثي إذ لا تكاد تخلو أية منطقة بيئية في اليمن من محصول الذرة الرفيعة ، فهو لتأقلمه الواسع يوجد مزرعاً في دلتا السهل الساحلي ، في الأودية وفي الهضاب و المدرجات على سفوح الجبال.

مواد و طرق البحث

أوضح علي (1988) وكذلك الخشن (1970) طريقة الانتخاب الكمي (الإجمالي) Mass selection أنها تتم بانتخاب النباتات في هذه الطريقة على أساس الشكل الظاهري Phenotype بانتخاب مجموعة من النباتات ذات مظهر متشابه لصفة معينة أو لمجموعة صفات ، وبعد حصادها يتم خلط البذور المخلوطة الناتجة من هذه العملية يطلق عليها بانتخاب التراكيب الوراثية الممتازة . و الغرض من ذلك تحسين المستوى العام للصفة القديم على أساس انتخاب التراكيب الوراثية الممتازة الموجودة أصلاً في الصنف القديم . وتمتاز طريقة الانتخاب الإجمالي بأنها سريعة النتائج تعمل على تحسين غلة الصنف المنتخب و تجانس نباتاته.

زرعت بذور الصنف جراحة الواسع الانتشار في المرتفعات الوسطى ولصلاحيته للخلط ببذور القمح بنسبة 30-40% في الخبز المركب المصلي (2005).

كانت الزراعة في حقول التجارب لمزرعة كلية الزراعة و الطب البيطري- جامعة نمار في الموسمين 2006 و 2007 في الأسبوع الأول من شهر مايو من كل موسم في خطوط المسافة بين خط و آخر 70سم وبين جوره (حفرة) وأخرى 30سم بزراعة أربع بذرات في كل جوره ثم خففت إلى نبات واحد بعد أسبوعين من الإنبات لضمان حصول عمليات الانتخاب مكرد (1998).

سمدت التجربة سماد سوبر فوسفات الكالسيوم 46% خامس أكسيد الفسفور بمقدار 150 كجم/هـ وسماد اليوريا 46% نتروجين بمقدار 150 كجم/هـ أيضاً على دفعتين متساويتين الأولى عند تحضير الأرض مع السماد الفسفوري و الثانية بعد شهر من الإنبات اليونس وعون (2008) ، الري و التعشيب حسب الحاجة.

وعند الحصاد تم انتخاب 100 نبات من مجموعة 1000 نبات ، ثم جرى انتخاب آخر في المعمل للحصول على 10 سبولات جيدة الصفات كما أشار علي (1988).

في الموسم الثاني 2007 زرعت البذور المنتخبة وكذلك بذور من الصنف الأصل القديم كل منها في لوح وبنفس طريقة الزراعة للموسم الأول . وعند الحصاد جرى انتخاب للمرة الثالثة وأجريت الدراسات التالية على عشرة نباتات القاضي (2002) وهي :

ارتفاع النبات (سم) من سطح الأرض حتى قاعدة السبولة ، وزن القصبية للنبات (جم) ، عدد حبوب السبولة ، وزن الف حبة (جم) ، وزن الحبوب طن/هـ ، وزن القصب طن/هـ استخدمت معادلة t – in-groups لدراسة المعنوية وكذلك معامل الارتباط (r) حسبما أوضحهما قاسم وآخرون (1975) لغرض معرفة الصفة الأكثر أهمية في زيادة الحاصل.

النتائج و المناقشة

أظهرت نتائج الجدول (1) وجود فروق معنوية بين الصنف المنتخب الجديد و الصنف الأصل القديم ، و كانت الفروق عالية المعنوية في كل من الصفات: طول النبات/سم ، عدد حبوب النبات (السبولة) ، وزن حبوب النبات (السبولة) جم ، حاصل القش (طن/هـ) وحاصل الحبوب (طن/هـ) ، حيث أعطى الصنف الأصل القديم 3.39 طن/هـ في حاصل القصب وصلت إلى 3.83 طن/هـ في الصنف المنتخب وهذه تمثل زيادة مقدارها 11.49% . وبالنسبة لحاصل الحبوب فإن الصنف الأصل أعطى 2.67 طن/هـ ، ارتفع إلى 3.50 طن/هـ في الصنف المنتخب ، وهذه تمثل زيادة مقدارها 23.71% في حاصل الحبوب. أما الزيادة الأكثر وضوحاً فهي في عدد حبوب سبولة الصنف المنتخب التي وصلت إلى 1472 حبة في المعدل بينما كانت في الصنف الأصل 1093 حبة بزيادة مقدارها 379 حبة في المعدل للسبولة وهذه الزيادة بلغت 25.75% الشكل (1) .

جدول (1): يوضح الصفات المدروسة لكل من الصنف جراحة الأصل و الصنف جراحة المنتخب

الصنف رقم العينة	الأصل	المنتخب	الأصل	المنتخب	الأصل	المنتخب	الأصل	المنتخب	الأصل	المنتخب
	طول النبات (سم)	عدد الحبوب /نبات	وزن الف حبة (جم)	حاصل القش طن/هـ	حاصل الحبوب طن/هـ	حاصل القش طن/هـ	حاصل الحبوب طن/هـ	حاصل القش طن/هـ	حاصل الحبوب طن/هـ	حاصل الحبوب طن/هـ
1	140	150	53.3	3.52	2.76	3.71	2.76	3.52	2.76	3.52
2	130	165	53.4	3.24	2.71	4.10	2.71	3.24	2.71	3.57
3	135	145	52.4	3.19	2.86	3.62	2.86	3.19	2.86	3.43
4	130	150	51.8	3.10	2.52	3.81	2.52	3.10	2.52	3.33
5	170	160	49.4	3.81	2.38	3.90	2.38	3.81	2.38	3.52
6	145	165	50.5	3.52	2.67	3.57	2.67	3.52	2.67	3.67
7	140	145	48.7	3.48	2.62	3.71	2.62	3.48	2.62	3.62
8	130	175	50.6	3.05	2.90	4.19	2.90	3.05	2.90	3.48
9	155	150	52.1	3.52	2.71	3.76	2.71	3.52	2.71	3.38
10	150	165	51.2	3.48	2.57	3.95	2.57	3.48	2.57	3.48
المتوسط	142.5	157.0	51.34	3.39	2.67	3.83	2.67	3.39	2.67	3.50
قيمة (t)	2.77	9.87	1.47	4.49	13.98					
المعنوية	**0.013	**<0.001	NS0.158	**<0.001	**<0.001					

وعند دراسة معامل الارتباط بين الحاصل و الصفات المدروسة ، وبين الصفات نفسها، فقد أوضح الجدول (2) وجود علاقة ارتباط عالية المعنوية بين كل من عدد حبوب السبولة و الحاصل الحبوب (**0.964) ، كذلك كانت العلاقة عالية المعنوية بين طول النبات وحاصل القش (القصب) (**0.701) وهي حالة متوقعة إلا أن العلاقة بين عدد حبوب السبولة ووزن الف حبة كانت عالية المعنوية سالبة (**-0.987) وهذا ناتج عن الزيادة الكبيرة في حبوب السبولة التي سببت انخفاضاً في حجم الحبة الذي انعكس سلباً على وزن الف حبة.

جدول (2): يوضح نتائج تحليل الارتباط للصفات المدروسة للصف جراحة المنتخب

			.284	طول النبات
		.164	.964**	عدد الحبوب/نبات
	-.987**	-.101	-.910	وزن 1000 حبة
.148	-.154	.701**	-.125	حاصل القصب
وزن 1000 حبة	عدد الحبوب/نبات	طول النبات	حاصل الحبوب	



شكل رقم (1): مقارنة بين صف جراحة المنتخب الجديد و صف جراحة الأصل.

الإستنتاجات والتوصيات

- ضرورة الحفاظ على المصادر الوراثية للذرة الرفيعة في اليمن لكونها متأقلمة ، مقاومة للظروف البيئية وذات صفات حقلية جيدة قابلة للتحسين.
- ضرورة استخدام طريقة الانتخاب الكمي لأنها من طرق تربية النبات سريعة النتائج وفي زيادة الحاصل الحبوبي .
- أن الصف الجديد جراحة المنتخب قد حقق زيادة في الحاصل الحبوبي بلغت 24.39% وكذلك زيادة مقدارها 11.49% في حاصل القصب.
- ضرورة تكثير بذور الصف جراحة المنتخب الجديد لتعميم زراعته على المزارعين ، و الاستفادة منه في برامج تربية النبات مستقبلاً .

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Production of New Sorghum (Sorghum Bicolor) Cultivar

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Abstract

A field trial was conducted to produce a new cultivar from the local variety jera'a. The experiment was carried out for two seasons, 2006 and 2007 using mass selection plant breeding method.

The results showed highly significant increase in stock for the new selected cultivar over the local variety amounted to 11.49 % for stock yield, and highly significant increase in grain yield giving 3.50 t/h. for the new cultivar over the local variety 2.67 t/h. This increase represented 23.71% and 25.75% in grain yield and kernel/head respectively.

Highly significant correlation coefficient was obtained between grain yield and kernels/head (0.964**) and between plant length and stock yield (0.701**).

It is recommended to multiply the new selected cultivar seeds to be distributed to farmers, as well as to use them for further breeding work.

دراسة علاقة الارتباط بين الحاصل ومكوناته لتحسين أصناف من القمح في اليمن

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ملخص

إن لتقدير الارتباطات بين الصفات أهمية في تخطيط برامج تربية النبات وصولاً إلى معرفة الصفة الأكثر أهمية في تحقيق زيادة في إنتاج أصناف جديدة من القمح. وقد أظهرت الدراسة علاقة الارتباط بين الحاصل الحبوبى للصنف سبأ والصفات الأخرى المدروسة حيث أن الحاصل الحبوبى له علاقة موجبة معنووية مع كل من وزن ألف حبة ودليل الحصاد، كذلك كانت العلاقة موجبة إلا أنها غير معنووية بين الحاصل وكل من المساحة الورقية وعدد حبوب السنبله وعدد السنابل في المتر المربع، وتظهر هذه الدراسة أن هذا الصنف يمتلك صفات ممتازة يمكن تحسينها مستقبلاً بالاعتماد على وزن ألف حبة في الانتخاب. أما الصنف سونالیکا فإن التركيز على وزن الف حبة والحاصل البيولوجي يعتبران الأكثر أهمية لوجود علاقة ارتباط عالية المعنووية بين الحاصل الحبوبى وكل من هاتين الصفتين ويبقى وزن ألف حبة هو الأكثر سهولة من الناحية التطبيقية أما الصنف بحوث 14 فقد أظهر علاقة ارتباط موجبة معنووية بين الحاصل الحبوبى ودليل الحصاد كما أظهرت أيضاً علاقة موجبة بين الحاصل الحبوبى والمساحة الورقية وطول السنبله إلا أنها لم تصل مستوى المعنووية بينما الصنف بحوث -37 فإن الحاصل الحبوبى يرتبط ارتباطاً موجباً عالي المعنووية مع كل من الحاصل البيولوجي ودليل الحصاد.

المقدمة

يُعد محصول القمح من محاصيل الحبوب الرئيسية في اليمن وهو من أكثر المحاصيل الغذائية استهلاكاً وطلباً عليها في الأسواق المحلية والعالمية. وقد ازدادت أهمية القمح والحاجة إليه في اليمن بسبب الزيادة المستمرة للسكان ومعدل النمو الذي يبلغ 3.2% وهي زيادة تعتبر مرتفعة، فالإحصاءات تشير إلى أن سكان اليمن حسب إحصاءات 1994 كان 14.6 مليون أصبح الآن 23 مليون والمتوقع أن يصل العدد إلى 30 مليون بحلول عام 2015. (1)

إن هذه الزيادة سوف تزيد في الفجوة الغذائية مع زيادة مستمرة في استيراد القمح الذي تصل الفجوة فيه 92% فقد استورد اليمن عام 2001 نحو مليوني طن من القمح وصلت إلى 2.9 مليون عام 2007م، وأسعار القمح وصلت أرقاماً خيالية بحيث أصبح سعر الطن 504 دولار بعد أن كان لا يتجاوز 170 دولار للطن في المعدل. (3).



إن سبب هذه الفجوة الواسعة في نسبة الاكتفاء الذاتي ترجع إلى عدة أسباب لعل أبرزها هو تدني الإنتاجية في الأصناف المزروعة محلياً مقارنة بالعالمية وبالذات المنتجة للقمح، فالمتوسط العالمي 2.7 طن للهكتار تصل إلى 7.5 طن/ هكتار في فرنسا بينما في اليمن 1.5 طن/هكتار في المعدل (3).

ولغرض رفع الإنتاجية المتدنية لا بد من الاتجاه إلى البحث العلمي لإيجاد الوسيلة الأفضل في معالجة هذا التدني ورفع معدل الإنتاجية من خلال استخدام طرق تربية النبات والطرق الإحصائية التحليلية العلمية لرفع كفاءة الإنتاجية لأصناف القمح المتداولة في الزراعة.

هدف البحث

يهدف البحث إلى الكشف عن تحديد المكون الأكثر أهمية من مكونات الحاصل الذي يؤدي إلى رفع الإنتاجية في كل من الأصناف المتداولة وإنتاج أصناف جديدة محسنة.

الدراسات السابقة

أوضح Quail et. al. (1989) عند دراستهم على عدة أصناف من القمح أن الارتباط بين الحاصل وكل من عدد الحبوب في السنبله وكثافة السنابل في المتر المربع ودليل الحصاد كان موجباً وعالي المعنوية على التوالي في حين كان الارتباط بين الحاصل وارتفاع النبات سالباً.

ذكر shamsuldin (1997) أن الارتباط بين الحاصل ودليل الحصاد كان معنوياً موجباً. وجد Amin et. al. (1992) في دراستهم على القمح أن الارتباط بين الحاصل وعدد حبوب السنبله كان موجباً معنوياً، أما الارتباط بين الحاصل ودليل الحصاد فكان عالي المعنوية، بينما كان الارتباط بين دليل الحصاد مع كثافة السنابل /م² سالباً معنوياً في حين كانت علاقة الارتباط بين عدد حبوب السنبله ووزن ألف حبة سالبة لكنها غير معنوية.

أشار Kim (1978) إلى وجود ارتباط موجب بين الحاصل وعدد حبوب السنبله لكنه كان سالباً بين وزن ألف حبة وعدد حبوب السنبله.

وجد Parasad et. al. (1979) ارتباطاً موجباً معنوياً بين حاصل الحبوب وكل من طول السنبله وعدد حبوب السنبله ودليل الحصاد، في حين كان الارتباط سالباً بين حاصل الحبوب وارتفاع النبات، أما Frimmel (1981) فقد وجد أن الارتباط بين مساحة الورقة والحاصل يتغير بتغير المنافسة بين مكونات الحاصل.

وجد Joshi et. al. (1982) ارتباطاً موجباً بين الحاصل ومساحة الورقة، بينما لم يجد Polihamer (1974) علاقة معنوية بين مساحة ورقة العلم وكل من حاصل الحبوب والحاصل البيولوجي.

لاحظ Hamdo (1995) علاقة ارتباط موجبة مع كل من الصفات عدد الحبوب للسنبله وطول السنبله ووزن ألف حبة وكثافة السنابل في المتر المربع ودليل الحصاد.

ذكر Vorbew (1976) عند دراسة الارتباط بين عدة صفات والحاصل كان موجباً عالي المعنوية.

حصل Sharma and Smith (1986) على ارتباط موجب وعالي المعنوية بين الحاصل ودليل الحصاد.

أوضح Sandhu and Margat (1985) أن الارتباط بين حاصل الحبوب في قمح الخبز ووزن ألف حبة وعدد الحبوب/ السنبله كان ارتباطاً موجباً عالي المعنوية، كذلك ارتبط الحاصل

ارتباطاً موجياً معنوياً مع عدد السنابل في المتر المربع ومع ارتفاع النبات، أما الارتباط بين وزن ألف حبة وطول السنبل فكان موجياً عالي المعنوية أيضاً، بينما الارتباط بين حبوب السنبل وعدد السنابل في المتر المربع فكان سالباً ومعنوياً في حين أظهر طول السنبل ارتباطاً موجياً ومعنوياً مع ارتفاع النبات.

المواد وطرق البحث

نفذت التجربة في مزرعة كلية الزراعة والطب البيطري/ جامعة ذمار خلال الموسم الشتوي 2008م ، طبق تصميم القطاعات العشوائية الكاملة (RCBD) بأربعة تكررات على أربعة أصناف من القمح هي سبأ وسونالیکا وبحوث -14 وبحوث -37. حيث زرع كل صنف في كل مكرر بأربعة خطوط، طول الخط ثلاثة أمتار والمسافة بين الخطوط 25 سم، وكانت الزراعة في 2008/2/9م وبمعدل 120 كجم/ هكتار بذار لكل صنف، سمدت كافة التجربة بمقدار 250 كجم/هكتار يوريا، 46% نتروجين على دفعتين متساويتين الأولى عند الزراعة والثانية بعد 45 يوماً، كما أضيف 160 كجم/ هكتار سوبر فوسفات الكالسيوم الثلاثي 48% خامس أكسيد الفوسفور دفعة واحدة عند الزراعة.(2) أخذت عشرة نباتات عشوائياً من كل صنف ومن كل وحدة تجريبية وأجريت الدراسات التالية وهي:

ارتفاع النبات/سم . (2) المساحة الورقية/سم² (2.3 طول السنبل/سم.4) عدد حبوب السنبل.
وعند الحصاد أخذ متر مربع عشوائياً من كل صنف ومن كل وحدة تجريبية لأخذ القراءات على كل من الصفات:

(5) وزن ألف حبة/جم. (6) عدد السنابل في م². (7) وزن الحاصل الحبوبى طن/هكتار.

(8) وزن القش طن/هكتار. (9) الوزن البيولوجي طن/هكتار. (10) دليل الحصاد.

استخدمت معادلة Falconer 1981 لحساب معامل الارتباط (R) (Correlation coefficient) بين الحاصل وكل من الصفات تحت الدراسة وكذلك بين أزواج الصفات نفسها.

النتائج والمناقشة

الصنف سبأ: أظهرت النتائج الموضحة في جدول (1) وجود ارتباط موجب بين كل من الحاصل الحبوبى ومساحة الورقة وعدد حبوب السنبل وعدد السنابل في المتر المربع ووزن ألف حبة ودليل الحصاد. أما العلاقة بين الحاصل الحبوبى وارتفاع النبات وطول السنبل ووزن القش والحاصل البيولوجي فكان سالباً إلا أن هذه العلاقة لم تكن معنوية، ويمكن الاستنتاج بأن هذا الصنف ذو صفات جيدة قد خضعت لضغط انتخابي بحيث لم تظهر معنوية ارتباط بين هذه الصفات، إلا أنه إذا أريد عمل برنامج في تربية النبات فيمكن العمل على تحسين هذا الصنف من خلال التركيز على انتخاب النباتات التي تمتاز بوزن ألف حبة ودليل الحصاد لأن دليل الحصاد المعنوي * (0.752) دليل على زيادة الحاصل الحبوبى على وزن القش من خلال معادلة دليل الحصاد التي هي النسبة بين الحاصل الحبوبى والحاصل البيولوجي.

جدول (1): علاقة الارتباط بين الحاصل ومكوناته للصنف سبأ.

HI %	Bwt t/h	Hwt t/h	TK Wt/g	SN M ²	SK No	SL Cm	LA Cm ²	P.H Cm	SABA
0.752*	-0.479	-0.439	0.687*	0.334	0.323	-0.177	0.618	-0.68	GY
-0.215	-0.543	0.425	-0.140	-0.349	-0.332	-0.379	-0.458	-	PH
0.439	-0.274	-0.352	-0.396	0.621	0.608	0.224	-	-	LA
-0.369	0.615	0.309	-0.250	0.353	0.698*	-	-	-	SL
-0.056	0.073	0.109	-0.594	0.692*	-	-	-	-	SK
-0.214	-0.266	0.355	0.142	-	-	-	-	-	SN
-0.501	0.176	0.247	-	-	-	-	-	-	TKwt
-0.412	-0.092	-	-	-	-	-	-	-	Hwt
-0.189	-	-	-	-	-	-	-	-	Bwt

* معنوي عند 5%، ** معنوي عند 1%، GY الحاصل الحبوبى ط/ه، PH ارتفاع النبات /سم، LA المساحة الورقية/سم²، SL طول السنبله/سم، SK عدد حبوب السنبله، SN عدد السنابل/م²، TKwt وزن ألف حبة/جم، Hwt وزن القش ط/ه، Bwt الحاصل البيولوجي ط/ه، HI دليل الحصاد %.

وبالنسبة لدراسة معامل الارتباط بين أزواج الصفات الأخرى، فقد ظهر ارتباط موجب ومعنوي بين طول السنبله وعدد حبوب السنبله، وهذا يشير إلى أن زيادة عدد حبوب السنبله ينعكس إيجاباً على طول السنبله، كما ظهرت علاقة ارتباط موجبة ومعنوية بين عدد حبوب السنبله وعدد السنابل في المتر المربع.

الصنف سوناليكا:

أظهر الجدول (2) عند دراسة معامل الارتباط بين الحاصل و الصفات الأخرى لهذا الصنف، أن كلا من وزن ألف حبة والحاصل البيولوجي أظهر ارتباطاً موجباً ومعنوياً عالياً، بينما كانت العلاقة بين الحاصل الحبوبى وكل من حاصل القش ودليل الحصاد موجبة معنوية، ويمكن اختيار أي واحدة من هذه الصفات للانتخاب على أساسها بحيث تؤدي إلى زيادة حاصل هذا الصنف، إلا أنه يلاحظ بأن صفة وزن ألف حبة وكذلك الحاصل البيولوجي قد أعطيتا أعلى قيمة لمعامل الارتباط وهي (0.931** و 0.924**).

ويمكن التركيز على وزن ألف حبة لأنها سهلة التنفيذ من الناحية العملية. أما معامل الارتباط بين الصفات الأخرى فقد كانت موجبة معنوية بين المساحة الورقية وكل من عدد السنابل في المتر المربع، ووزن ألف حبة، حاصل القش والحاصل البيولوجي، وبين طول السنبله ودليل الحصاد. كما كانت العلاقة موجبة عالية المعنوية بين وزن ألف حبة والحاصل البيولوجي وبين حاصل القش والحاصل البيولوجي مما يدل على أن زيادة القش تؤدي طردياً إلى زيادة الحاصل البيولوجي الذي يمثل كلا من القش وحاصل الحبوب. وقد أظهرت النتائج أن لمساحة الورقة أثر إيجابي بالإضافة إلى عدة صفات مهمة لكونها ذات أهمية في عملية التركيب الضوئي.

الصنف بحوث -14:

عند دراسة معامل الارتباط للصفات تحت الدراسة الموضحة في جدول (3) ظهرت علاقة موجبة بين حاصل الحبوب وكل من المساحة الورقية وطول السنبله وعدد السنابل في المتر المربع ودليل الحصاد، إلا أن دليل الحصاد كان معنوياً بلغ (0.749*)، كما أن معامل الارتباط لمساحة

الورقة يليه طول السنبله كان عالياً إلا أنها لم تصل إلى مستوى المعنوية ورغم ذلك يبقى دليل الحصاد هو الأكثر تأثيراً في زيادة الحاصل الحبوبى.
وعند دراسة العلاقة بين أزواج الصفات الأخرى فقد كانت العلاقة موجبة بين عدد حبوب السنبله وكل من وزن ألف حبة وحاصل القش والحاصل البيولوجي، وحيث أن وزن ألف حبة هو الأعلى قيمة (*0.763) فإنه يبقى الأكثر تأثيراً على عدد حبوب السنبله.

جدول (2): علاقة الارتباط بين الحاصل ومكوناته للصنف سونالیکا.

HI %	Bwt t/h	Hwt t/h	TK Wt/g	SN M ²	SK No	SL Cm	LA Cm ²	P.H Cm	SONA LIKA
0.741*	0.924**	0.771*	0.931**	0.569	0.006	0.524	0.678	0.537	GY
0.187	0.614	0.611	0.620	0.239	0.016	0.495	0.326	-	PH
0.285	0.738*	0.716*	0.703*	0.732*	0.115	0.204	-	-	LA
0.782*	0.261	0.028	0.653	0.312	0.401	-	-	-	SL
0.106	-0.042	-0.082	-0.007	0.332	-	-	-	-	SK
0.293	0.581	0.536	0.575	-	-	-	-	-	SN
0.739*	0.835**	0.678	-	-	-	-	-	-	TKwt
0.146	0.956**	-	-	-	-	-	-	-	Hwt
0.429	-	-	-	-	-	-	-	-	Bwt

* معنوي عند 5%، ** معنوي عند 1%، GY الحاصل الحبوبى ط/هـ ، PH ارتفاع النبات /سم، LA المساحة الورقية/سم²، SL طول السنبله/سم، SK عدد حبوب السنبله، SN عدد السنابل/م²، TKwt وزن ألف حبة/جم، Hwt وزن القش ط/هـ ، Bwt الحاصل البيولوجي ط/هـ ، HI دليل الحصاد %.

أما العلاقة بين وزن ألف حبة وكل من حاصل القش (*0.889) والحاصل البيولوجي (*0.834) فكانت معنوية عالية، مما يشير إلى أن الحبوب الممتلئة تعمل على زيادة حاصل القش والحاصل البيولوجي، كما ظهرت علاقة طردية موجبة عالية المعنوية بين حاصل القش والحاصل البيولوجي حيث أن حاصل القش هو جزء من مكونات الحاصل البيولوجي.

جدول (3) علاقة الارتباط بين الحاصل ومكوناته للصنف بحوث -14.

HI %	Bwt t/h	Hwt t/h	TK Wt/g	SN M ²	SK No	SL Cm	LA Cm ²	P.H Cm	BOH 14
0.749*	-0.139	-0.381	-0.439	0.347	-0.275	0.452	0.505	-0.079	GY
-0.238	0.196	0.202	0.355	0.093	-0.091	-0.562	0.062	-	PH
0.173	0.105	-0.028	0.188	0.066	0.119	-0.508	-	-	LA
0.508	-0.202	-0.302	-0.513	0.244	-0.404	-	-	-	SL
-0.639	0.722*	0.743*	0.763*	-0.123	-	-	-	-	SK
0.487	-0.374	-0.436	-0.201	-	-	-	-	-	SN
0.882**	0.834**	0.889**	-	-	-	-	-	-	TKwt
-0.874**	0.969**	-	-	-	-	-	-	-	Hwt
-0.744	-	-	-	-	-	-	-	-	Bwt

* معنوي عند 5%، ** معنوي عند 1%، GY الحاصل الحبوبى ط/هـ ، PH ارتفاع النبات /سم، LA المساحة الورقية/سم²، SL طول السنبله/سم، SK عدد حبوب السنبله، SN عدد السنابل/م²، TKwt وزن ألف حبة/جم، Hwt وزن القش ط/هـ ، Bwt الحاصل البيولوجي ط/هـ ، HI دليل الحصاد %.

الصنف بحوث 37:

من الجدول (4) نلاحظ علاقة ارتباط موجبة معنوية لحاصل القش مع الحاصل الحبوبى إلا أن العلاقة الموجبة كانت عالية المعنوية بين الحاصل وكل من الحاصل البيولوجي (**0.849) ودليل الحصاد (**0.926). ومن العلاقات الموجبة مع الحاصل الحبوبى هو طول السنبله التي كانت (0.603) إلا أنها لم تصل مستوى المعنوية.

أما العلاقة بين ارتفاع النبات ووزن ألف حبة فكانت موجبة عالية المعنوية (**0.939) وهذا يدل على أن وزن ألف حبة يؤثر إيجاباً على ارتفاع النبات. أما العلاقة بين حاصل القش والحاصل البيولوجي فهي عالية المعنوية (**0.976) وهذا يعود إلى أن القش هو أحد مكونات الحاصل البيولوجي الأساسية.

جدول (4) علاقة الارتباط بين الحاصل ومكوناته للصنف بحوث 37.

HI %	Bwt t/h	Hwt t/h	TK Wt/g	SN M ²	SK No	SL Cm	LA Cm ²	P.H Cm	BOH 37
0.926**	0.849**	0.718*	0.247	0.027	0.159	0.603	0.174	-0.396	GY
-0.265	-0.465	-0.425	0.939**	-0.393	-0.452	-0.759	0.509	-	PH
0.273	-0.041	-0.098	0.446	-0.028	0.176	-0.493	-	-	LA
0.335	0.812**	0.811*	-0.744*	0.025	0.458	-	-	-	SL
-0.034	0.311	0.343	-0.621	0.011	-	-	-	-	SK
-0.058	0.176	0.212	-0.444	-	-	-	-	-	SN
-0.036	-0.458	-0.481	-	-	-	-	-	-	TKwt
0.404	0.976**	-	-	-	-	-	-	-	Hwt
0.591	-	-	-	-	-	-	-	-	Bwt

GY الحاصل الحبوبى ط/ه ، PH ارتفاع النبات /سم ، LA المساحة الورقية/سم²، SL طول السنبله/سم ، SK عدد حبوب السنبله ، SN عدد السنابل/م² ، TKwt وزن ألف حبة/جم ، Hwt وزن القش ط/ه ، Bwt الحاصل البيولوجي ط/ه ، HI دليل الحصاد %.

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التحري عن متبقيات المضادات الحيوية في اللحوم الحمراء وتأثير المعاملات الحرارية عليها

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ملخص

هدفت هذه الدراسة إلى الكشف عن متبقيات المضادات الحيوية في اللحوم الحمراء الناتجة من الأبقار والأغنام والماعز المعروضة للبيع في محلات بيع اللحوم في مدينة ذمار ، ودراسة تأثير كل من التبريد والتجميد والطبخ على تواجد هذه المتبقيات. شملت الدراسة (300) ذبيحة وبواقع (100) ذبيحة لكل من الأبقار والأغنام والماعز ، ومن كل ذبيحة تم أخذ أربعة نماذج تمثل العضلات والكبد والكليتين والقلب . أجري الفحص باستخدام الطريقة المايكروبيولوجية المباشرة ، واستعملت لهذه الدراسة جرثومة *Bacillus subtilis* لاختبار الكشف عن متبقيات المضادات الحيوية في هذه الأنواع من اللحوم الحمراء . أظهرت النتائج أن أعلى نسبة لتواجد متبقيات المضادات الحيوية كانت في ذبائح الأبقار تليها الأغنام ثم الماعز ، حيث بلغت النسبة (8.5%) ، (6.0%) و (4.3%) على التوالي . وأتضح من الدراسة أن هنالك تأثيراً واضحاً لدرجة حرارة التبريد على بعض المتبقيات من المضادات الحيوية المتواجدة في اللحوم الحمراء ، في حين لم تتأثر هذه المتبقيات بدرجة حرارة التجميد (- 18 م) ولمدة (30) يوماً . وعند إجراء عملية الطبخ (درجة الغليان) ولمدة (60) دقيقة على العينات الموجبة لتواجد متبقيات المضادات الحيوية لوحظ التأثير الواضح لهذه العملية على تلك المتبقيات . وقد تمت مناقشة الأهمية الصحية لتواجد متبقيات المضادات الحيوية في اللحوم الحمراء والدور الذي تقوم به هذه المتبقيات في إحداث الكثير من المخاطر الكبيرة على صحة الإنسان سواء تلك المخاطر الناجمة عن التفاعلات السمية أو عن الحالات السرطانية أو عن المقاومة الجرثومية أو غيرها من المخاطر الأخرى .

المقدمة

المضادات الحيوية عبارة عن مواد عضوية كيميائية معقدة التركيب يتم إنتاجها كلياً أو جزئياً بواسطة الأحياء المجهرية سواء البكتيريا أو الفطريات ، والتي لها القابلية وبتراكيز مختلفة على تثبيط أو قتل البكتيريا والأنواع الأخرى من الأحياء المجهرية ، كما أنها ذات فعالية فسلجية عالية حتى في التراكيز القليلة جداً ، فعلى سبيل المثال يؤثر البنسلين على الجراثيم الحساسة له حتى عندما يكون ذا تركيز 0.000001 غم / مل ، وكذلك فإن عمل المضادات الحيوية يكون اختيارياً أي بمعنى أن قوتها الحيوية تكون ضد جراثيم محددة (1 ، 2) .



ومن الجدير بالذكر أن غالبية الأغذية وبخاصة الأغذية ذات المنشأ الحيواني ومنها اللحوم الحمراء يمكن أن تتعرض إلى العديد من الملوثات سواء الملوثات الجرثومية أو المبيدات الحشرية أو المعادن الثقيلة أو المواد الكيميائية ولاسيما المضادات الحيوية ، حيث أن اللحوم الحمراء يمكن أن تتعرض إلى المضادات الحيوية سواء أثناء فترة التربية للحيوانات المنتجة لهذه اللحوم أو بعد عمليات الذبح وبخاصة عند استخدام المضادات الحيوية في عمليات الحفظ وإطالة مدة الخزن لهذه اللحوم أو عند استعمال المضادات الحيوية أثناء عمليات التعليب أو غيرها من المعاملات الأخرى (3 ، 4) .

وعلى الرغم من الفوائد المتوخاة من استعمال المضادات الحيوية في المجالات المختلفة وبخاصة في مجال الوقاية أو في مجال المعالجة من الأمراض الناتجة عن مسببات البايولوجية سواء المسببات الفايروسية أو المسببات البكتيرية أو غيرها من المسببات ، إلا أن بقاء كميات حتى ولو قليلة جداً من هذه المضادات في جسم الحيوان وبالتالي في اللحوم الناتجة منه تؤدي إلى حدوث الكثير من المخاطر الصحية البالغة على صحة الإنسان وحياته (5 ، 6) .

وتجدر الإشارة إلى أن وجود المضادات الحيوية في اللحوم يمكن أن يحدث إما بصورة عرضية (Accidental) ، أي أن تواجد المتبقيات في هذه الحالة يكون ناتجاً عن طريقة عرضية وغير مقصودة فقد يكون نتيجة لخطأ من الشخص القائم بالعمل كأن يعطي مثلاً جرعة أكثر من الجرعة المقررة أو أنه يلتزم بحدود الجرعة المقررة ولكنه يستمر في استعمال المضاد الحيوي لمدة طويلة ، ومن الممكن أن يحدث نتيجة لعدم ترك مدة كافية للتخلص من متبقيات هذه المضادات قبل عملية الذبح ، أو قد يحدث عند تعريض الغذاء أو الماء للتلوث بهذه المضادات (7 ، 8) .

وقد يحدث بصورة مقصودة (Intentional) ، أي أن تواجد المتبقيات في هذه الحالة يكون ناتجاً عن طريقة مقصودة ، حيث تستعمل في هذه الحالة المضادات الحيوية إما للعلاج والوقاية ، وأما أن يكون القصد من استعمالها هو لغرض تحفيز النمو في الحيوانات ، أو ربما تستخدم لغرض حفظ اللحوم ومنتجاتها (9 ، 10) .

يعتمد تواجد المضادات الحيوية في الأنسجة العضلية على العديد من العوامل ، من أهمها نوع وتركيز المضاد الحيوي ، طريقة إعطاء الدواء ، وقت إيقاف استعمال المضاد الحيوي قبل عملية الذبح ، ومدى حساسية الطريقة المستعملة للكشف عن هذه المتبقيات (11 ، 12) .

وفي العقود الأخيرة زاد الاهتمام بشكل كبير بموضوع متبقيات المضادات الحيوية في الأنواع المختلفة من الأغذية وبخاصة اللحوم سواء اللحوم الحمراء أو اللحوم البيضاء ، لما تسببه هذه المضادات من مخاطر صحية واقتصادية على المستوى العالمي ، ولذلك فقد قامت منظمة الصحة العالمية (WHO) بتحديد المعدلات القصوى لمتبقيات المضادات الحيوية في الأنسجة الحيوانية المختلفة والتي تشمل العضلات ، الأرباب ، الكلى ، الدهون (13 ، 14) .

وبالنظر لخطورة هذه المتبقيات على صحة الإنسان ، فقد صممت هذه الدراسة لمعرفة نسبة تواجد هذه المتبقيات في اللحوم الحمراء الناتجة من الأبقار والأغنام والماعز المتداولة في مدينة ذمار ، بالإضافة إلى دراسة مدى تأثير المعاملات الحرارية على تواجد متبقيات المضادات الحيوية في هذه اللحوم .

المواد وطرائق العمل**أولاً:- جمع العينات**

تم جمع العينات من (300) ذبيحة من محلات بيع اللحوم في مناطق متفرقة من مدينة ذمار ، وبواقع (100) ذبيحة لكل من ذبائح الأبقار والأغنام والماعز ، ومن كل ذبيحة تم أخذ أربعة نماذج تمثل العضلات والكبد والكليتين والقلب ، وذلك خلال الفترة الممتدة ما بين يناير 2008 م ولغاية نهاية شهر سبتمبر 2008 م .

جمعت العينات وفقاً لما ورد في (15) ، حيث تم أخذ كمية تتراوح ما بين (25 – 50) غم لكل نموذج من النماذج المشار إليها في أعلاه ، ووضعت كل عينة في كيس نايلون معقم ، ثم نقلت العينات في صندوق مبرد (Cool Box) إلى مختبر الصحة العامة و الأمراض المشتركة / كلية الزراعة والطب البيطري / جامعة ذمار ، وفي المختبر قسم كل نموذج إلى أربعة أجزاء .

الجزء الأول:- أجري عليه اختبار الكشف عن متبقيات المضادات الحيوية باستخدام الطريقة المايكروبيولوجية المباشرة (Direct method) وطبقاً لما أشار إليها كل من (16 ، 17) ، وأن النماذج التي اظهرت نتيجة موجبة أخضعت للخطوات التالية .

الجزء الثاني:- حفظ بدرجة حرارة التلاجة (4 م) ولمدة ثلاثة أيام ، وأجري الفحص على العينات بعد انتهاء فترة الحفظ ، حيث أجري الفحص على النماذج التي أعطت نتيجة موجبة وباستخدام الطريقة المايكروبيولوجية المباشرة .

الجزء الثالث:- حفظ بدرجة حرارة التجميد (- 18 م) ولمدة (30) يوماً ، وأجري الفحص على العينات بعد انتهاء فترة الحفظ ، حيث أجري الفحص على النماذج التي أعطت نتيجة موجبة وباستخدام الطريقة المايكروبيولوجية المباشرة .

الجزء الرابع:- حفظ بدرجة حرارة التجميد (- 18 م) ولمدة (30) يوماً ، بعدها ترك في درجة حرارة الغرفة ولمدة ساعة واحدة ، وبعد ذلك تم تعريضها لدرجة حرارة الطبخ (درجة الغليان) ولمدة (60) دقيقة ، حيث أجري الفحص على النماذج التي أعطت نتيجة موجبة وباستخدام الطريقة المايكروبيولوجية المباشرة .

ثانياً:- تحضير معلق الأبواغ

استعملت لهذه الدراسة جرثومة *Bacillus subtilis* بوصفها جرثومة اختبار ، حفظت هذه الجرثومة على الوسط الزرع المائل (Slant) ، ثم حفظت بدرجة حرارة التلاجة ، وجرى تجديد وتنشيط الجرثومة مرة كل أسبوعين للمحافظة على نشاطها ، استعملت صبغة كرام للتأكد من عدم حصول أي تلوث جرثومي آخر ، حضر محلول قياسي لمعلق الأبواغ والموصوف من قبل (18) وكما ورد في (19) .

ثالثاً:- تحديد تركيز الأبواغ

تم أجرائه باستعمال طريقة العد القياسي بالأطباق (Standard Plate Count Method) ، وبحسب ماورد في (20) باستعمال الوسط الزرع الصلب والمعقم الخاص للعد القياسي ، وقد ثبت التركيز على 107 بوغ / مل من المعلق الجرثومي وفقاً لما ورد في (16) .

رابعاً:- تحضير الأطباق الزرعية المبدورة

تم أجرائه بوضع (0.1) مل من معلق الأبواغ في طبق بتري معقم وأضافه (13 – 15) مل من وسط الميلر – هنتون (Mueller – Hinton Agar) بدرجة (45 – 50) م لذلك الطبق مع التحريك المستمر لضمان انتشار الأبواغ بصورة جيدة داخل الوسط الزرع ، ثم ترك ليبرد ويتصلب بدرجة حرارة الغرفة .

خامساً:- الكشف عن متبقيات المضادات الحيوية

استخدمت لهذا الغرض الطريقة المايكروبيولوجية المباشرة ، حيث أخذت قطعة اللحم وقطعت إلى أقراص ذات قطر (8) ملم وذات سمك (2) ملم من كل نموذج ، ووضعت مباشرة على سطح الوسط الزرع المبدور ، وبعدها تركت الأطباق بدرجة حرارة الغرفة لمدة (2) ساعة للسماح للمضاد الحيوي أن وجد بالانتشار داخل الوسط الزرع المبدور ، وبعدها حضنت الأطباق على درجة (32) م ولمدة (18 – 20) ساعة ، ووفقاً لما ورد في (16 ، 17) .

قرأت النتائج عن طريق قياس هالة تثبيط النمو (Inhibition zone of growth) من نهاية حافة قطعة اللحم إلى بداية النمو الجرثومي أي إلى نهاية منطقة التثبيط ، حيث تعد النتيجة موجبة إذا كان قطر هالة التثبيط يساوي (2) ملم أو أكثر من ذلك ، وتعد النتيجة قليلة (Trace) إذا كان قطر هالة التثبيط (1) ملم ، في حين تعد النتيجة سالبة إذا كان قطر هالة التثبيط أقل من (1) ملم وذلك وفقاً لما ورد في (16 ، 21 ، 22) .

سادساً:- المعاملات الحرارية

أجري الكشف عن تأثير المعاملات الحرارية المختلفة على النماذج التي أظهرت نتيجة موجبة فقط لفحص الكشف عن متبقيات المضادات الحيوية والذي أشرنا إليه في الفقرة (خامساً) في أعلاه ، حيث تم إجراء المعاملات الآتية:-

1- التبريد

حفظت النماذج في الثلاجة بدرجة (4 م) ولمدة (3) أيام ، وأخذت قطعة على هيئة قرص من النموذج المراد فحصه ، ووضعت مباشرة على سطح الوسط الزرع الصلب المبدور.

2- التجميد

حفظت النماذج في المجمدة (- 18 م) ولمدة (30) يوماً ، وأخذت قطعة على هيئة قرص من النموذج المراد فحصه ، ووضعت مباشرة على سطح الوسط الزرع الصلب المبدور.

3- الطبخ

حفظت العينات بدرجة التجميد (- 18 م) ولمدة (30) يوماً ، وبعدها تم اخراج العينات وتركت في درجة حرارة الغرفة لمدة ساعة واحدة ، ثم وضعت في دورق زجاجي معقم سعة 400 مل يحتوي على 300 مل ماء مقطر معقم ، وبعد ذلك وضعت في حمام مائي (Water bath) بدرجة الغليان ولمدة (60) دقيقة ، حيث تم حساب الوقت بعد حصول الغليان داخل الدورق الزجاجي ، بعد ذلك تركت لتبرد بدرجة حرارة الغرفة ، وأجري فحص الكشف عن متبقيات المضادات الحيوية بالطريقة المايكروبيولوجية المباشرة .

النتائج

أظهرت النتائج أن أعلى نسبة لتواجد متبقيات المضادات الحيوية كانت في ذبائح الأبقار تليها الأغنام ومن ثم الماعز ، حيث بلغت نسبة تواجد متبقيات المضادات الحيوية في هذه الذبائح (8.5%) ، (6.0%) و (4.3%) على التوالي ، وكما هو موضح في الجدول رقم (1) . كما يتضح من الجدول أعلاه بأن نسبة تواجد متبقيات المضادات الحيوية في الأكباد الناتجة من الأبقار والأغنام والماعز كانت هي الأعلى ، حيث بلغت (13 %) ، (9 %) و (7 %) على التوالي .

وأشارت النتائج الموضحة في الجدول رقم (2) إلى وجود تأثير واضح لدرجة حرارة التبريد (4 م) ولمدة ثلاثة أيام على بعض المتبقيات من المضادات الحيوية المتواجدة في العينات . وعند إجراء عملية التجميد على (- 18 م) ولمدة (30) يوماً ، أشارت النتائج إلى عدم وجود تأثير واضح لهذه العملية على متبقيات المضادات الحيوية المتواجدة في اللحوم الحمراء ، وكما هو موضح في الجدول رقم (3) .

أما النتائج الموضحة في الجدول رقم (4) فأنها تشير إلى العدد والنسبة المئوية للنماذج المفحوصة والنماذج المتأثرة المأخوذة من ذبائح الأبقار والأغنام والماعز .

جدول (1):- يوضح نتائج الطريقة المايكروبايولوجية المباشرة للكشف عن متبقيات المضادات الحيوية في ذبائح الأبقار والأغنام والماعز .

نوع النماذج	عدد النماذج المفحوصة	النتائج الموجبة			
		ذبائح الأبقار	ذبائح الأغنام	ذبائح الماعز	العدد النسبة المئوية
العضلات	100	8	6	3	8 % 6 % 3 %
الأكباد	100	13	9	7	13 % 9 % 7 %
الكلى	100	7	5	4	7 % 5 % 4 %
القلوب	100	6	4	3	6 % 4 % 3 %
المجموع	400	34	24	17	8.5 % 6.0 % 4.3 %

جدول (2):- يوضح تأثير درجة حرارة التبريد (4 م) ولمدة ثلاثة أيام على العينات الموجبة لمتبقيات المضادات الحيوية في اللحوم الحمراء الناتجة من ذبائح الأبقار والأغنام والماعز .

نوع النماذج	ذبائح الأبقار			ذبائح الأغنام			ذبائح الماعز		
	عدد النماذج الموجبة	عدد النماذج المتأثرة*	النسبة المئوية	عدد النماذج الموجبة	عدد النماذج المتأثرة*	النسبة المئوية	عدد النماذج الموجبة	عدد النماذج المتأثرة*	النسبة المئوية
العضلات	8	3	37.5 %	6	2	33.3 %	3	2	66.7 %
الأكباد	13	4	30.8 %	9	3	33.3 %	7	3	42.9 %
الكلى	7	1	14.3 %	5	1	20.0 %	4	1	25.0 %
القلوب	6	1	16.7 %	4	2	50.0 %	3	1	33.3 %

التحري عن متبقيات المضادات الحيوية في اللحوم الحمراء وتأثير المعاملات الحرارية عليها ضاري عليوي المشهداني
جدول (3):- يوضح تأثير درجة حرارة التجميد (- 18 م) ولمدة (30) يوماً على العينات الموجبة لمتبقيات
المضادات الحيوية في اللحوم الحمراء الناتجة من ذبائح الأبقار والأغنام والماعز .

نوع النماذج	ذبائح الأبقار			ذبائح الأغنام			ذبائح الماعز		
	عدد النماذج الموجبة	عدد النماذج المتأثرة*	النسبة المئوية	عدد النماذج الموجبة	عدد النماذج المتأثرة*	النسبة المئوية	عدد النماذج الموجبة	عدد النماذج المتأثرة*	النسبة المئوية
العضلات	8	0	% 0	6	0	% 0	3	0	% 0
الأكباد	13	0	% 0	9	0	% 0	7	0	% 0
الكلى	7	0	% 0	5	0	% 0	4	0	% 0
القلوب	6	0	% 0	4	0	% 0	3	0	% 0

الجدول رقم (4):- يوضح تأثير درجة حرارة الطبخ (درجة الغليان) ولمدة (60) دقيقة على العينات الموجبة
لمتبقيات المضادات الحيوية في اللحوم الحمراء الناتجة من ذبائح الأبقار والأغنام والماعز .

نوع النماذج	ذبائح الأبقار			ذبائح الأغنام			ذبائح الماعز		
	عدد النماذج الموجبة	عدد النماذج المتأثرة*	النسبة المئوية	عدد النماذج الموجبة	عدد النماذج المتأثرة*	النسبة المئوية	عدد النماذج الموجبة	عدد النماذج المتأثرة*	النسبة المئوية
العضلات	8	8	% 100	6	6	% 100	3	3	% 100
الأكباد	13	13	% 100	9	9	% 100	7	7	% 100
الكلى	7	7	% 100	5	5	% 100	4	4	% 100
القلوب	6	6	% 100	4	4	% 100	3	3	% 100

* النماذج المتأثرة:- ويقصد بها النماذج التي أظهرت نتائج سلبية لفحص الطريقة المايكروبيولوجية المباشرة ، أي النماذج التي تم فيها استنزاف متبقيات المضادات الحيوية.

المنافشة

إن وجود متبقيات المضادات الحيوية في الأنواع المختلفة من الأغذية ومنها اللحوم الحمراء يؤدي إلى حدوث الكثير من المخاطر الكبيرة على صحة الإنسان ، ومن أهم هذه المخاطر هي حدوث التفاعلات السمية سواء التسمم المباشر الذي تسببه هذه المتبقيات أو حدوث حالات سرطانية حيث أشارت الكثير من الدراسات إلى أن متبقيات المضادات الحيوية لها فعالية مسرطنة للإنسان ، وكذلك حدوث التفاعلات ذات العلاقة بموضوع الحساسية وهي من المخاطر التي تهدد حياة الإنسان عند وجود المضادات الحيوية في الأنواع المختلفة من الأغذية ومنها اللحوم الحمراء فقد تحصل الحساسية لدى بعض الأشخاص عند وجود كمية قليلة من المضادات الحيوية ولاسيما البنسلين ، بالإضافة إلى حدوث المقاومة الجرثومية للمضادات الحيوية حيث أكدت الأبحاث التي أجريت في هذا المجال إلى أن تعرض المستهلك لجرعات قليلة وغير فعالة من المضادات الحيوية يؤدي إلى ضعف مقاومة جسم الإنسان لأية عدوى يتعرض لها وكذلك ظهور عترات (Strains) وأجيال من الجراثيم المختلفة مقاومة لهذه الجرعات من المضادات الحيوية (23 ، 24 ، 25) .
وقد أكدت هذه الدراسة وبشكل واضح على المخاطر الصحية الناجمة عن تواجد متبقيات المضادات الحيوية في الأنواع المختلفة من اللحوم الحمراء ، حيث أظهرت النتائج التي حصلنا

عليها أن نسبة تواجد هذه المتبقيات في ذبائح الأبقار بلغت (8.5 %) ، والأغنام (6.0 %) ، والمعاز (4.3 %) ، وكما هو موضح في الجدول رقم (1) .

كما يتضح من الجدول أعلاه أن ذبائح الأبقار معرضة لتواجد متبقيات المضادات الحيوية أكثر من ذبائح الأغنام والمعاز ، وأن ذبائح الأغنام معرضة لتواجد متبقيات المضادات الحيوية أكثر من ذبائح المعاز ، وربما يعود السبب في ذلك إلى انتشار تربية الأبقار والأغنام في مناطق مختلفة وعلى مساحات واسعة من الارض ، على العكس من المعاز الذي ربما تكون تربيتها محدودة إلى حد ما وأقل بالمقارنة مع الأبقار والأغنام ، وبالتالي فإن الأبقار والأغنام يمكن أن تتعرض أكثر من غيرها من الحيوانات الحقلية لأنواع المختلفة من المسببات المرضية وبخاصة الأصابات التنفسية والتسممات المعوية والطفيليات الخارجية والداخلية وغيرها من المسببات الأخرى التي تستوجب أستمعلاً واسعاً للمضادات الحيوية سواء في مجال المعالجة أو في مجال الوقاية من هذه الأمراض والسيطرة عليها عند حدوثها .

وبهدف اجراء المقارنة للنتائج التي حصلنا عليها مع بعض الدراسات المحلية ، فقد حاولنا البحث عن دراسات محلية سابقة حول هذا الموضوع في الجمهورية اليمنية ، إلا أننا لم نحصل على ذلك ، حيث يبدو أن هذه الدراسة هي الأولى من نوعها في الجمهورية اليمنية .

وعند اجراء المقارنة للنتائج التي حصلنا عليها مع بعض الدراسات التي اجريت في وطننا العربي وفي بعض المناطق الأخرى من العالم ، فأنا نلاحظ بأن نسبة تواجد متبقيات المضادات الحيوية التي حصلنا عليها مقارنة إلى النسب التي وجدها بعض الباحثين (26) ، إلا أن هذه النسب اختلفت مع البعض الأخر من الباحثين (15) .

ومن خلال هذه الدراسة تبين أن نسبة تواجد متبقيات المضادات الحيوية في الأكياد كانت هي الأعلى بالمقارنة مع نسبة تواجد هذه المتبقيات في الأجزاء والأعضاء الأخرى من الذبائح ، حيث يتضح من الجدول (1) أن نسبة تواجد متبقيات المضادات الحيوية في أكباد الأبقار والأغنام والمعاز بلغت (13 %) و (9 %) و (7 %) على التوالي ، وجاءت هذه النتائج متقاربة مع النتائج التي حصل عليها بعض الباحثين (27 ، 28 ، 29) ، حيث أشار هؤلاء الباحثون إلى أن أكثر الأعضاء المعرضة لظهور متبقيات المضادات الحيوية هي الأكياد ، ولذلك يفضل أن تؤخذ النماذج من الأكياد التي تعود إلى الذبائح المراد التعرف على مدى تواجد متبقيات المضادات الحيوية فيها ، إلا أن هذه النتائج لا تتفق مع ما أشار إليه الباحث (30) .

ولقد اثبتت النتائج الموضحة في الجدول (2) التي تخص تأثير درجة حرارة التبريد (4 م°) ولمدة ثلاثة أيام على العينات الموجبة لمتبقيات المضادات الحيوية في اللحوم الحمراء الناتجة من ذبائح الأبقار والأغنام والمعاز ، بأن هنالك تأثيراً واضحاً لدرجة حرارة التبريد على بعض المتبقيات من المضادات الحيوية المتواجدة في العينات التي تمت دراستها ، وهذا يتفق مع ما ذكره بعض الباحثين (15 ، 31 ، 32 ، 33) ، إذ أشار هؤلاء الباحثون إلى أن هنالك هبوطاً واضحاً في مستوى متبقيات البنسلين ، وأن هنالك تأثيراً متبايناً على بعض الأنواع الأخرى من متبقيات المضادات الحيوية في اللحوم عند تخزينها بدرجة حرارة التبريد (4 م°) .

وتجدر الإشارة إلى أن فقدان فعالية البعض من المضادات الحيوية يكون بسبب التغير الكيميائي الحاصل ، وأن أكثر أنواع هذه التغيرات هو التحلل المائي ، الأكسدة ، الأختزال والأنحلال بالضوء ، وهذا ما أكدته (34) ، إذ وجد أن خزن مجموعة البيتا لاكتام (B – Lactams) بدرجة حرارة (4 م°) يؤدي إلى تحللها (Deterioration) وبالتالي فإن درجة حرارة التبريد تؤدي إلى هبوط في فعالية هذه المجموعة من المضادات الحيوية ، أما الاوكسي تتراسايكلين فقد وجد الباحث بأنه أكثر مقاومة لدرجة حرارة التبريد .

وتشير النتائج الموضحة في الجدول رقم (3) التي تخص تأثير درجة حرارة التجميد (18 م°) ولمدة (30) يوماً على العينات الموجبة لمتبقيات المضادات الحيوية في اللحوم الحمراء الناتجة من ذبائح الأبقار والأغنام والماعز ، إلى أن هذه المتبقيات لم تتأثر بدرجة التجميد ، إذ لم يتأثر أي من هذه المتبقيات بدرجة حرارة (18 م°) ولمدة (30) يوماً ، إلا أننا لاحظنا حدوث نقص في قطر هالة التثبيت للنماذج المأخوذة من الأكباد ومن الكلى ، وهذا يتفق مع ما ذكره الباحث (32) حيث أشار إلى أن الخزن بدرجة حرارة (20 م°) لا يسبب أي فقدان من متبقيات الأوكسي تتراسايكلين ، كما لاحظ بأن اللحوم الحاوية على متبقيات البنزويل بنسلين والمخزونه بدرجة (20 م°) وخلال (10) أيام من الخزن تعاني من فقد بنسبة (20%) من هذه المتبقيات تحت تأثير درجة حرارة التجميد ، كما أشار الباحث (33) إلى أن درجة (20 م°) لم تؤثر بصورة كبيرة في متبقيات الكلورامفينيكول ، وبصورة أقل في متبقيات التتراسايكلين والسفاديميدين لمدة (5) أسابيع من الخزن .

وفي هذا المجال فأننا نود الإشارة إلى ضرورة قيام الجهات المسؤولة عن الرقابة الصحية على اللحوم بأجراء الدراسات المستقبلية حول هذا الموضوع ، والتركيز في ذلك على تأثير درجة حرارة التجميد (18 م°) ولفترات أطول ، بحيث يتم تجميد العينات الموجبة لمتبقيات المضادات الحيوية على هذه الدرجة لمدة (6) أشهر على سبيل المثال أو حتى أكثر من هذه الفترة ، ويتم أخراج أجزاء من هذه النماذج كل شهر للوقوف على تأثير درجة التجميد على هذه المتبقيات.

وأما النتائج الموضحة في الجدول (4) التي تخص تأثير درجة حرارة الطبخ ولمدة (60) دقيقة على العينات الموجبة لمتبقيات المضادات الحيوية في اللحوم الحمراء الناتجة من ذبائح الأبقار والأغنام والماعز ، فأنها تشير إلى أن درجة حرارة الطبخ تؤثر في هذه المتبقيات وتؤدي إلى تحللها ، وهذا يتفق مع ما ذكره (15 ، 33 ، 34) حيث أشار هؤلاء الباحثين إلى أن درجات الحرارة العالية تؤدي إلى حصول تثبيط في مستوى متبقيات المضادات الحيوية ، وأن تعريض اللحوم الحاوية على متبقيات الأوكسي تتراسايكلين على درجة حرارة (70 م°) ولمدة ساعتين يؤدي إلى انخفاض هذه المتبقيات .

وتجدر الإشارة إلى أن هذا التباين في تأثير عمليات الطبخ على متبقيات المضادات الحيوية في اللحوم وبحسب ما أشار إليه (15 ، 33) يعود إلى العديد من العوامل ، ومن أهم هذه العوامل هي مقدار درجة الحرارة التي يتعرض لها المضاد الحيوي ، مدة التعرض لتلك الدرجة الحرارية في أثناء عمليات الطبخ ، طبيعة المضاد الحيوي وتركيبه الكيميائي ، بالإضافة إلى شكل وحجم قطعة اللحم المعرضة لعملية الطبخ حيث يؤثر كل من الشكل والحجم على درجة نفاذية الحرارة إلى داخل قطعة اللحم ، وبالتالي على تحطيم متبقيات المضادات الحيوية المتواجدة داخل هذه القطعة من اللحوم.

وعموماً فأن النسبة العالية لتواجد متبقيات المضادات الحيوية في الأنواع المختلفة من اللحوم الحمراء المتداولة في مدينة دمار التي أظهرتها هذه الدراسة تعد مؤشراً مهماً للجهات المسؤولة عن الرقابة الصحية على اللحوم ، وضرورة قيام هذه الجهات بأخذ نماذج عشوائية من اللحوم الحمراء المعروضة للبيع في الأسواق المحلية بين فترة وأخرى وإجراء الكشف عن تواجد متبقيات المضادات الحيوية في هذه اللحوم ، وذلك بهدف الوقوف على المعدلات القصوى لمتبقيات المضادات الحيوية (MRL) (Maximum Residue Limits) في الأجزاء والأعضاء المختلفة من ذبائح الأبقار والأغنام والماعز ومقارنة النتائج التي تحصل عليها هذه الجهات مع الجداول العالمية ذات العلاقة وبخاصة تلك الجداول الصادرة عن منظمة الصحة العالمية (WHO).

ومما يزيد المشكلة تعقيداً هو الاستخدام العشوائي للمضادات الحيوية طوال فترة حياة الحيوان سواء لأغراض المعالجة أو لأغراض الوقاية ، بالإضافة إلى كونها مواد مضافة للأعلاف ، أو مواد حافظة للحوم أو المنتجات الحيوانية المختلفة ، مما يؤدي إلى تواجد متبقيات لهذه المضادات الحيوية في السوائل وفي الأنسجة المختلفة لجسم الحيوان ، وبالتالي تواجد هذه المتبقيات في الأجزاء والأعضاء المختلفة للذبائح الناتجة .

ومن الأمور التي نود الإشارة إليها في هذا المجال ، هي أن تواجد المضادات الحيوية في الأنسجة العضلية للحيوان وبالتالي في الأنسجة العضلية للذبيحة يعتمد على العديد من العوامل ، ومن أهم هذه العوامل هي نوع وتركيز المضاد الحيوي ، طريقة إعطاء الدواء ، وقت إيقاف استعمال المضاد الحيوي قبل عملية الذبح ، بالإضافة إلى مدى حساسية الطريقة المستعملة للكشف عن هذه المتبقيات .

ومن الجدير بالذكر أن غالبية الأدوية المستخدمة في مجال الطب البيطري هي من الأدوية التي تتميز بكونها ذات آثار تراكمية (Accumulation) في الأنسجة والأعضاء الداخلية المختلفة في جسم الحيوان وبالتالي في الذبيحة ، كما أن هذه الأدوية تتميز بأنها لا تتأثر كثيراً بالمعاملات المختلفة التي يتم إجرائها على اللحوم الناتجة من هذه الحيوانات ، وفي المحصلة النهائية فإن متبقيات الأدوية البيطرية تلعب دوراً خطيراً على صحة المستهلك إذا لم يتم الالتزام والتقييد الدقيق بفترة سحب الدواء من جسم الحيوان (Withdrawal period) قبل عملية الذبح .

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Detection of Antibiotic Residues in Red Meat and the Effect of Heat Treatment on Them

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Abstract

The aim of this study was to investigate the occurrence of antibiotic residues in the Carcasses of Cattle, Sheep, and goats, three hundred samples of carcasses of cattle, sheep and goats (one hundred each) were collected from meat retail markets in Thamar city.

Samples were taken from meat retail markets in Thamar city, specifically from muscles, liver, Kidneys and heart. The test was done by direct microbiological method, *Bacillus subtilus* was selected for detection of antibiotic residues. The effects of different thermal treatment on antibiotic residues were studied.

Results revealed that the presence of antibiotic residues in cattle carcasses, was more than that in sheep and goat carcasses; also the detection of antibiotic residues in sheep carcasses was more than that for goat carcasses, and the percentage of detection of antibiotic residues in these carcasses were (8.5%), (6.0%) and (4.3%) respectively .

This study registered that the cooling temperature at 4 C° for a period of 3 days gave the effect on the remains of antibiotic in different percentages for all examined samples.

As for the freezing at -18C° for a period 30 days it was found that all samples under going examination for remains of antibiotic were unaffected by freezing at -18 C° for the stated period, but there was a reduction in the radius of discouragement with regards to samples taken from livers and kidneys .

As for the cooking (boiling) temperature for 60 minutes showed full effect on the presence of remains of antibiotics for all samples under going examination in all cattle, sheep, and goats 100%, the result of examination was negative.

CONTENTS

Papers in English

الأبحاث باللغة الإنجليزية

- 1 Detection of Extracellular Enzymes Produced by Fungi Isolated from Dried Fruits
Saeed M. Alghalibi and Maysoun A. Al Zubairy (Biology)
- 11 Mycoflora and Total Aflatoxin Isolated From Dry Dates in Sana'a, Yemen
Abdul-Rahman M. Shatar, Saeed M. Alghalibi and Maysoun A. Al-Zubairy (Biology)
- 21 Synthesis and Characterization of Some New Uracil Derivatives and their Biological Activity
Saieba S. Hassan, Abdulkarim H. Al-syari and Muhsen O. Muhammed (Chemistry)
- 35 Effect of Silica Fume on the Hydration Kinetics of Atbra Cement Pastes Produced in Sudan
Fatima A. Al-Qadri (Chemistry)
- 43 Studies on the Effect of mono - alkoxy pyrophosphato coupling agent on the Mechanical Properties of Magnesium hydroxide Filled HDPE/EVA/Mg(OH)₂ / Composites
Nabil A. N. Alkadasi (Chemistry)
- 53 Distribution of Aliphatic Hydrocarbon in Coastal Surface Sediments from the Red Sea of Yemen
Nabil A. A Al-Shwafi (Earth and environmental)
- 61 Development of Advanced Monitoring System in Real Time Environment
Khalil Saeed M. Al-Wagih (Information Technology)
- 75 A Study of α β Tracker With Some New Algorithms For Selecting α and β Parameters
Khalil S. M. Al-Wagih and Mohammed F. Abdulla (Information Technology)
- 93 Abu-Ali Mohammad Ibn-Al-Haytham [Alhazen] 965-1039
Ahmed M. Al-Hadrani (Medicine)
- 99 The Occurrence of Gallbladder Carcinoma in Yemeni Patients Undergoing Cholecystectomy in Two Hospitals
Saeed H. Al-Bahlooli, Ahmed S. Soltan and Ahmed Al-Malahi (Medicine)
- 107 A Study on the Prevalence of Mange Among Arabian Camels in Najaf Province / Iraq
Hidar M. Ramahi and Ahmed M. Khalaf (Veterinary Medicine)

Papers in Arabic

الأبحاث باللغة العربية

- 1 إنتاج صنف جديد من الذرة الرفيعة
عبد الحميد أحمد اليونس
(زراعة)
- 9 دراسة علاقة الارتباط بين الحاصل ومكوناته لتحسين أصناف من القمح في اليمن
عبد الحميد أحمد اليونس و محمد علي حسن فارح
(زراعة)
- 17 التحري عن متبقيات المضادات الحيوية في اللحوم الحمراء وتأثير المعاملات الحرارية عليها
ضاري عليوي المشهداني
()

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