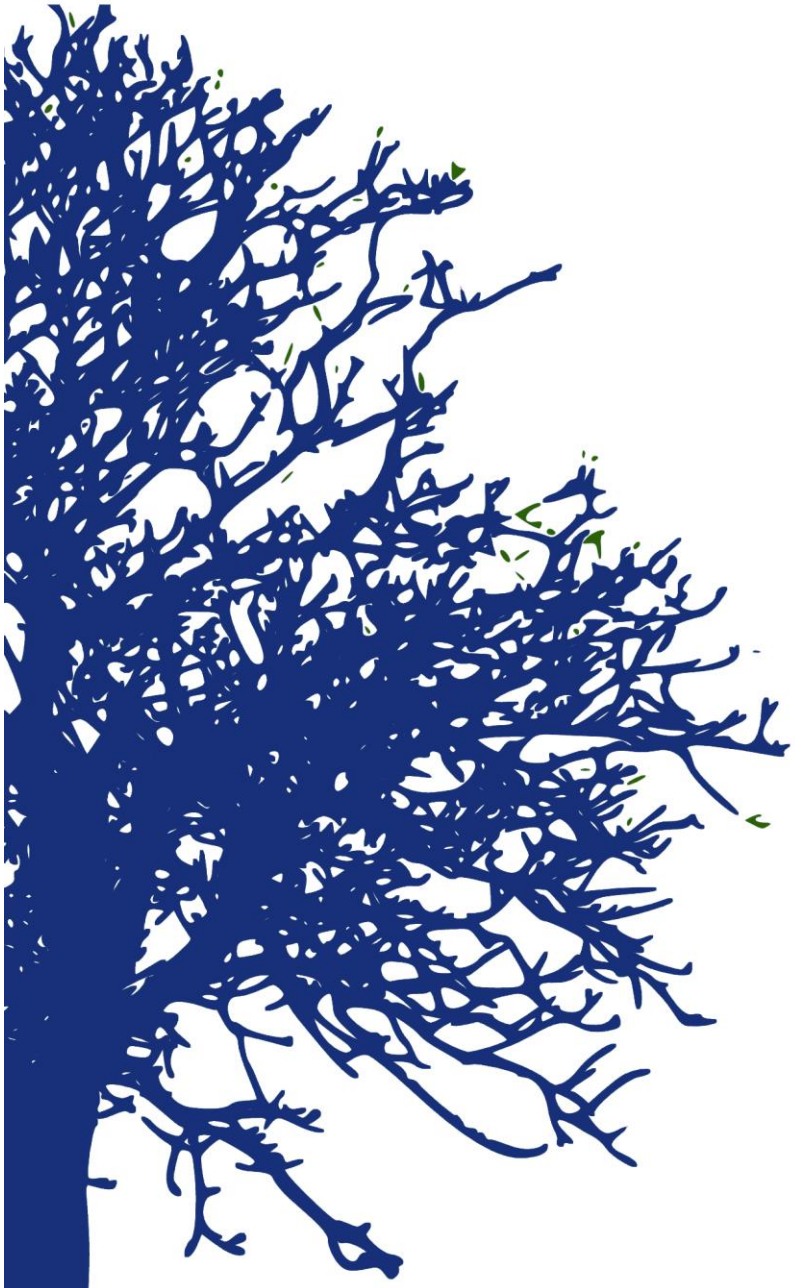

ICBE

The 8th International Conference of Biotechnology, Environment and Engineering Sciences

18th October 2020, Stockholm, Sweden

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Abstracts

The Impact of COVID-19 Pandemic Thread on Agriculture Sector

Ahasan Ullah Khan[1*] and Anayat Ullah Khan[2]

[1]Climate-Smart Agriculture Lab, Department of Agroforestry and Environmental Science, Faculty of Agriculture, Sylhet Agricultural University, Sylhet-3100, Bangladesh.
[2]Department of Mathematics, Faculty of Science, Jashore University of Science and Technology, Jashore 7408, Bangladesh.

Email: *Ahasan Ullah Khan (ahasanullahsau@gmail.com), and Anayat Ullah Khan (justianmath171521@gmail.com)

Abstract

The Corona virus is spreading at a very rapid leap, inflicting great damage to all sectors without any exclusion, but however the extent of the damage and losses resulting from the epidemic different from one sector to another sector and one country to another country. In this situation, most of the farmers meet crises in agriculture sector, so the aim of the study is to analyze and discuss the impact of Covid-19 on agriculture. In lockdown condition, effect on all the sector of agriculture and the farmers say that no one is coming from outside to buy cows and goats and they are not able to sell big cows and goats in Eid, 2020. Even though it is being sold, there are many losses. Despite good yield fruits, the lockdown char coronavirus has also affected local markets in Bangladesh. the findings also indicate that the COVID-19 has had a huge impact on food. Additionally, COVID-19 threatened agriculture even in some developed, whereas developing countries are the most pretentious due to their high dependency in securing their food supplies. Finally, based on the findings, we have suggested some recommendations that would help boosting sustainable agriculture sector.

Keywords: Coronavirus disease, agriculture, food security, and animal.

Seaweed Growth Detection in Aquaculture Environment Using Simple Linear Iterative Clustering Method

Çağdaş Doğan, Güven Yücetürk, Uğur Acar

Department of Innovative Applications Design and Development
Netaş Telecommunications Inc. Co.
cdogan@netas.com.tr

Abstract

Estimating the total biomass of cultivates in aquaculture plantations (fisheries, mussel plants, seaweed farms and compound sites) remains to be an issue for the industry and the researchers alike. There has been a diverse array of approaches towards this issue, like using markers, manually stapling the leaflets, weighting the actual mass of the organism and calculating the total mass by extrapolation. Seaweed growth detection is a subset of this problem. Our goal is to introduce a solution by automatically detecting the ratio of the target object in images of seaweed taken from an underwater environment. Researchers/operators then can evaluate the total mass of seaweed. This study is aimed to function as essentially a decision support system. The system is built based on an image segmentation algorithm named Simple Linear Iterative Clustering (SLIC) which is a kind of superpixel segmentation. This paper conveys the results obtained from our approach towards the seaweed growth detection, elaborates on the usage and feasibility of our solution in seaweed sites and showcase the economic impact in the industry. Other dimensions of the growth detection methods in current practice for seaweed growth is also discussed, such as lack of automation in the current best-practices while focusing on the difficulties accompanying this status-quo.

Keywords: Aquaculture, Seaweed, Growth Detection, SLIC

Mutation Effect of Chemical Mutagen Ethymthane sulfonate (EMS) on Some Local Yeasts

Badia Abdul Razzak Malla Obaida, Rehan Nashwan Abul-Rahman and Maha Azad Hamid

College of Science/ Biology Department/ University of Mosul

E-mail : Badia_Jamal@yahoo.com

Abstract

Thirty samples of orange juice were collected from local markets in Mosul / Iraq. Isolates were diagnosed after performing phenotypic, culture and biochemical tests. The results showed that the yeasts belong to the following species: *Rhodotorula rubra* 36%, *Trichosporon asahii* 16%, *Cryptococcus laurentii* 28%, and *Candida tropicalis* 20%. The susceptibility of isolates to six antibiotics Candizole (Cd), Clotrimazole (Ct), Fluconazole (Fc), Ketoconazole (Kc), Lamisil (Ls), and Nystatin (Nys) was studied. The results of the sensitivity test showed that *R. rubra* was resistant to all antibiotics used except for Lamisil (Ls). The rest of the yeasts varied among themselves in their resist antibiotics.

The chemical mutagen Ethyl Methanesulfate (EMS) at a concentration of 0.2 mg / ml on the vitality of the yeasts showed that the highest effect in the yeast *Crypto. laurentii*, with the killing severity reaching 4.47% while the lowest effected yeast was *Tricho. asahii*, with killing severity reaching 72%.

***Key words*:** Yeasts, Isolate, Orange juice, EMS.

On-site detection of saliva-alcohol as a function of blood alcohol concentration using colorimetric biosensor based on deposited Chromium (VII) Oxide Nanoparticles on filter paper

Jalal N. Jeber[1*], Maher Ahmed Abed[2], Ausama Abbas Faisal[3]

[1]*Universities of Baghdad, College of Science, Department of Chemistry, Baghdad, Iraq.*

[*]*Corresponding author email: jalalanalyticalchemistry@scbaghdad.edu.iq*

[2]*University of Anbar, College of Agriculture, Department of Food Sciences, Anbar, Iraq*

[3]*University of Anbar, College of medicine, Department of biochemistry, Anbar, Iraq*

Abstract

Alcohol intoxication is usually associated with drowning, falls, overdoses, fires, occupational accidents, physical and sexual abusements, domestic violence and traffic accidents. Therefore, alcohol considered an important factor for the explanation of the occurrence of many types of injuries. For many purposes such as forensic, it is important to establish a detection method to ensure whether the subject or the patient have consumed alcohol at a level that would be the reason for the accidents or injuries occur. Therefore, in this work, a simple, rapid and low-cost method was developed and validated for the detection of the alcohol in saliva as a function of blood alcohol concentration (BAC). The method is based on fabricated a biosensor consisted of chromium oxide nanoparticles deposited on filter paper. The validation of the biosensor was tested on 50 participants which categories into two selected groups (1 and 2). Group 1 consisted of 20 subjects from an organized party (no alcohol), they usually consumed three to four drinks as an average per week while Group 2 consisted of 30 subjects from an organized party the local bar (alcohol group), usually consumed two to three drinks per day. The results of the present study have shown that 95% of group 1 demonstrated positive results with variable colour intensities of the BAC in comparison to the 80% only of subjects from group 2. The present study has approved that the fabricated biosensor can effectively detect 0.02% or more of BAC which can be a useful test for many purposes such as medical, forensic, research and workplace.

Keywords: Biosensor, Blood alcohol concentration, Chromium oxide nanoparticles (CrONPs), On-site alcohol detection

Health behavior evaluation in women with multiple pregnancies

Alaa Ibrahim Ali

Department of Obstetrics &Gynecology / College of Medicine /. Al-Mustansiriyah University
Iraq- Baghdad.

EMail: alaa.Ibrahim@uomustansiriyah.edu.iq.

Abstract

Objective: The main purpose of this study is to evaluate the impact of the health behavior in pregnant Iraqi women in multiple pregnancies, and which one had characteristic influence in examined Iraqi women.

Patients and Methods:

The study was carried out in ***Al-Yermook Hospital*** for a period of one year from July 2019 to July 2020. It was included 40 pregnant women in multiple pregnancies, completed the 22 weeks gestational age, with taking their verbal consent for the participation in this investigation. Selected questioner used to collect data, as well as using Juczyński's Health Behavior Inventory questionnaire to evaluate the health behaviors of recruited women involved in this study.

Results:

The heath behaviors rate was high particularly for Iraqi pregnant women in multiple pregnancies, as the health behaviors indicators were high among all the identified categories.

Conclusion:

The higher health behavior rate of pregnant women in multiple pregnancies, as the preventative behaviors had the highest rate.

Key words: health behavior, multiple pregnancies, Iraqi women.

Antifungal Effectiveness of Different Denture Cleansing Methods.

Maha A. Al-Rejaboo[1] Asmaa A. Abdulkadir[2] Omar Abdul Mohsin sheet[3]

[1]*Department of Biology Collage of Science/University of Mosul*
mahaalrejaboo2@uomosul.edu.iq
[2] *Collage of Dentistry / University of Mosul*

asmaa2006@uomosul.edu.iq
[3]) *Collage of Dentistry / University of Mosul*

omaras2006@uomosul.edu.iq

Abstract: Cleansing of denture is a patient responsibility, effectiveness of cleansing method depend on type of cleanser used by patient and method of cleansing .A**im** : evaluate the popular denture cleansing against oral fungi responsible in oral stomatitis of denture wearer in Mosul city. **Materials and methods:** Selection of type of cleanser to be studied was done after pilot surveying in Mosul city denture wearer. The cleansing methods were immersion in 30%salty water, brushing, immersion in 0.25% sodium hypochlorite and immersion in commercially available effervescent tablet. Rodotorula mucilaginosa\Candida parapsilosis\Candida albicans are the isolated fungi from infected patients in Mosul city were used in this study. One hundred fifty acrylic base samples were prepared divided to three groups according to type of fungi and subdivided into four subgroups according to type of cleansing plus control group . After contamination with specific microorganism cleansing done with different methods and then fungal count in cfu unit was done. **Results :** Cleansing samples with sodium hypochlorite or polygrip or immersing in salty water decrease mean cfu of *Candida albicans* when compared with control group with. While mechanical cleansing group showed increasing in candida growth when compared with other groups. The highest suppression in *Candida parapsilosis* growth was shown in sodium hypochlorite group followed by polygrip group followed by mechanical cleansing . *Rhodotoryla mucilaginosa* were affected with all cleansing. **Concusion:** Commonly available patient home care methods used by Mosul city patients is very effective against studied fungi ,mainly immersion in 0.25% sodium hypochlorite then immersion in 30% salty solution then mechanical cleansing.

***Keywords*:** Disinfection, Denture cleanser, Antifungal, Candida, Denture stomatitis.

Glyphosate Degradation by four Plant Growth Promoting Bacteria (PGPB) detected in HPLC assay

Nibal Kh. Mousa[1]* , Nazar R. Merzah[2] , Maysoon M. Abdul Hassan[1]

[1]*Remediation Pollutants Center, Environment and Water Directorate, Ministry of Science and Technology, Baghdad, Iraq*

[2]*Plant protection Directorate, Iraqi Agriculture Ministry, Baghdad, Iraq.*

Nibal.Mousa@mail.co.uk

Abstract

The best eco-friend, cost-effective and efficacious nature is pesticides bioremediation. Four plant Growth Promoting Bacteria (PGPB) used in Glyphosate biodegradations: Bacillus megaterium, Bacillus. Subtilis, Rhizobium sp. and Azotobacter sp. were grown on Mineral salt media for (2-60) days with Glyphosate concentrations (5, 10, 15, 20, and 25) ppm. The glyphosate degradation ratio showed (70.9-91) %, (25 ppm/60 days) for B. megaterium, B. subtillis respectively. The best Rhizobium spp. degradation ratio was for 25ppm in (2-60) days reached (96.24 - 96.25) %, while Azotobacter sp. best degradation ratio was for (25 ppm / 54.16%), at 60 days. The HPLC residues for each B.megaterium at 25 ppm (30-60)days , (89-91)%, B. Subtillis (15ppm/14 days) reaches (90.3)%, Rhizobium sp. at(25) ppm / 30-60days reached (93-78)% , while Azotobacter sp. the (25) ppm at(30-60) days reached (82-79%) respectively for (25 ppm).The conclusion that all bacteria types tend to the 25 ppm for 30 days was Rhizobium sp.93% while for 60 days was Bacillus megaterium (91)% , improve Glyphosate biodegradation as a source for phosphorus and suggest could be well exploited for bioremediation or biodegradation of Glyphosate contaminated soil or water for the period reached 30-60 days.

Keywords: Microorganisms, Bioremediation, Organophosphorus pesticides.

The effect of prealbumin on liver in rheumatoid arthritis in iraqi patient

Alaa A. Abdulrazzaq, Teba F. Hadi,[2] Shahad A. Hawi[3]

AL-Rasheed University college

Alaa.Abd@alrasheedcol.edu.iq

Abstract

Rheumatiod arthritis (RA) is a long-term autoimmune disorder that represent by many symptoms like swollen and painful joints, as well as other organ may be affected like liver and other overlapping pathological and clinical manifestations. As a result, this disease can shows a great clinical challenges and many questions about diagnostic criteria for liver diseases .The present study was designed to investigate if the prealbumin effect in the liver progression in rheumatoid arthritis patients , so this study was conducted in the department of Biology, Al-Rasheed University College, comprising of n= 80 subjects known rheumatoid arthritis patients with an age range 30 - 60 years and healthy controls n=80 with age range 30 - 60 years attending the Baghdad Teaching Hospital (Educational Laboratory), Al-Kindy Teaching Hospital and Al-Imamian Al-Kadhimyain Medical City in Baghdad with disease duration was 6.5 ± 1.4 years. In this study some chemistry measurements included the (CBC) and the levels of CRP protein and prealbumin were estimated to investigate their correlation with liver abnormalities in RA patients. The mean (± S.D.) serum CRP level in the control group was found to be 7.60 ± 24.61while it expressed higher levels in RA patients 25.05± 51.19 and the mean (± S.D.) serum prealbumin level in the controls was found to be 210.3± 51.2 whereas in RA patients, its level was found to be 168.2 ± 50.6 .This study suggested that c-reactive protein (CRP) could be a good inflammatory markers that were reported to have a fatal value for the valuation of systemic inflammatory disease and the serum prealbumin (PA) concentration might be a more sensitive indicator in assessing liver abnormalities in rheumatoid arthritis patients.The aim of the study was to find the effect of prealbumin protein on liver sufficiency in rheumatoid arthritis ptients.

Keywords: Rheumatoid arthritis (RA), c-reative protein (CRP) , prealbumin (PA), comblete blood count (CBC),White blood cell (WBC).

Groundwater recharge estimation in semi-arid zone: a study case from the region of Tebessa (Algeria)

Amel Hibia [a], Layachi Gouaidiaa [a], Omar Guefaifiaa [a], Djamel bengsmia [b]

[a]Laboratoire environnement et ressources minérales et énergétiques dans l'Est Algérien, Université Larbi Tebessi, Tébessa, Algérie ; +213 (0)557019975 +213(0)672596564 +213(0)779321524
[b]Water and environmental engineer
amel.hibi@univ-tebessa.dz

Abstract:

In arid zones where rainfall is below the threshold of isohyets 200, and given the the scarcity of surface water resources, the exploitation of groundwater remains the only way to meet the various needs. In order to ensure the sustainability of this resource, which is becoming increasingly scarce in the face of increasing demand, it is imperative to establish adequate management. From this point of view, knowledge of the recharge rate of aquifers is of particular interest in any quantification study and management. This work constitutes a contribution to the determination of the potential recharge of a basin located in a semi-arid zone in eastern Algeria, and to follow its evolution over a period of 10 years. The proposed methodology is an approach for estimating the value of groundwater recharge using a soil and rock-dependent hydrogeological model. It is based on the infiltration coefficient and annual precipitation. Given the absence of a ground meteorological station in the Telidjene basin, remote sensing was used to estimate precipitation at a finer scale.

Keywords: Groundwater recharge, Hydrogeological model , semiarid area, remote sensing, Tebessa.

A Numerical computation of airflow over Iraq regions

Firas Sabeeh Basheer, Wedyan Ghalib Nsaif and Hazim H.Hussain Al-Saleem

Atmospheric Science Department, College of Sciences, Mustansiriyah University

Abstract

The best way to understand the general atmosphere system is to collect and analyze data, identify the variables that occur in the upper and lower classes, and compare them with other values in favor of comparing them to other studies and research. Studies have been conducted in this research by analyzing the wind speed and direction and comparing it with the surface roughness to reach a concept by dividing the regions of Iraq on the basis of the surface roughness that affects the wind speed near the surface of the earth. The methods used in the study depend on the hourly rates of surface roughness, wind speed and direction taken from the European-Mediterranean Weather Forecast (ECMWF) for a full year (2016(For 34 stations over Iraq. Results obtained from wind speed analysis and trend data. The highest value of wind speed (6.5 m / s) in the less rough areas (0-50 m) is concentrated in the semi-desert in the southern and western regions of the country (Anbar, Najaf and Smawa) and the lowest wind speed (1.8 m / s) for the rough areas (11- 72 m) in the mountainous regions in the northern part of the state in the governorate (Erbil, Zakho, Sulaimaniyah).

Keywords*:* climate change, wind speed, wind direction, surface roughness, airflow.

Great benefits of *Conocarpus erectus*

Wafaa Mohamed Masoud El Faras

Alexandria University -Egypt

Email: welfaras@yahoo.com

DOI: 10.46617/icbe8006

Abstract

Plants still an important source of omega oils that help to healthy for human and animals. Fresh leaves, stem, flower and fruits samples from Conocarpus erectus family Combretaceae has been analyzed using (GLC) Gas and liquid chromatography. The results indicated high levels of Omega oils which related to growth of nerve cells in brain. PH of fresh leaves determined and alkaloids with 8.2 which could help patients of diabetic type II

Key words: Conocarpus, Omega oils, diabetic type II

Full Papers

Mutation Effect of Chemical Mutagen Ethymthane sulfonate (EMS) on Some Local Yeasts

Badia Abdul Razzak Malla Obaida, Rehan Nashwan Abul-Rahman, Maha Azad Hamid

College of Science/ Biology Department/ University of Mosul
Corresponding author: Badia_Jamal@yahoo.com

DOI: 10.46617/icbe8002

Abstract

Thirty samples of orange juice were collected from local markets in Mosul / Iraq. Isolates were diagnosed after performing phenotypic, culture and biochemical tests. The results showed that the yeasts belong to the following species: *Rhodotorula rubra* 36%, *Trichosporon asahii* 16%, *Cryptococcus laurentii* 28%, and *Candida tropicalis* 20%. The susceptibility of isolates to six antibiotics Candizole (Cd), Clotrimazole (Ct), Fluconazole (Fc), Ketoconazole (Kc), Lamisil (Ls), and Nystatin (Nys) was studied. The results of the sensitivity test showed that *R. rubra* was resistant to all antibiotics used except for Lamisil (Ls). The rest of the yeasts varied among themselves in their resist antibiotics.

The chemical mutagen Ethyl Methanesulfate (EMS) at a concentration of 0.2 mg / ml on the vitality of the yeasts showed that the highest effect in the yeast *Crypto. laurentii*, with the killing severity reaching 4.47% while the lowest effected yeast was *Tricho. asahii*, with killing severity reaching 72%.

***Key words*:** Yeasts, Isolate, Orange juice, EMS.

1.Introduction

Yeasts are microscopic eukaryotic organisms located within the fungi kingdom. Their dimensions range from 3-10 µm in diameter to 5-30 µm in length [1]. It belongs to three fungal subdivisions: Ascomycota, Basidiomycota, and Deuteromycota, 1.500 species have been described today [2]. It spreads in many foods and can have a major or secondary role in the spoilage of some foods. The latter case results from the fact that it grows at a relatively low rate compared to bacteria [3]. It is considered one of the microorganisms that some of them can be found in extreme conditions, whether in terms of high glucose concentrations, as yeasts grow in them that like high permeability pressures Saccharophiles, including *Saccharomyces rouxii*, which causes spoilage of foodstuffs with high concentrations of sugars [4]. The low pH is 5.5 or less, and thus leads to significant economic losses as well as health problems, as it causes food poisoning and cancers due to the secretion of mycotoxins such as Aflatoxins [8]. Several studies and research have been conducted to investigate yeasts in juices. Obasiet *et al*. [5] observed the presence of *Candida kruesi*, *C. zeylanoides*, *C. parapsilosis*, *C. norvegensis*, *C. lusitaniae*, *C. parapsilosis*, *Rhodotorula minuta*, *Kodamea* sp. , *Geotrichum* sp. in natural and canned orange juice. Mathla *et al*. [6] was able to isolate the yeast *C. guilliermondi*, *C. lusitaniae*, *C. fumata*, *C. zeylanoides*, *C. krusei*, *Geot. capitatum* and *S. cerevisiae* from foodstuffs with high sugars content included fruits juice, apple jam, apricot jam, cherry jam, dates and others. While Chatterjee *et al*. [7] different types of yeasts in orange juice, apple juice, pineapple juice, grapes juice, mango juice and sugarcane juice.

Mutagenes work to bring about a change in the arrangement of nitrogenous bases, whether by deletion, addition, or substitution [8], Which is either substituting a base for another place from the same group and this is called a transition, such as replacing a purinian base with another place in the same group or a base Primidine is another place, but if a purine base is replaced by a premidine base or vice versa, the process is called transversion, which leads to a change in the composition of the gene and thus a change in the composition of the protein that encodes it, which may result in a change in characteristics such as the inability to synthesize some amino acids or vitamins or the emergence of allergy or resistance to some antibiotics and others [9]. Chemical mutagens are among the most important substances in causing genetic mutations. These mutagens include Nitrogen mustards, Nitrous Acid, Hydroxylamine, Hypoxanthine, Ethymthane sulfonate (EMS), Acridines, Nitrosoguanidine, 5-Bromouracil, and some other substances such as caffeine, Mitomycin, and others. EMS is one of the alkyating agents that attack the (CH3CH2) and methyl (CH3) group at the 7-site on the purine ring. This removes the base by displacing the DNA without affecting the sugar-phosphate column [10].

Due to the importance of this product to the consumer and the seriousness of its contamination with these microorganisms and the financial losses it causes, the current study focused on isolating and diagnosing some types of yeast that contaminate it. And studying the effect of chemical mutagens EMS on its vitality.

2.Materials and Methods

2.1Isolation of Yeasts

Colleted 30 samples of orange juice from local markets in Mosul city/ Iraq. From each sample, 1 gm was taken and a series of dilutions was performed from 10^{-1}-10^{-6}, after which 1 ml of the 10^{-5} and 10^{-6} dilutions were taken and implanted on Yeast Extract Malt Extract Medium (YM Agar) as it was spread on the surface using a glass diffuser (Spreader). With a volume of 3 plates for each sample, the plates were incubated at 28 C for 7 days until the emergence of yeast colonies.

2.2Diagnostic Tests

Morphological Characters of Colonies and Microscopic Examination

Isolates were grown on Malt Extract Agar (MEA) Medium and incubated at 28°C for 48 hours. Observations of phenotypic characteristics were recorded, and examined under a light microscope at 40X powers to observe the shape of the yeast cells.

Biochemical Tests

1. **Growth Test at 25 and 37 °C**

   The yeasts were grown on MEA medium by streaking method, and incubated at 25 and 37 ° C for 3-7 days. Record a negative result in the absence of growth or positive in the presence of growth.

2. **Test the usability of Nitrates as The Only Source of Nitrogen**

   The test was performed by cultivating the yeasts on the MEA solidified medium by Streaking method, incubated at 25 and 37 ˚C for 3-7 days.

3. **Determination of Ability to Resistance Glacial Acetic Acid**

   Inoculated Petri dishes containing solid medium Malt Acetic Acid (MAA) with part of pure culture of yeasts studied by Streaking method and incubated at 28 ˚C for 3-7 days. Infer the positive result with weak, medium, or dense growth and the negative result that no growth occurred.

4. Grow ability in low levels of water with higher levels of carbohydrates

Inoculated Petri dishes containing Malt Extract Yeast Extract 50% Glucose Agar (MY50G) medium with part of the culture of each isolate from the studied isolates by Streaking method and incubated at 28 °C. Observed the results after 3-7 days, the absence of growth indicates the negative result, while the occurrence of weak, medium or dense growth indicates the positive result.

5. Grow Ability in Low Levels of Water With Increased Level of Sodium Chloride

Isolates were inoculated by Streaking method on Malt Extract Yeast Gxtract 5%(or 10%) Salt 12% Glucose Agar (MY- 10-12) medium (MY10-12). And incubated at 28 ° C for 3-7 days. The result is positive when weak, medium, or dense growth occurs, and negative when no growth occurs at all [11].

6. The Ability of Yeasts to Form Mycelium Test

The test was conducted to find out ability of yeast to form the true mycelium and Pseudomycelium. Small flasks containing 20 ml of Sabouraud's Glucose Broth (SGB) medium were inoculated with a portion of pure culture yeasts. The flasks were incubated for 48 hours at 28 ° C. The yeasts were examined microscopically at force (40X) to note budding, cell morphology and mycelium type whether true or Pseudomycelium [12].

7. Diazonium Blue B (DBB) Color Test

Is one of the important tests to distinguish between Ascomycetes and Basidiomycetes yeasts. The isolated yeasts were grown on YM Agar food medium and incubated at 28 C for 10 days until the cysts were in the Ascomycetes. One to two drops of Diazonium Blue B reagent (DBB) reagent was added to the surface of the developing colonies and left for 2-3 minutes at laboratory temperature. The coloration of the colonies to a dark reddish-purple color indicates that they belong to the Basidiomycetes, which is (the positive result), but if the colonies were colored orange, this indicates that they belong to the Ascomycetes, which is the negative result [13].

2.3 Mutation Effect of Chemical Mutagen Ethyl Methanesulfate (EMS)

The method of Gjermansen [14] was used, 0.02 g of the EMS mutagen was dissolved in 100 ml sterile distilled water so that the mutagen concentration was 0.2 mg/ ml, 5 ml of which were transferred to a test tube containing 1 ml of the yeast cell suspension and the tubes were incubated in a vibrating water bath in Temperature 30 C for 70 minutes. Cells were deposited by central centrifugation using a Centrifuge for 15 minutes at 9000 rpm. Wash the precipitate with 10 ml of sodiumthia sulfate (2%) and centrifuge for 15 minutes at 9000 rpm. The washing process was repeated three times, then the sediment was suspended by adding 10 ml sterile distilled water, the tubes were shaken well and 0.2 ml of the suspension was spread by a sterile glass rod on the surface of the Petri dishes containing the solidified Sabourd's Glucose Agar Medium (SGA) medium at a rate of 5 dishes/ treatment with the presence of comparison dishes (control). The plates were incubated at a temperature of 28 C for a period of 7 days. The number of developing colonies in the five plates was calculated to be the total number of surviving colonies in 1 ml of mutated yeast suspension and the percentage killed according to the following equations:

$$\% \text{ Survivors} = \frac{\textit{The resulting number from mutagenesis treatment}}{\textit{The resulting number of non-mutagenic dishes}} \times 100$$

% kill = 100 - Percentage of surviving individuals

2.4 Antibiotic Resistance Test of Wild Type and Mutant Yeasts Isolates

For the purpose of determining the resistance of non-mutagenic and mutagenic yeast isolates using an EMS. The solidified Yeast Extract Peptone Glucose Agar (YPG) medium was prepared with the addition of antibiotics at the final concentrations mentioned in Candizole (Cd) ,Clotrimazole (Ct), Fluconazole (Fc), Ketoconazole (Kc), Lamisil (Ls), and Nystatin (Nys) at a concentration of 30 µg/ ml, the isolates were inoculated by Streaking method and the plates were incubated at a temperature of 28 °C for 48 hours .Isolates are antibiotic, as mutant isolates that are resistant to the antibiotics used here can be identified [15].

3.Results and Discussion

The current study demonstrated the presence of 25 isolates of yeasts out of a total of 30 orange juice samples belonging to four different genera. The most frequent yeast was *R. rubra*, which appeared in 9 isolates by 36%, and the least appeared was 4 isolates in *Trichosporon asahii* with 16%, Table (1). The reason for the high number of yeasts in juices can be explained by the speed of growth of yeasts, their possession of genes responsible for fermentation of sugars such as xylose, as well as their ability to grow in foods with a high level of glucose concentrations. These results are in agreement with other studies done on the juices [16], where they isolated strains of *S. cerevisae* from date juice and raisins, as well as Khattab *et al*. [17] who isolated the largest number of yeasts *Debaryomyces hansenii*, *S. cerevisiae*, *C. tropicalis*, and *Pichia kudriavzevii* from Orange juice, mandarin juice, mango juice, and sugarcane juice.

Table (1): Number of isolates and rates of infection with yeasts in orange juice samples

Yeast	The number of isolates	% Recurrence
R. rubra	9	36
Tricho. asahii	4	16
Cryptococcus laurentii	7	28
C. tropicalis	5	20
Total	25	100

3.1 Diagnostic Tests for Yeasts

Culture Characteristics and Microscopic Examination

The results of the diagnostic tests showed that the isolated yeasts belong to four different genera, which are as follows:

1. *R. rubra*: The color of their colonies was orange, 1.5 mm in diameter, round, mucous in texture, convex soft to the touch, shiny, with smooth edges, it appears under the microscope in the form of a spherical-lemon cell with a diameter of 12 µm with the presence of eosinophilic mycelium (1-A and B).

2. *Tricho. asahii* : Its colonies are white to pink, 3 mm in diameter, irregular, dry, diaphragmatic, cotton in texture, rough, opaque, flat, elevated center, with fringed edges, appear under the microscope a lemon-shape, with a diameter of 10 µm and are distinguished by their formation of true mycelium (1- C and D).

3. *Crypto. laurentii* : White colonies convex 1.2 mm in diameter, circular, leathery in texture, not mucous, shiny, with lobed edges and rough surface, under the microscope they appear oval to elongated in shape (7.0×2.0) µm with true mycelium (1-E and F).

4. *C. tropicalis* : White, frothy colonies 2.5 mm in diameter are round and convex, the edges of the colonies are smooth and shiny, under the microscope they look oval, their dimensions are (7.5×3.5) µm, Pseudomycelium formation (1-G and H).

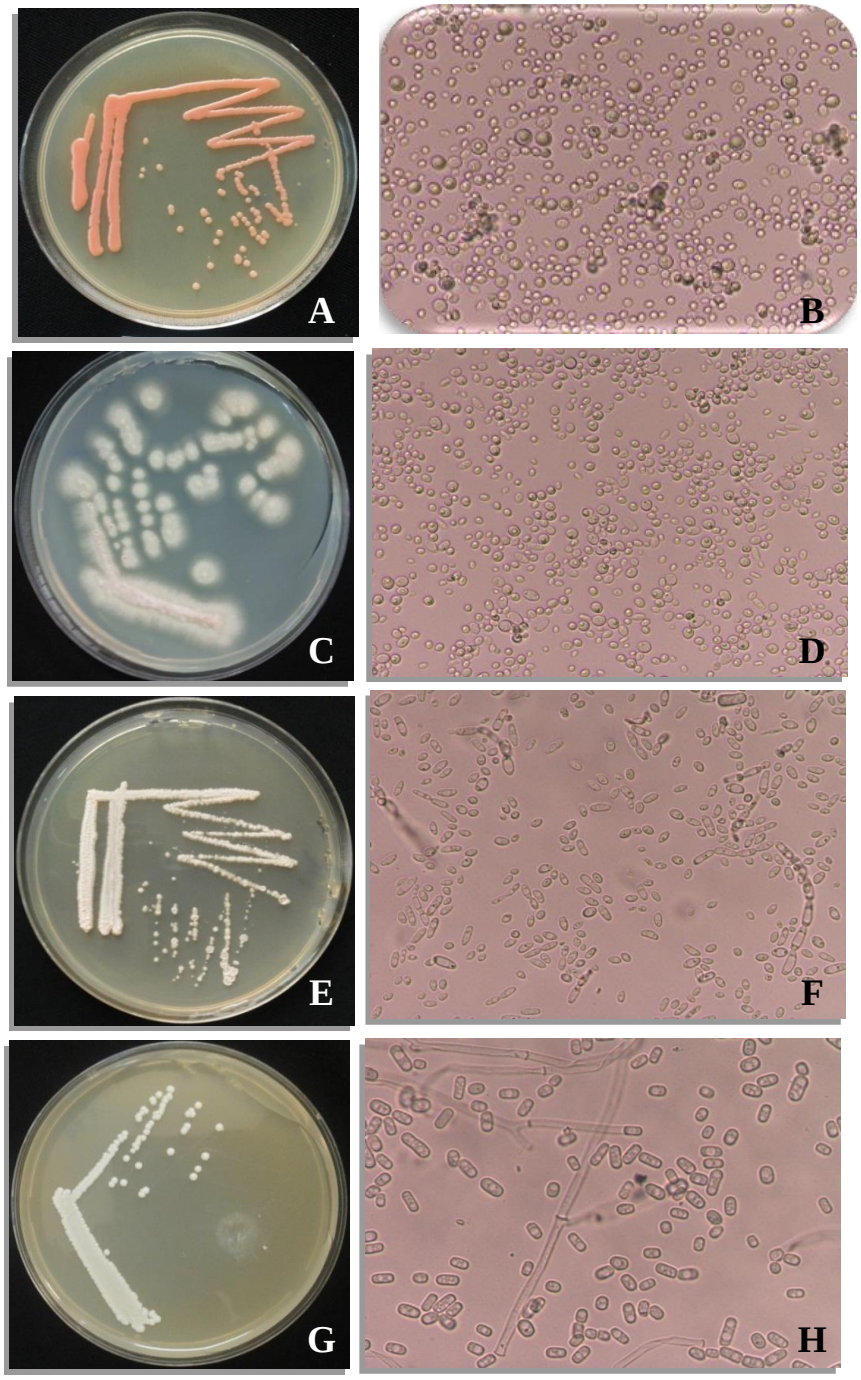

Figure (1): The Characteristics and Microscopic of yeasts

Where, A and B: *R. rubra*, C and D: *Tricho. asahii*, E and F: *Crypto. laurentii*, G and H: *C. tropicalis.*

Biochemical Tests

Growth Test at 25°C and 37 °C

The results of this test showed the ability of 3 isolates to 75% to grow at the tested temperatures with the exception of isolates *Crypto. laurentii* 25%, which showed a negative growth result at a score of 37˚C, Table (2), and this is consistent with what was reported by [18].

Test the usability of Nitrates as The Only Source of Nitrogen

The results came to demonstrate the ability of the two isolates *R. rubra* and *Crypto. laurentii*, 50%, weakly benefit from nitrate as the only source of nitrogen, as for the two isolates, *Tricho. asahii* and *C. tropicalis* (50%) was test negative in Table (2), and this result is consistent with what was indicated by [12].

Determination of Ability to Resistance Glacial Acetic Acid

It is noted from Table (2) that 3 isolates, 75%, were not able to resist snow acetic acid, except for *R. rubra* 25% as it grew thicker and this is consistent with the classification key mentioned by [12].

Grow ability in low levels of water with higher levels of carbohydrates

The results shown in Table (2) showed that 2 of the isolates (50%) were negative in this test, while the other two isolates (50%) were positive for the test and had weak growth.

Grow Ability in Low Levels of Water With Increased Level of Sodium Chloride

The current results revealed that *Crypto. laurentii* isolate 25% were test negative, while 5 isolates (75%) tested positive, and it ranged from *R. rubra* to weakly grown *Tricho. asahii* and *C. tropicalis* (Table 2). This is in agreement with [12].

Diazonium Blue B (DBB) Color Test

We notice in Table (2) that out of 4 isolates from the tested yeasts, only one isolate (25%) was positive in this test while 3 isolates were negative (75%). These traits are consistent with what Kurtzman and Fell [13] describe. It was mentioned that this test is widely used to distinguish between cystic and Yeasts.

Table (2): Biochemical Diagnostic Tests for Yeast Isolates

Diagnosed yeasts	Type of test						
	The ability to grow in						
	25˚C	37˚C	Presence of nitrates as a source (N)	Presence of glacial acetic acid	Low water level High for carbohydrates	Low and high water level for NaCl	DBB
R. rubra	+++	+	+	+++	+	+++	▪
Tricho. asahii	+++	+	-	-	+	+	▫
Crypto. laurentii	+++	-	+	-	-	-	▫
C. tropicalis	+++	+	-	-	-	+	▫

(-):There is no growth, (+): weak growth, (++):Medium growth, (+++):Dense good growth, (▪): Basidiomycetes, (▫):Ascomycetes

3.2 Mutation Effect of Chemical Mutagen Ethyl Methanesulfate (EMS) on The Vitality of Some Yeasts

The results showed mutagenesis of the four yeast isolates *R. rubra*, *Tricho. asahii*, *Crypto. laurentii*, and *C. tropicalis* using an EMS chemical mutagen at a concentration of 0.2 mg / ml had a clear effect on the viability of all susceptible yeasts, with the killing percentage being 61.72, 72, 47.4, and 60.5 % respectively (Table 3). It should be noted that chemical mutagens affect the nitrogenous bases that make up the basic unit for building the DNA (nucleotide), leading to permanent changes in the structural unit during its interaction with the nitrogenous bases. Its effect is to replace one nitrogen base with another, or alter the binding properties of the nitrogen base in such a way that it changes the binding forces with another base [19]. Fariss *et al*. [20] confirmed that the compound is one of the most effective substances in causing mutagenesis, as it does not act as a mutagen only, but rather is considered a toxic substance that kills yeast cells. And its effect on cell viability is directly proportional to its concentration in the medium [21]. Also, Anandarajah *et al*. [22] in their study that using a 0.28 M concentration of EMS increased the killing percentage to 50%. EMS was found to have a fatal effect on *S. cerevisiae* cells, and exposure to them for 45 minutes led to an increase in the killing rate to 43% [23]. Bessadok *et al*. [24] observed in their study that exposing the yeast cells *C. tenuis* CtTun15, *D. hansenii* DhTun 2015, *Tricho. Asahii* TaTun15, *Yarrowia lipolytica* YlTun15, and *R. mucilaginosa* RmTun15 for the EMS chemical mutagen for different time periods (15, 30, 45 and 60) min. The percentages of killed cells are directly proportional to the increase in exposure to all types of yeasts.

Table (3): The effect of the chemical agent EMS at a concentration of 0.2 mg / mL on the viability of the isolated yeasts

Yeasts	The Average of alive yeast		% Survivors	% Death
R. rubra	Control	32.4	38.27	61.72
	Treatment	12.4		
Tricho. asahii	Control	27.8	28	72
	Treatment	7.8		
Crypto. laurentii	Control	22.8	52.6	47.4
	Treatment	12		
C. tropicalis	Control	32.4	39.5	60.5
	Treatment	12.8		

3.3Resistance of Yeast isolates Studied to Antibiotics

It is evident from the observation of Table (4) that *R. rubra* was resistant to all antibiotics used except for Ls. The rest of the yeasts differed among themselves in their ability to resist antibiotics.Yeast resistance is due to its possession of a group of mechanisms that give the cell the characteristic of antibiotic resistance [25], including the inhibition of Ergosterol, which enters the synthesis of the cell membrane and the formation of holes that increase the loss of important substances to the outside of the cell [26]or the occurrence of a mutation in the genes encoding the enzymes that transport the drug to the cell as well as changes in the target protein that lead to an increase in its production and thus reduce the toxic effect of the drug [27]. This is in agreement with the Malla Obaida *et al*. [28]study that yeasts *R. minuta* BA78, *R. glutinis* BA83, *R. graminis*

BA1, *R. mucilaginosa* BA58, *R. mucilaginosa* BA75, *R. mucilaginosa* BA61, and *S. cervisiae* BA179 were resistant to Candizole, Fluconazole, Lamisil, and Nystatin and sensitive to Clotrimazole and Ketoconazole. The results of the susceptibility test for *C. colliculosas* showed resistance to all antibiotics used (Candizole, Clotrimazole, Fluconazole, Ketoconazole, Lamisil, and Nystatin) with the exception of the antibiotic Nystatin [29]. In another study conducted by Malla Obaida [30],*C. krusei* showed resistance to Candizole and Nystatin and sensitivity to the antibiotics Clotrimazole, Fluconazole, Ketoconazole, and Lamisil, while the yeast *C. utilis* showed resistance to all antibiotics except Nystatin, and the rest of the yeasts varied in their resistance and sensitivity to antibiotics.

Table (4): Sensitivity and Resistance Test of Yeast Isolates for Antibiotics

Yeasts	Antibiotics					
	Cd	Ct	Fcz	Kc	Ls	Nys
R. rubra	R	R	R	R	S	R
Tricho. asahii	S	R	R	S	R	S
Crypto. laurentii	R	S	S	R	S	R
C. tropicalis	R	S	R	S	R	S

R : Refer to the resistance status
S : Refer to the sensitivity status
Candizole (Cd) ,Clotrimazole (Ct), Fluconazole (Fc), Ketoconazole (Kc), Lamisil (Ls), and Nystatin (Nys).

3.4 Antibiotic Suceptibility Test of Mutated Yeasts Strains

The results of the study of resistance and sensitivity of yeast isolates mutagenic to the chemical mutagen EMS are shown in Table (5), as it is evident from the observation of the table that the yeast *C. tropicalis* has become resistant to all antibiotics. The yeast *Tricho. Asahii* showed sensitivity to all antibiotics except for Ct. Whereas, *R. rubra* and *Crypto. laurentii* became sensitive to all antibiotics used and retained the Cd antibiotic resistance status. Compared with non-mutagenic isolates, mutated isolates showed sensitivity or resistance to some antibiotics, and this may be due to a change in the genetic structure of parental isolates, which may have occurred in the genes carried on the plasmid or chromosome in the way that made these isolates sensitive or resistant to these antibiotics and here it is worth noting To the effect of mutagens used [31]. The EMS mutagen causes a single mutation of the type of mutations, double mutations of the type of transmission, transversion mutation, or transition mutations [32].

Table (5): Resistance and sensitivity of yeast mutant isolates using MNS for antibiotics

Yeasts	Antibiotics					
	Cd	Ct	Fcz	Kc	Ls	Nys
R. rubra	R	S	S	S	S	S
Tricho. asahii	S	R	S	S	S	S
Crypto. laurentii	R	S	S	S	S	S
C. tropicalis	R	R	R	R	R	R

R : Refer to the resistance status
S : Refer to the sensitivity status
Candizole (Cd) ,Clotrimazole (Ct), Fluconazole (Fc), Ketoconazole (Kc), Lamisil (Ls), and Nystatin (Nys).

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On-site detection of saliva-alcohol as a function of blood alcohol concentration using colorimetric biosensor based on deposited Chromium (VII) Oxide Nanoparticles on filter paper

Jalal N. Jeber[1*], Maher Ahmed Abed[2], Ausama Abbas Faisal[3]

[1]*Universities of Baghdad, College of Science, Department of Chemistry, Baghdad, Iraq.*

[*]*Corresponding author email: jalalanalyticalchemistry@scbaghdad.edu.iq*

[2]*University of Anbar, College of Agriculture, Department of Food Sciences, Anbar, Iraq*

[3]*University of Anbar, College of medicine, Department of biochemistry, Anbar, Iraq*

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Abstract

Alcohol intoxication is usually associated with drowning, falls, overdoses, fires, occupational accidents, physical and sexual abusements, domestic violence and traffic accidents. Therefore, alcohol considered an important factor for the explanation of the occurrence of many types of injuries. For many purposes such as forensic, it is important to establish a detection method to ensure whether the subject or the patient have consumed alcohol at a level that would be the reason for the accidents or injuries occur. Therefore, in this work, a simple, rapid and low-cost method was developed and validated for the detection of the alcohol in saliva as a function of blood alcohol concentration (BAC). The method is based on fabricated a biosensor consisted of chromium oxide nanoparticles deposited on filter paper. The validation of the biosensor was tested on 50 participants which categories into two selected groups (1 and 2). Group 1 consisted of 20 subjects from an organized party (no alcohol), they usually consumed three to four drinks as an average per week while Group 2 consisted of 30 subjects from an organized party the local bar (alcohol group), usually consumed two to three drinks per day. The results of the present study have shown that 95% of group 1 demonstrated positive results with variable colour intensities of the BAC in comparison to the 80% only of subjects from group 2. The present study has approved that the fabricated biosensor can effectively detect 0.02% or more of BAC which can be a useful test for many purposes such as medical, forensic, research and workplace.

Keywords: Biosensor, Blood alcohol concentration, Chromium oxide nanoparticles (CrONPs), On-site alcohol detection

1.Introduction

The consumption of alcohol leads to a risk factor for morbidity and mortality which related to both unintentional and intentional injuries[1]. Globally, 16% of deaths and 13% of disabilities from injuries were estimated in 2000 to be attributed to alcohol [2]. Alcohol can coordinately affect many individual psychomotor skills such as brain-hand, foot and eye [3]. The effects of alcohol on the human body are several such as visual focus, long reaction time and delay in judgment leading to injuries from causes like falls and motor vehicle accidents[4, 5]. The individual's cognitive skill can also be affected by alcohol intoxication. Exposition of persons to alcohol can place them in a real dangerous situation, less averse to risk-taking and be more aggressive which lead to both intentional injuries as either victims or perpetrators and unintentional injuries such as burns and drowning. A large number of studies have illustrated the involvement of alcohol among nonfatal and fatal injuries. For example, in the analysis of more than 65 articles which were published between 1975 to 1995, has reported that the high percentage cases of intoxicated with alcohol were 31.5% among homicide deaths, 31.0% among non-traffic and 22.7% among suicide[2, 6]. While a moderate percentage of cases of involvement of alcohol in injuries events have suggested that alcohol is a risk factor even if the consumption of alcohol doesn't provide information about the actual risk. Several factors have contributed to increase the alcohol use from the people such as increased availability, urbanization, high-intensity mass marketing, changing in the social norms, poorly awareness and relaxation in rules of overseas trade. According to the WHO report in 2018 which has stated that the harmful using of alcohol results in approximately 3 million deaths each year which represents

5.3% of all deaths[7]. Also, the disability and the death in the early age group 20-39 years increased to 13.5% of all deaths which are caused by alcohol consumption. The harmful use of alcohol has caused lots of behavioural disorders, noncommunicable conditions and mental behaviour. In addition to causal relationships between infectious diseases such as tuberculosis, HIV/AIDS and harmful drinking[8]. The BAC (Blood alcohol concentration) considered as the most useful measurement for determining the concentration of alcohol in blood which is used for different purposes such as medical, forensic, research settings and forensic. Lots of methods, techniques and methodologies have been developed for the quantitative determination of BAC in whole blood such as electrochemical [9, 10], spectrometry [11], HNMR [12], colorimetric[13, 14] and amongst all these methods the gas chromatography is the preferable one[15-17]. However, the GC method has many disadvantages such as time-consuming, requires skills and expensive. Therefore, cheap, fast, rapid, non-invasive method is required for the quantitative determination of BAC. The BAC has been determined in urine[18, 19], breath [19, 20] and blood [21, 22]. Till now, the breath method for estimation of BAC is used the breath meter[19, 23]. Despite this method provides a rapid result but is requires calibration and person cooperation which may be sometimes very difficult in comatose or combative persons. In this paper, we aimed to develop an analytical method based on synthetic alcohol-saliva biosensor which may permit a cheap, rapid, and simple detection of ethanol content in saliva. This test provides an accurate estimate of BAC saliva. This method was validated by the serum BAC method [24]. The saliva disk consists of deposited chromium oxide nanoparticles on filter paper. The new technique is successfully applied for the detection of ethanol as a function of BAC and can be used in various purposes.

2.Materials and Methods

Reagents

All chemicals and standards were of analytical grade and all preparations were conducted using double distilled water. Potassium Dichromate, Mulberry leaves extract, filter paper and double-distilled water was used throughout the experiment. Beers, wine and liquors were purchased from the local markets.

Table 1. ***The classification of intoxication levels of BAC [25]***

Blood alcohol concentration in g/100 ml	**Level**	**Symptoms**
0.00-0.05	Sober	1- People appear clinically normal. 2- Some of them have euphoria. 3- Reaction time is slowed down two times
0.06-0.09	Light	1- Self-criticism 2- Loss of inhibitions 3- Loss of concentration and normal judgement
0.10-0.15	Moderate	1- memory problems 2- Further loss of self-criticism 3- emotional instability 4- Early ataxia, apraxia and agraphia
0.15-0.25	Strong-very strong	1- Loss of orientation 2- Apathy and emotional eruptions 3- Emotional instability 4- Partial amnesia 5- Ataxia, agraphia, apraxia
0.25-0.35	Stupor -Coma	1- Total loss of muscle coordination 2- Total loss of orientation. 3- Amnesia 4- Worsening of the abovementioned
0.35-higher	Coma-Death	1- General anaesthesia and paresis 2- Stupor followed by a comma 3- Suppression of the vital centres in the brain with cardiorespiratory collapse and death

Synthesis of chromium oxide nanoparticles

The chromium oxide nanoparticles were synthesized using the extractor of mulberry leaves which were used as a reduction agent. 20 gm of mulberry fresh leaves were mixed and boiled with 150 ml of double-distilled water in 250 ml round bottom flask at 60 C° for 1 hour. 0.7 M of potassium dichromate ($K_2Cr_2O_7$) solution was prepared by dissolving 10.3 g with 50 ml of double-distilled water. The Whatman filter paper (No 40) was used to filter the potassium dichromate solution. 10 ml of 0.7 M of potassium dichromate solution was added to the 10 ml extractor of mulberry leaves. Then the mixture solution was stirred for 15 min. After 5 minutes of stirring, the colour of the solution was changed from orange to colourless. The solution mixture was dried in two stages, first at 100 C° for 6 hours using hot air oven and second at 650 C° for 10 minutes using muffle furnace. The synthesized chromate oxide nanoparticles were then scrubbed, grinded and collected in a sealed container for further characterization.

Characterization of Chromium oxide nanoparticles (CrONPs)

The prepared nanoparticles have been characterized using AFM and UV-spectrometer Shimadzu, 1800). Several experiments of AFM were performed to image CrONPs at a range of salt concentrations (0.2M-0.8M). The results of AFM have shown that most of the CrONPs surfaces diameter have in the range of 40-70nm. Some larger of CrONPs structures >70 nm have also been observed (Fig 1). While, the prepared nanoparticles absorbance was measured in the range 190-900 nm in order to determine the wavelength. The nanoparticles have shown maximum absorbance peak at 320 nm wavelength.

a. Loading the chromium oxide nanoparticles onto the filter paper

0.5 gm prepared solid CrONPs were dissolved in the concentrated solution of sulfuric acid (15%). The prepared chromium oxide nanoparticles were impregnated into the Whatman filter paper (No 40 with paper dimension 4 length x 1 width cm) by a simple dipping process, followed drying the paper using a hairdryer. The impregnation process was repeated 5 times at least in order to ensure a full close-packed of chromium oxide nanoparticles assembly on the filter paper. The deposited filter paper is flexible, and shape and size can be adjusted based on the requirement. The loading of CrONPs onto the filter paper is easily observed by the light yellow colour which is visible to the naked eye. The light colour of the close-packed CrONPs into the filter paper is originated from the assembling and absorption of nanoparticles on the callous fibers. The desorption of nanoparticles from the filter paper has been examined by immersion in various solvents and particles have shown strong adsorption via hydrophobic interactions and van der Waals forces between the nanoparticles and the cellulose fibres. The concentrated sodium borohydride was used to wash the surface of prepared CrONPs loaded filter paper in order to facilitate access of the reactant molecules to the metal surface as shown in Fig 2.

a. Samples

In this study, a total of 50 participants with ages ranged from 25-45 years were taken apart. Twenty participants (10 male and 10 female) were chosen to form the no-alcohol group (1), while 30 participants (20 male and 10 female) were taken to form the alcohol group (2). All the participants in this study were drinkers, healthy with no medical history, volunteers, who have consumed three to four drinks per week as an average.

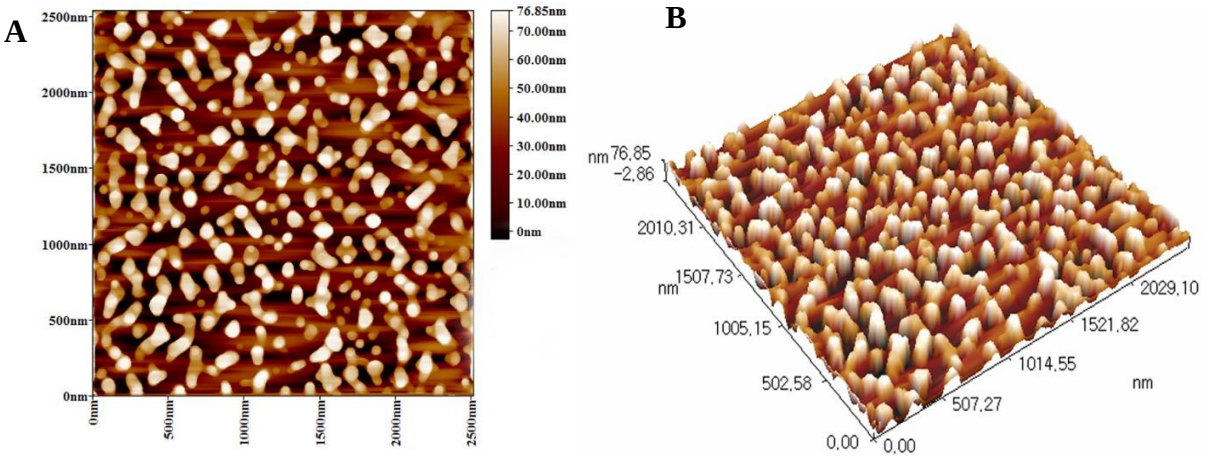

Figure 1. ***Morphological characterization of chromium oxide nanoparticles. (A) Global AFM-registration of nano-CrO, and (B) three-dimensional image of nano-CrO.***

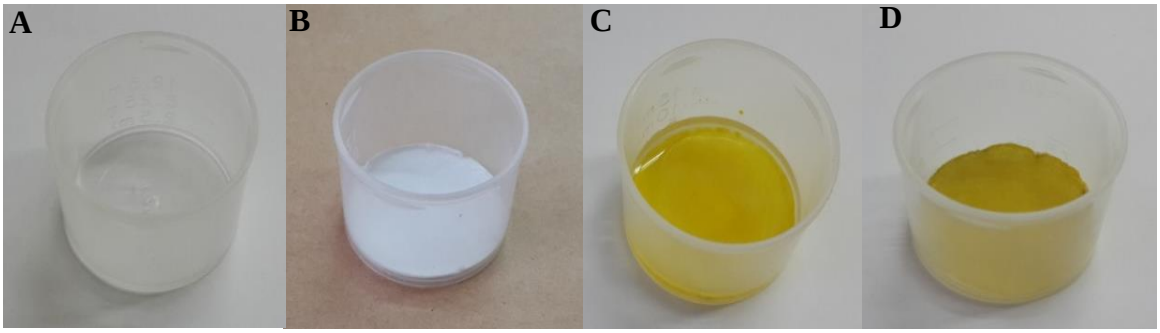

Figure 2. ***The steps of preparation of the biosensor. A is an empty cup; B is the cap with a filter paper; C is the CrO nanoparticles solution (acidic medium, 15% sulfuric acid) is added to the filter paper; D is the final shape of the biosensor.***

General Procedure

All the participants were given an instruction which included not drink any kind of drinks that contains alcohol 24 hours before the assay. While the alcohol group, their instruction included not to eat 3 hours before the assay. The alcohol assays were preformed to all participant at the same time. First, study information was given to all participants to ensure that they understood the effects of alcohol, for those with an alcohol group they were given detailed information about the alcohol dosages, assay time and types of drinks. The alcohol group participants were weighted and based on their weights, they were administered the alcohol doses at 0.8 ml/Kg of body weight. According to these doses, the blood alcohol concentrations were expected to be in the range of 0.01-0.03 g/100 ml. The participants drank 25 ml every 2 minutes for 15 minutes of standard alcohol drink which was mixed of water. Then, the alcohol assays were recorded every 0.5 hours for 3 hours. After complete the experiments, all the participants were remained in the laboratory to ensure their safety by decrease the BAC level to the safety level (Below 0.02%).

*Validation of the proposed method (*biosensor*) and Interpretation of it results*

Before using the disk in the real samples (saliva), the validity of it need to be check. Therefore, a series of alcoholic and non alcholic drinks such as whisky, vodka, wine, beer, barbican and water have been used to check whether is the prepared disk is capable to use as a detection tool for alcohol or not. The experiment has shown excellent results since all the alcholic drinks have shown positive results (positive results mean to change the colour of the disk from yellow

to greenish) as shown in Fig 3a, while the non-alcohol drinks have shown negative results (the disk remans yellow color) as shown in Fig 3 b.

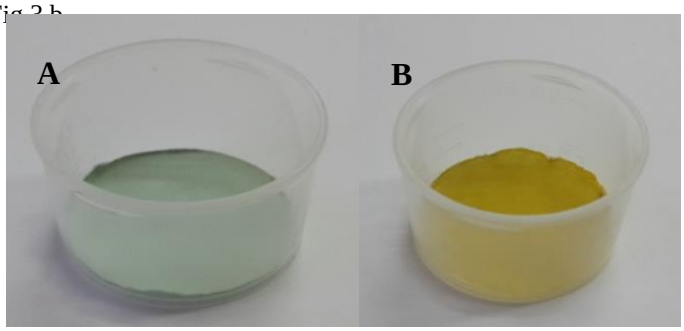

Figure 3. ***The interpretation of the results. (A) indicates of positive results; (B) indicates to the negative results***

3.Results and Discussion

As mentioned above, the development of rapid, cheap and on-site detection method of ethanol in the saliva is required. The proposed method is based on changing the colour of the composited filter paper by chromium oxide nanoparticles from colourless to the light green as a result of the following reaction:

$$2CrO_3 + 8H_2SO_4 + 3CH_3CH_2OH \rightarrow 2Cr_2(SO_4)_3 + 3CH_3COOH + 11\ H_2O$$

Yellow Colorless Green-bluish

Therefore, in this paper, the results are considered as a positive when the colour of composited filter paper becomes light green, while a negative result when the filter paper remains colourless as shown in Fig 1.In the current study, 19 out of 20 of group (1) samples (95 %) have demonstrated positive results with variable intensity of green-bluish colour, while the remaining %15 showed negative results. While group (2), 24 out of 30 samples (80%) have shown positive results and the remaining 10 % showed negative results as shown in Table 2. All of the blood alcohol concentration estimation techniques depend on the amount of alcohol in the body which is, in turn, depends on many factors such as sex, food, type, quantity of beverage and the rate of alcohol elimination. Using the above techniques has many of disadvantages such as time consuming, requires laboratory skills and expensive. Therefore, the proposed method has overcome all previous difficult and it can rapid, reliable, non-invasive and valid method in comparison with the reported methods. Some of the results 5% and 20% for groups 1 and 2 respectively have shown negative results although all the samples have followed the same procedures, this can be explained by the following reasons. First, the proposed method is capable to detect the blood alcohol concentration of 0.02% or higher than this with excellent accuracy. Thus, any level of blood alcohol concentration less than 0.02% cannot be detected by the proposed method. Second, some alcoholic drinks have a very low alcoholic concentration (less than 5% like beer). While the positive results have shown variable intensities of green-bluish colour and this can be explained by the following facts: these high colour intensities due to consumption of drinks contain high alcohol concentration (>40%). Also, there are many of facts can also influence significantly on the detection method. For example, both of small intestine and the stomach are responsible for the alcohol absorption, therefore, empty stomach and beverage with higher alcohol concentration can lead to fast absorption rate and vice versa. Based on the literature, the maximum of absorption rate can be obtained when the beverage contains 20-45% of alcohol concentration , while the absorption rate is decreased due to consumption beverage with low alcohol concentration like beer (less 5%), high fluid or consume the beverage with food which were all noticed in the groups (1 & 2). Finally, group 1 has shown a high percentage of positive results (95%) than the group 2 (80%) which might be explained as the following: group 1 consumed the alcohol in a short period of time which means there is no enough time for alcohol to be metabolized and hence the high concentrations of alcohol in the blood will be released. While group 2 consumed alcohol with a long period of time which provides ample time for metabolism and lead to slow the absorption rate. All of obtained results are tabulated in Tables 2&3.

Table 2. ***The obtained results by the proposed and reference methods of group 1***

Beverage type	Total number (20)	No of positive sample	No of negative sample	Found in serum of positive samples g/100 ml[a] [24]	Found in serum of negative samples g/100 ml[a]
Whisky	5	5	0	0.02-0.3	NA
vodka	5	5	0	0.09-0.25	NA
Wine	5	5	0	0.06-0.21	NA
Beer	5	4	1	0.02-0.08	0.018

Table 3. ***The obtained results by the proposed and reference methods of group 2***

[a] Determination of Blood alcohol concentration by GC.[24]

Beverage type	Total number (30)	No of positive sample	No of negative sample	Found in serum of positive samples g/100 ml[a] [24]	Found in serum of negative samples g/100 ml[a]
Whisky	8	8	0	0.09-0.25	NA
vodka	8	6	2	0.07-0.23	0.016-0.018
Wine	8	6	2	0.06-0.22	0.016-0.019
Beer	6	4	2	0.02-0.09	0.016-0.018

[a] Determination of Blood alcohol concentration by GC[24].

4.Conclusion

In this paper, a colourimetric saliva-alcohol biosensor has been described, which is prepared by deposition of chromium (VII) Oxide Nanoparticles on the filter paper using an acidic medium (sulfuric acid). The disk is a rapid, practical and feasible method for detect of blood alcohol concentration of 0.02 % or higher throughout saliva for all types of samples even though for those with unconscious state. The above described procedure exhibits the capability of the method for detection of alcohol in saliva on site without any unnecessary pretreatment. The disk is simple low cost and rapid and it must be used in forensic or medicine purposes as a powerful tool for blood alcohol detection. Also, the it can be helpful for screening of individuals or identification of those who might be at risk due to alcohol consumption, which might be serve as a powerful tool against inappropriate consumption of alcohol.

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Health behavior evaluation in women with multiple pregnancies

Alaa Ibrahim Ali

Department of Obstetrics &Gynecology / College of Medicine /. Al-Mustansiriyah University
Iraq- Baghdad.

Corresponding author: alaa.Ibrahim@uomustansiriyah.edu.iq.

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Abstract

Objective: The main purpose of this study is to evaluate the impact of the health behavior in pregnant Iraqi women in multiple pregnancies, and which one had characteristic influence in examined Iraqi women.

Patients and Methods:The study was carried out in ***Al-Yermook Hospital*** for a period of one year from July 2019 to July 2020. It was included 40 pregnant women in multiple pregnancies, completed the 22 weeks gestational age, with taking their verbal consent for the participation in this investigation. Selected questioner used to collect data, as well as using Juczyński's Health Behavior Inventory questionnaire to evaluate the health behaviors of recruited women involved in this study.

Results:The heath behaviors rate was high particularly for Iraqi pregnant women in multiple pregnancies, as the health behaviors indicators were high among all the identified categories.

Conclusion: The higher health behavior rate of pregnant women in multiple pregnancies, as the preventative behaviors had the highest rate.

Key words: health behavior, multiple pregnancies, Iraqi women.

1.Introduction:

One of the important subjects to bear in our mind the influencing effect of the health behavior on the lifestyle of the human being due to the dependence of the of the pregnancy course, fetal development, and the child development on the women's health throughout the duration of preconception and the pregnancy period.[1]

There is a significant proportion between the women health and other factors that leads to adverse pregnancy outcome such as; the lifestyle (smoking, drinking alcohol during pregnancy that lead to increase the risk of preterm delivery, lower birth weight), the physical environment, genetic role, maternal obesity, gestational hypertension, gestational diabetes, deficient folic acid supplementation, which can result in the increasing risk of pre-eclampsia development, cardiac defects, still birth, orofacial defect.[2, 3]

Actually, on the other hand there is a negative relation between the women health behavior and nutrition, such as lack of sleep, alcohol abuse, and psychoactive medicine abuse; so that the pregnancy is regarded as the perfect time for the correction of this negative relationship by changing the lifestyle of the pregnant women as well as the eating habit [4]. Multiple pregnancies are regarded as one of important subjects in obstetrics, as the pregnancy of the women with multiple pregnancy is considered as a high risk pregnancy, because it is associated with increased risk of obstetrical and neonatal complications. Thus, the aim of this investigation is to reduce complications especially the associated miscarriage, premature delivery, intrauterine growth retardation, higher incidence of nausea and vomiting, infant and neonatal mortality [5, 6].

Therefore, there is a need for preventive care particularly the women health behavior.[7] There are many causes and risk factors in multiple pregnancies, such as advanced maternal age, assisted reproductive technology, hereditary and environmental factors, it accounts around 3% of the birth in the world. As the multiple pregnancy incidence is of increasing over the past period [8, 9]. The pregnant women should be alert to these changes and problems that occur during this period, trying to prevent those complications by close observation as well as good health care [10].

2.Patients and methods:

This study included 40 pregnant women in multiple pregnancies; recruitment of women was done throughout the period from June 2019 to June 2020 at Al-Yarmouk hospital in Baghdad, Iraq, after taking a verbal and written consent from all women enrolled in this study. All of them completed their 22 weeks and that was our inclusion criteria for the study group, depending on the questionnaire survey including the physical activity of pregnant women, social habits such as alcohol and smoking, coffee drinking, and narcotics intake, as well as the taking hospitalization, reproductive histories, and the gestational age of their pregnancies. The Health related behavior inventory (HBI) was used to evaluate the indicator of the health behavior, and that's called Juczyński's questionnaire, which consists of 24 questions about health behavior according to these following factors; (1) Positive mental attitude [PMA]; ability of the women to control her emotions, tension , stress, and depressive states (2) preventive behaviors [PBs], (3) health practices [HPs]; sleep, fitness activities (4) proper nutritional habits (PNH) ; types of food that the pregnant women had taking like vegetables, fruit, whole wheat bread. Additionally, the benefit of HBI is covering the women behaviors: (1) those who need medical recommendation. (2) Those behaviors that increase or decrease the disease risk. (3) Those behaviors that maintain health, by using the 5

point scale as following; 1 means almost never, 2 means rare, 3 means from time to time, 4 means rottenly, 5 means almost always. The subjects in this study was asked to answer this questioners, the assessing the intensifier for each one of the health behavior indicator, then ranging the scores between 24-120, the higher augmentation of the health behavior then the scores classified depending on values, and converted to standardized scores from 1-10, as 1-4 means low HBI, 5-6 scores means average HBI, and 7-10 means high HBI. Questioner sheets were given for all enrolled women and the results recorded after reading and signing informed sheets.

Statistical analysis:

The descriptive methods were used in the analysis of quantitative variables, including the mean, median, standard deviation, maximum, and minimum values. The existence of significant differences was at P value< 0.05. The Mann-whitney U test and IBM SPSS statistical software version 21 were used for statistical analysis.

3.Results:

The paramount cause of hospitalization in those women was the threatened premature contractions in about 48% and other causes include diabetes, hypertension, anemia, and one of enrolled women admitted due to twin-twin transfusion syndrome. Table 1 has showed that there was a high rate of women health behaviors as the values for each indicator had been illustrated in this table clearly, and the health behaviors indicators were; residence, level of education, marital status, successful pregnancy's number, that were illustrated in table 2, and those indicators were all high in women with multiple pregnancies in all categories, and the preventive behaviors had the highest rate.

The average age of the enrolled women in our study was (30.2 ± 4.4) as their age ranged from 24-45 years, and their gestational age, Majority of women were from urban areas, as the M ± SD was (98.3 ± 10.30) and the remaining were from rural area(90.2 ± 12.9), with p value 0.09.As well as the majority of women were married, with the M ± SD of (95.3 ± 10.9), while others were single (90.1 ± 11.9), with significant statistical difference as the P value was 0.038. Most of our participants were in their second pregnancy, more than first pregnancy, as the M ± SD (99.4 ± 7.6, 90.2 ± 10.4) respectively. More than half of pregnant participants were not having any physical activity before hospitalization, they got pregnant naturally, and few of them had undergone in vitro fertilization, for pregnant current pregnancy was not the first, were individualized by health behavior indicators for categories of health practices, and positive mental attitude, their statistical significance was on average, and only it was significant for the health practices.

Discussion:

The incidence of the multiple pregnancies increase over the last years especially for the developed countries, as a result of increasing the assisted reproductive techniques, with advanced age of women in her first pregnancy, ovulation induction without IVF, all can lead to dramatic increase in the rate of dizygotic twins [11]. Actually, most of the women in this study had got pregnancy naturally 32%, and around 3.2%got pregnant by IVF.

The multiple pregnancies are regarded as a high risk pregnancy for that the pregnant women needs frequent visits and the frequent leading cause for hospitalization of those women was the premature uterine contraction in half of women, and 11% as a result of co morbidities, such as anemia, diabetes, hypertension. [12, 13]

Many different reports mentioned that the powerful relation between the neonatal health and the lifestyle of pregnant women during her pregnancy, particularly the physical activity, and the eating habit, as they contributed in the reduction of back pain, which is common in pregnancy, beside the excessive weight gain prevention, and unfortunately the multiple pregnancies with its obstetric complications is considered as a contraindication to any excessive physical activity during pregnancy. In this study, our results had showed that there was a higher rate of health behavior among pregnant women, as well as the higher rate of the health behavior for the selected categories in those pregnant women in multiple pregnancies as all the values were greater than (3.22 ± 0.45).

Similar findings was reported by Juczyński et al., 1999 who found the same relation between the health behaviors and the high risk pregnancy in a 61 women, as the health behavior index was (90.18 ± 12.78) which was greater than seen in healthy women, as well as there were a higher rates of positive mental attitudes, and preventive behaviors ((3.94 ± 0.6)(3.91 ± 0.78)) respectively [14]. In contrast, the results of Boguszewski et al., 2018 on 268 control group (healthy women), and 214 pregnant women, showed an average rate of women's heath behaviors and they found an average rate of health behaviors, as the health practices, and the positive mental attitude ((3.68 ± 0.67)(3.69 ± 0.68)) respectively. [2]

4.Conclusion:

Our study found and highlighted about the health behaviors in Iraqi pregnant women in multiple pregnancies, beside the health behaviors indicators, as we found that they had a higher rate, particularly the preventive behaviors, but their values were not dependent on the variables in this study.

Table 1: Characteristics of Iraqi pregnant health behaviors for each pregnancy

Behavior categories	**M**	**MS**
Proper nutritional habits (PNH)	3.93	0.5
Preventive behaviors (PBs)	3.99	0.6
Positive mental attitude (PMA)	3.7	0.5
Health practices (HPs)	3.22	0.45

Table 2: Characteristics of Iraqi health behaviors according to selected variables

Variables	***M ± SD**	**Statistical analysis**
Education		
Secondary	92.3 ± 9.4	Z = –0.49; p = 0.62
Higher	94.2 ± 11.5	
Place of residence		
City	98.3 ± 10.3	P= 0.09
Rural	(90.2 ± 12.9)	
Marital state		
Married	99.3 ± 10.9	P = 0.038
Single	90.1 ± 11.9	
Pregnancy		
First	90.2 ± 10.4	P= 0.95
Another one	99.4 ± 7.6	

***** Data are expressed as the mean

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A Numerical computation of airflow over Iraq

Firas Sabeeh Basheer[1], Wedyan Ghalib Nassif* and Hazim H.Hussain Al-Saleem[2]

Department of Atmospheric Science, College of Science, Mustansiriyah University, Baghdad, Iraq
[1]b.firas@uomustansiriyah.edu.iq, wedyan.atmsc@uomustansiriyah.edu.iq,
[2]dr.hazim@uomustansiriyah
*Corresponding Author Email: wedyan.atmsc@uomustansiriyah.edu
DOI: 10.46617/icbe8005

Abstract

The best way to understand the general atmosphere system is to collect and analyze data, identify the variables that occur in the upper and lower classes, and compare them with other values in favor of comparing them to other studies and research. Studies have been conducted in this research by analyzing the wind speed and direction and comparing it with the surface roughness to reach a concept by dividing the regions of Iraq on the basis of the surface roughness that affects the wind speed near the surface. The research aims to know the effect of air flow on the nature of the earth's surface and its effect on the different regions in Iraq.

The methods used in the study depend on the hourly rates of surface roughness, wind speed and direction taken from the European-Mediterranean Weather Forecast (ECMWF) for a full year 2016 from 34 stations over Iraq. Results obtained from wind speed analysis and trend data. The highest value of wind speed (6.5 m / s) in the less rough areas (0-50 m) is concentrated in the semi-desert in the southern and western regions of the country (Anbar, Najaf and Smawa) and the lowest wind speed (1.8 m / s) for the rough areas (11- 72 m) in the mountainous regions in the northern part of the state. The importance of the results enables us to know the movement of air in this layer in terms of its weakness or strength according to the nature of the surface of the earth, as it has formed (barren lands, bodies of water, mountainous areas), which can be used in future studies to monitor the movement and speed of winds and to determine the natural properties of the air layer in contact with the surface of the earth. This requires knowledge of the impact of temperature, wind speed and direction in dividing the layers of Iraq on the basis of surface roughness.

Keywords: climate change, wind speed, wind direction, surface roughness, airflow.

1. Introduction

Surface roughness is an important factor in wind movement near the surface of the earth and in determining many physical factors such as dispersion of pollutants, values of evaporation rate, volatile energy rates, and various engineering fields in designing residential complexes and building airports, as well as selecting the best sites for installing wind power plants [1]. The roughness of the surface reduces the speed of the wind and that is why the winds in the lower atmosphere are completely stagnant. The direction of the surface winds is the result of three forces: Coriolis force and pressure that control the movement of the wind, while the third force is the frictional force that increases the speed Wind [2] Many of the previous studies dealt with the analysis of the change in the near-surface wind speed, where the researcher Zhang, R., Zhang, S., Luo, J reanalyzed and monitored the station data. Differences in the near-surface wind speed were

analyzed in China [3] As for the researcher Naglaa Muhammad analyzed the differences in the wind speed near the surface by studying the aerodynamic surface roughness of the area surrounding Al-Mustansiriya University for a circle of diameter (1 km), where the length of the surface roughness is one of the main variables in micro-metering studies and studies [4] Other researchers have studied the relationship of surface roughness to air temperature and perceived heat flow [5] Researcher LI Zhikun analyzed the characteristics of changing the wind speed and direction in the earth, as the results of his study showed that the expansion of the city causes an increase in roughness, pulls the northern winds, reduces the wind speed in Leeds and changes the wind field in Beijing. [6] Several studies analyzing observations of surface wind speed have found a decrease in wind speed over the past 30 to 50 years. Sometimes suggested cause is increased surface roughness, although the evidence to date that this is the primary factor remains inconclusive. In this study, changes in surface roughness were verified for 20 stations in the Netherlands and 137 stations in 7 other European countries, and this is what the researcher Wever found [7]. An analysis of the observed ground wind velocity trends in IP is to assess whether the atmosphere is still observed above this mid-latitude area. The main objectives are those studied by the researcher LOPEZ-BUSTINS [8]. The researcher used a new method to improve wind speed predictions. The outputs show that the GPR model improves prediction accuracy over the original NWP data, and taking into account atmospheric stability reduces prediction errors [9]. Therefore, GIS provides a flexible environment for entering digital data from various sources and it is a powerful tool [10]. By providing data from this center, it has become possible to conduct many research studies, identify many weather phenomena, analyze patterns and shapes of weather, and identify the variables that occur in the climate system. Since these centers provide data for many aerial variables, this study was a major step in analyzing wind speed and direction and compared to surface roughness for a whole year over the region of Iraq. The roughness of the surface plays an important role in the wind movement near the ground surface. All data are provided by the European Center that contains reliable data and the importance of this research is due to dividing the regions of Iraq into layers according to the nature of the geographical area.

2. Description of study area

Iraq was chosen for this study, it is located within the geographical coordinates between latitudes (29.5-37.5 N) north of the equator, and between longitudes (39.45 - 48.45 E) of the northern hemisphere. Iraq has a subtropical climate, Continental, dry, with a hot and dry summer and cold winter with some precipitation in the center and south country and more rain in northern Iraq.[11] In this study, data on wind speed and direction as well as surface roughness were provided by the ECMWF for eight full hours (2016),more than 34 station data were collected for different areas of the country as in figure (1), the stations are shown in table (1).

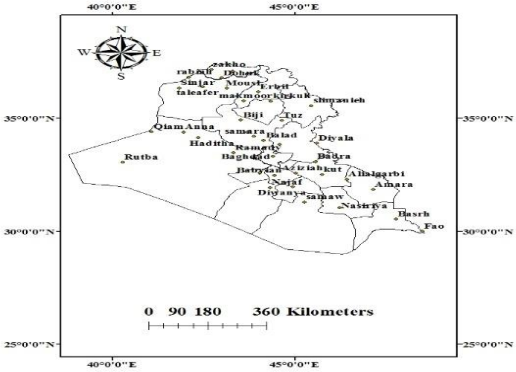


Figure 1. Meteorological stations in Iraq map.

Table 1. Meteorological stations [12]

Station	Longitude (Degree)	Latitude (Degree)	Station	Longitude (Degree)	Latitude (Degree)
Baghdad	44.4	33.3	zakho	42.72	37.13
Rutb	40.28	33.03	slimanieh	45.45	35.53
Basra	47.78	30.52	Erbil	44	36.15
Mousl	43.15	36.31	talafer	42.48	36.37
Najaf	44.32	31.92	Tuz	44.65	34.88
Diwania	44.95	31.95	samara	43.88	34.18
Ramady	43.32	33.45	Anna	41.95	34.37
kerballa	44.05	32.57	Qiam	41.07	34.38
kirkuk	44.35	35.74	Nukheb	24.28	32.03
Amara	47.17	31.83	Balad	44.15	34
Dyala	45.62	33.88	Sinjar	41.83	36.32
Smaw	45.27	31.27	Alialgarbi	46.43	32.28
kut	45.75	32.49	Badra	45.57	33.06

Nasiriya	46.23	31.02	Aziziah	45.04	32.55
Babylon	44.45	32.45	Haditha	42.35	34.13
Tikirit	43.7	34.37	Biji	43.53	34.9
Dohuk	43	36.78	Fao	48.5	29.98

3. Data Analysis

Data analysis was done using MATLAB software to convert Net CDF (network common data form) format file get it from European Centre for Medium-Range Weather Forecasts (ECMWF). was Hourly data of wind speed from 1/1/2016 to 31/12/2016 was collected at two height (2 meter and 10 meter). Data for surface roughness from the Iraqi region was collected as well.

the value of the friction velocity (U*) was calculated using the following equation :[13]

$$\frac{k(U(Z_2)-U(Z_1))}{\ln\left(\frac{Z_2-d}{Z_1-d}\right)} \qquad (1)$$

Where : k is the Von Karman constant (0.41)

U(Z2):Wind speed at 10 meter ,U(Z1):Wind speed at 2 meter .

d is the zero-plane displacement (in meters) ,which is calculate from canopy height (hc) and surface surface roughness(ZO) :[14]

$$Z_o = 0.07\, h_c \qquad (2)$$

$$\frac{d}{h_c} = \frac{2}{3} \qquad (3)$$

hence

$$d = \frac{2}{3} * \frac{Z_o}{0.07} \qquad (4)$$

from eq.1 and eq.3 to calculate U(z):[15]

$$U(z) = \frac{U_*}{k}\left[\ln\left(\frac{z-d}{Z_o}\right)\right] \qquad (5)$$

4. Results and Discussions

4.1 Analysis of wind speed and direction at 00:00 UTC over the Iraq region

Figure 2: shows an analysis of wind speed and direction at night at (00:00 UTC). The highest value of wind speed was (2.96 m / s) as the wind moved from northwest to southeast, and the lowest wind speed (0.25 m / s) was traveling in winds from northwest to east, where we notice that the movement of air in the desert areas was faster than mountainous regions, this is because winds tend to remain in open areas as a result of cooling and forming a layer of stable air. The desert causes winds to move faster than the desert (add reference that supports this statement).

4.2 Analysis of wind speed and direction at 03:00 UTC over the Iraq region

Figure 3: shows an analysis of the speed and direction of wind at night at (03:00UTC). The highest value of wind speed was (2.98 m / s), the wind moved from northwest to south and southeast. the lowest wind speed (0.25 m / s) was directed in wind from the eastern direction and during the day, in the daytime, the air in mountainous regions is always faster than air in desert regions .

4.3 Analysis of wind speed and direction at (06:00, 09:00) UTC over the Iraq region

Figure 4 and Figure 5: show an analysis of wind speed and direction at (06:00 / UTC). The highest value of the wind speed was ((2.92 m / s)) as the wind moved from north to southeast, and the lowest wind speed (0.23 m / s) was directed from the wind from the north in the western region. The movement of air in mountain regions differs from the movement of air in desert regions through the difference in speed and air currents in mountain regions, and the reason for this difference is that these areas are a rough surface that increases the friction factor with the movement of air, which leads to a decrease in its speed.

4.4 Analysis of wind speed and direction at (*12:00*)UTC over the Iraq region

Figure 6: shows an analysis of hourly wind speed and direction (12:00UTC). The highest value of wind speed was (3.86 m / s) as the wind blows from north to south and the lowest wind speed (0.6 m / s) The wind direction is from the northern direction from east to southwest, the day and night speed varies. In the daytime, air in mountainous areas is always faster than air In desert areas and at night, the opposite happens, as during the day the element of obstructing the peaks in the mountainous areas of wind overcomes, and thus the wind speed in the mountainous regions is less than its speed in the desert areas while at night and because the wind tends to dwell in open areas as a result of cooling and the formation of a layer of stable air, then Mountainous areas in this case are still warmer than desert regions, so the stability of air in them is less than desert areas, which leads to the movement of winds that are faster than desert areas.

4.5 Analysis of wind speed and direction at (*15:00*)UTC over the Iraq region

Figure 7: shows an analysis of the wind speed and direction at (15:00 / UTC). The highest value of the wind speed was the highest value of the wind speed (3.65 m / s) in which the winds go from northwest to southwest and the lowest wind speed (0.59 m / s). It heads in the wind from

north to south direction. Air stability in mountainous areas is less than desert areas, which leads to wind movement that is faster than desert areas.

4.6 Analysis of wind speed and direction at (*18:00*)UTC over the Iraq region

Figure 8: shows an analysis of wind speed and direction at 18:00 / UTC. The highest value of wind speed was (2.98 m / s) in which the wind went from northwest to southeast and the lowest wind speed (0.07 m / s). It winds in the direction from the north to the east, As the wind tends to remain silent during the day in the desert areas, this is why we notice that the fast wind is heading from the mountainous areas towards the desert.

4.7 Analysis of wind speed and direction at (*21:00*)UTC over the Iraq region

Figure 9: shows an analysis of wind speed and direction at (21:00 / UTC) and the highest value of wind speed was ((2.83 m / s)) as the wind runs from northwest to southwest, and the lowest wind speed is (0.24 m / s). From north to east, during the day the air in mountainous regions is always faster than air in desert regions.

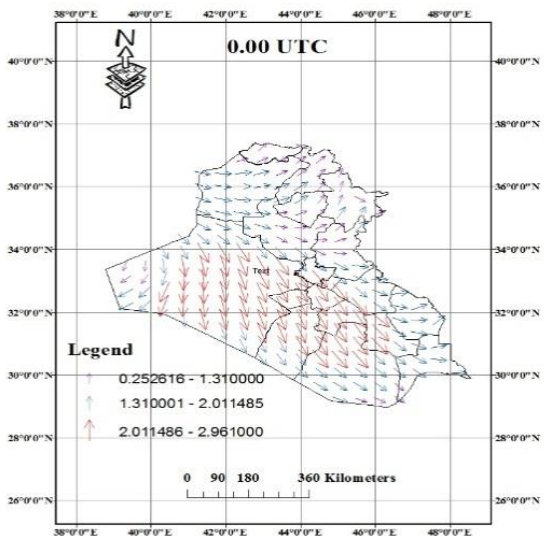


Figure2. The hourly average for one year (2016) of wind speed and direction at (00:00 UTC) over Iraq region.

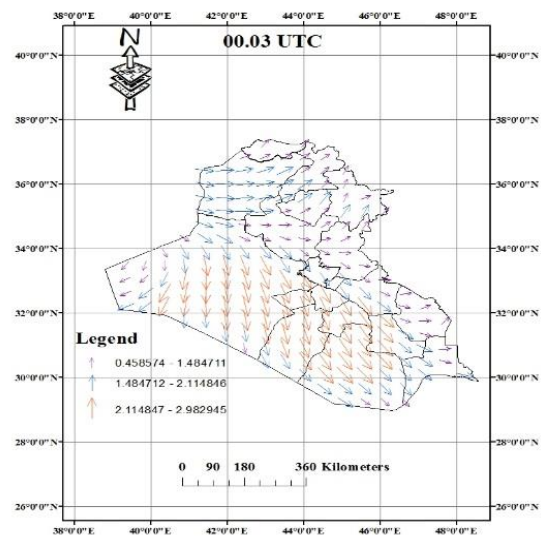


Figure3. The hourly average for one year (2016) of wind speed and direction at (03:00 UTC) over Iraq region.

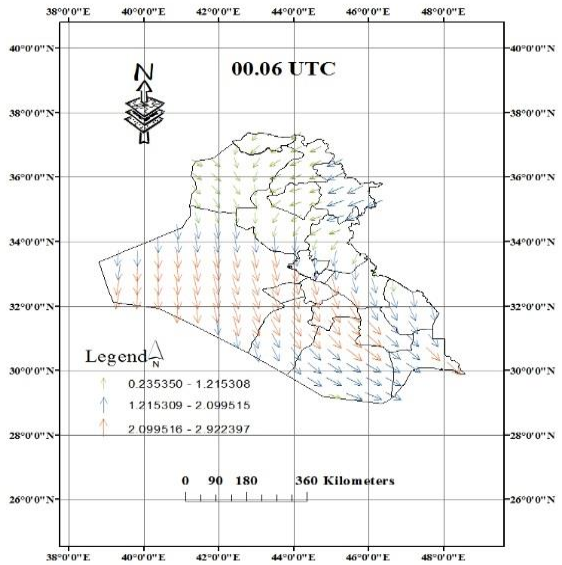


Figure4. The hourly average for one year (2016) of wind speed and direction at (06:00 UTC) over Iraq region

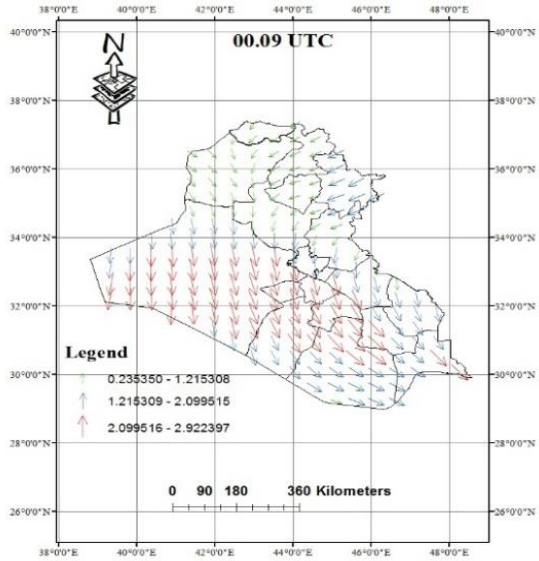


Figure5. The hourly average for one year (2016) of wind speed and direction at (09:00 UTC) over Iraq region

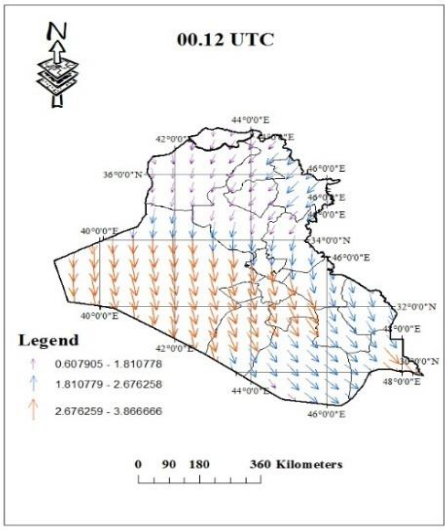


Figure6. The hourly average for one year (2016) of wind speed and direction at (12:00 UTC) over Iraq region

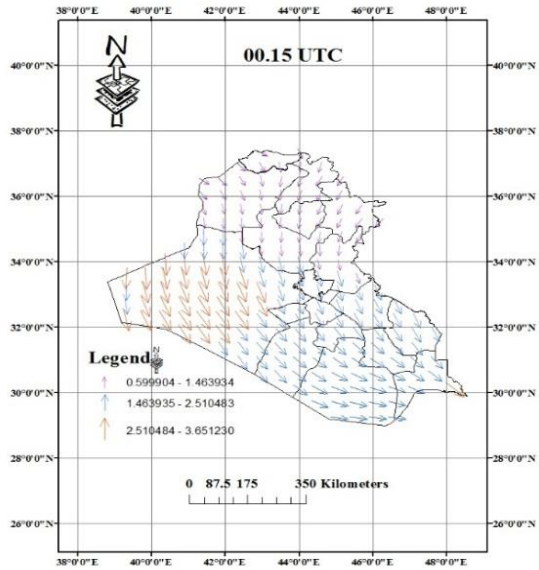


Figure7. The hourly average for one year (2016) of wind speed and direction at (15:00 UTC) over Iraq region

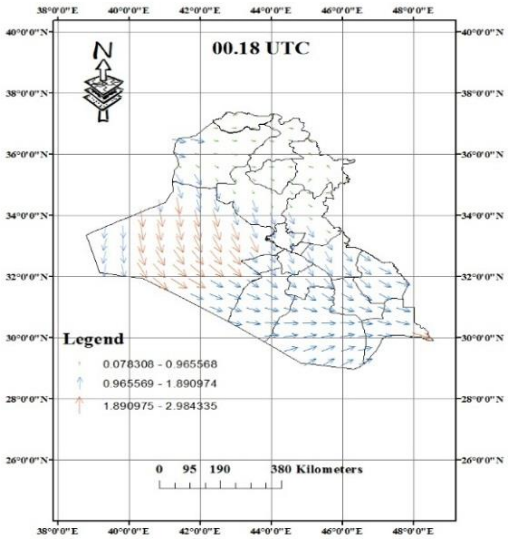


Figure8. The hourly average for one year (2016) of wind speed and direction at (18: 00 UTC) over Iraq region

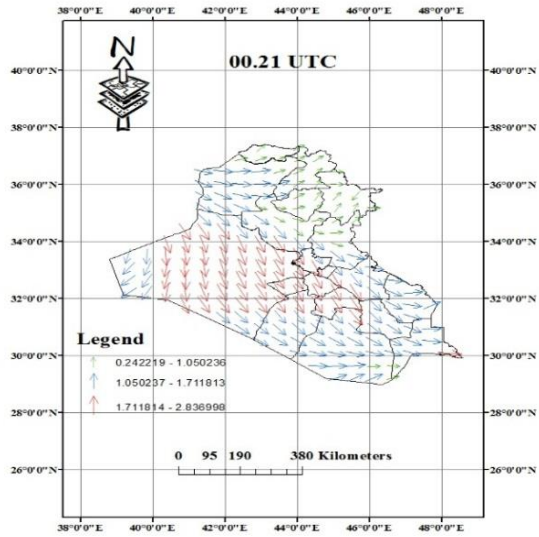


Figure9. The hourly average for one year (2016) of wind speed and direction at (21: 00 UTC) over Iraq region

4.8 Analysis of wind speed and direction data based on surface roughness over Iraq

Figures 10, and 11 and Table 2 show the analysis of wind speed data and direction during the study period for a full year (2016) for all regions of Iraq. The analysis showed that Iraq can be divided into four different rugged regions, where the highest wind speeds were found in Anbar, Najaf and Muthanna. In the less rough areas (0.50 m / s), there are surface roughness ranges (0.5-2 m) for eight governorates (Baghdad, Babil, Salah al-Din, Karbala, Qadisiyah, Dhi Qar, Maysan, Basra). The approximate topographical nature of roughness areas ranges from (2-11 m) represented in the governorates of (Mosul, Diyala and Wasit) and the lowest wind speed of 1.8 m / s in the hardest areas (11-72 m) which are the northern regions of Iraq, which are mountainous regions. Duhok, Erbil, and Sulaymaniyah).

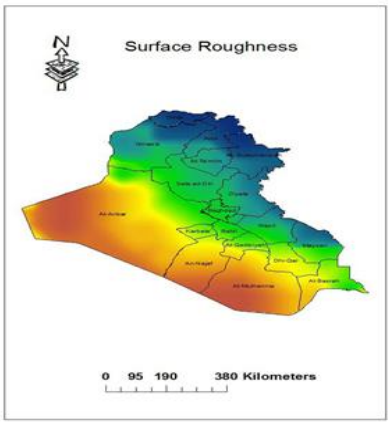


Figure 10. represents surface roughness for Iraq region.

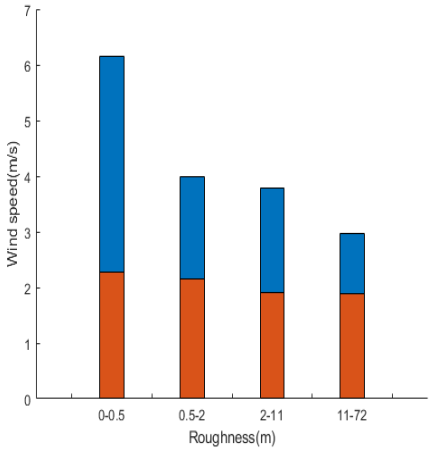


Figure 11. represents wind speed analysis according to surface roughness.

Table 2. The distribution of surface roughness in the different areas in Iraq

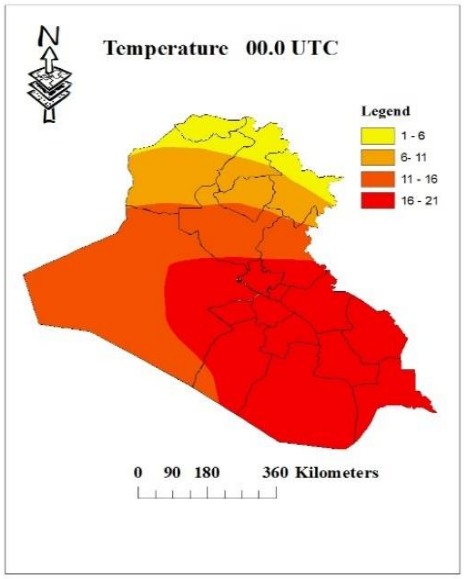
Roughness (m)	**Wind speed (m/s)**	**Station** (Iraqis' provinces)							
0-0.5 m	2.72-6.15	Al-Anbar	Al-Najaf	Al-Muthanna					
0.5-2 m	2.16-4	Baghdad	Babylon	Sala ad-Din	Karbala	Al-Qadisiyah	Dhi-Qar	Maysan	Al-Basrah
2-11 m	1.9-3.79	Ninawa	Al-Tamim	Diyala	Wasit				
11-72 m	1.89-2.97	Dohuk	Arbil	Sulaymaniyah					

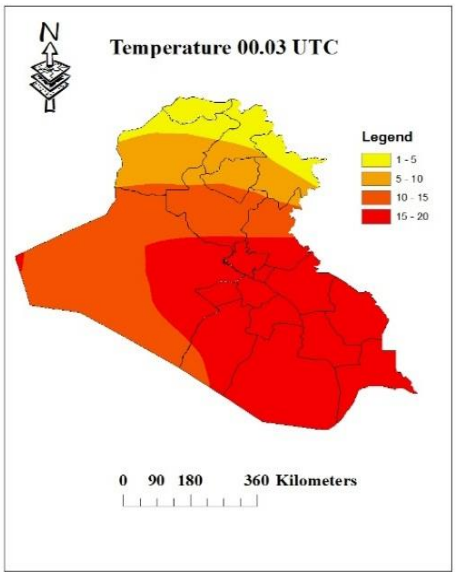
4.9 Analysis Average hourly for each year (2016) of temperatures over the regions of Iraq

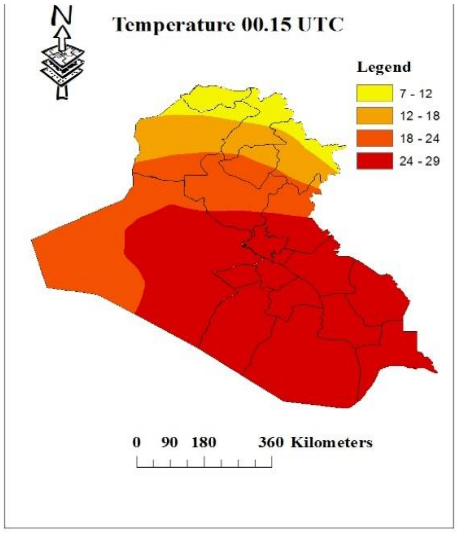
Figure 11 shows the distribution of hourly temperatures over the regions of Iraq, were it was found that the highest value of temperature was at 12:00 a.m. at 33 in the southern regions. These regions are characterized as dry desert areas due to lack of rain, high evaporation and low relative humidity in these areas. the lowest value was reached at 00:00 in mountainous areas, which are characterized by frequent rain, lack of evaporation and high relative humidity in the

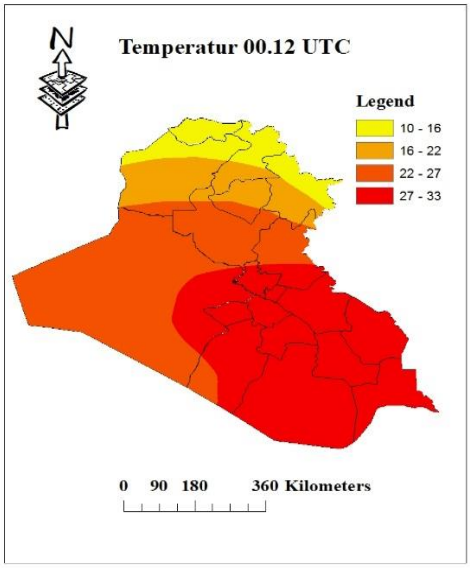
northern regions, where the speed and direction of the wind are associated with a decrease or increase in temperatures, and speed variation. During the day, the air of the mountainous regions is always faster than the air in the desert areas, and at night the opposite happens, as during the day the element of obstructing the peaks in the mountainous areas of wind overcomes, thus the wind speed in the mountainous regions is less than its speed in the desert areas while at night and because wind tends to remain in open areas as a result of cooling and forming a layer of stable air. In this case, mountainous areas are still warmer than desert regions, so the air stability there is less than desert areas, which leads to wind movement that is faster than desert areas. As the speed between the air in the mountainous and desert areas decreases as the air speed increases, in relation to the direction of the wind and the movement of air currents over the mountainous regions, the assumption is that the air over the mountainous regions warms more than the air in the desert areas, as it will be upward air currents that lead to a relative decrease in pressure that requires fetching air from the mountainous regions to replace the rising air, thus bringing relatively cool air from the mountainous

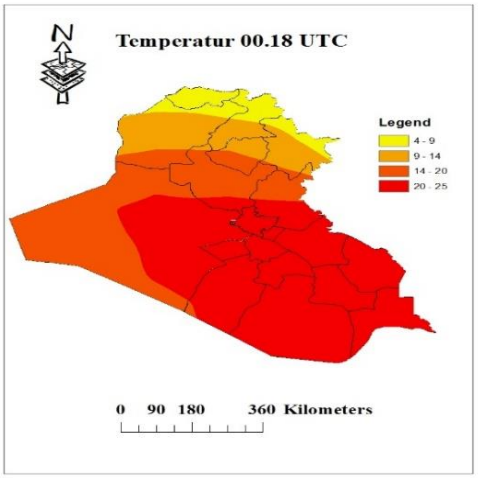
.

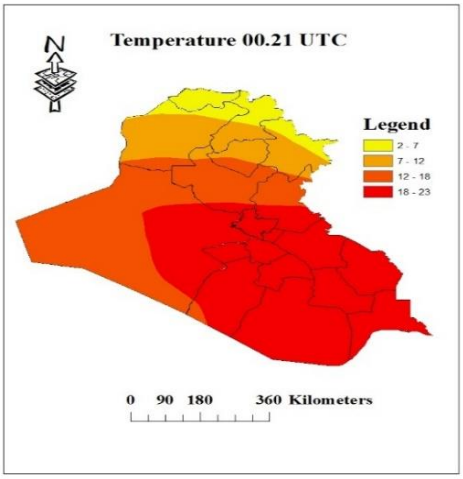


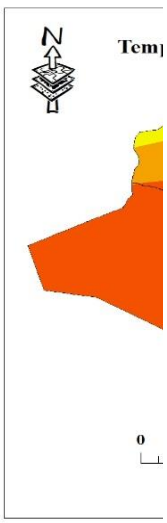





N
Temperatur 00.12 UTC
Legend
10 - 16
16 - 22
22 - 27
27 - 33
0 90 180 360 Kilometers


N
Temperatur 00.18 UTC
Legend
4 - 9
9 - 14
14 - 20
20 - 25
0 90 180 360 Kilometers


N
Temperature 00.21 UTC
Legend
2 - 7
7 - 12
12 - 18
18 - 23
0 90 180 360 Kilometers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Tem
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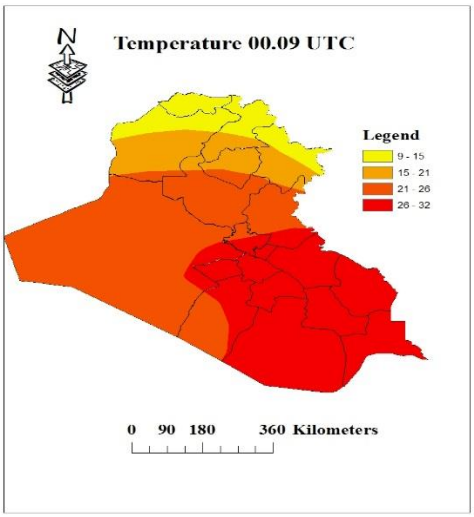
N
Temperature 00.09 UTC
Legend
9 - 15
15 - 21
21 - 26
26 - 32
0 90 180 360 Kilometers

Figure 12: The hourly average for one year (2016) Temperature over Iraq region

5. Conclusions

The results showed that the highest wind speed was (6.15 m / s) for the less rugged areas (0-0.5) add regions name, which are semi-desert areas, and the lowest wind speed was (1.8 m / s) in the rugged mountainous areas (add regions name). .It was also found that there is an inverse relationship between wind speed and surface roughness, as wind speed increases when the roughness values referred to by comparing wind velocity and surface roughness are reduced, and the prevailing winds are northwest and southeast to monitor wind movement and speed, as bumpers can be made to reduce air movement. finally, the highest temperature was in desert areas (add name of this areas/regions) and the lowest temperature in mountainous areas (add name of this areas/regions). In order to find out the effect of temperatures on the division according to the roughness of the surface and reduce the rise in temperature in desert areas, afforestation can be used.

6. Recommendations

Afforestation is one of the most important tools that must be provided for better understanding of climate conditions and accurate studies. Since the surface of the earth is covered with plants, it creates climatic conditions of its own, , especially during the night when the orchard climate is warmer than the open areas as the plants retain the long reflected rays. Mirrored from the Earth's surface, the vegetation also works to obstruct the wind and reduce its speed near the surface of the earth until it reaches its lowest speed inside the orchard or vegetation[16]

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Seaweed Growth Detection in Aquaculture Environment Using Simple Linear Iterative Clustering Method

Çağdaş Doğan*, Güven Yücetürk, Uğur Acar

Department of Innovative Applications Design and Development
Netaş Telecommunications Inc. Co.
**cdogan@netas.com.tr*
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Abstract: Estimating the total biomass of cultivates in aquaculture plantations (fisheries, mussel plants, seaweed farms and compound sites) remains to be an issue for the industry and the researchers alike. There has been a diverse array of approaches towards this issue, like using markers, manually stapling the leaflets, weighting the actual mass of the organism and calculating the total mass by extrapolation. Seaweed growth detection is a subset of this problem. Our goal is to introduce a solution by automatically detecting the ratio of the target object in images of seaweed taken from an underwater environment. Researchers/operators then can evaluate the total mass of seaweed. This study aimed to function as a decision support system. The system is built based on an image segmentation algorithm named Simple Linear Iterative Clustering (SLIC) which is a kind of superpixel segmentation. This paper conveys the results obtained from our approach towards the seaweed growth detection, elaborates on the usage and feasibility of our solution in seaweed sites and showcase the economic impact in the industry. Other dimensions of the growth detection methods in current practice for seaweed growth is also discussed, such as lack of automation in the current best-practices while focusing on the difficulties accompanying this status-quo.

Keywords: Aquaculture, Seaweed, Growth Detection, SLIC

1. Introduction:

Detection of growth is a necessity in seaweed cultures, however, it is labor-intensive and conventional methods mostly consist of manual measurement techniques [1]. A study that contain a computer vision component focuses on aerial detection of natural growth of harmful algae on lake and bay area surfaces [2]. Another paper on automatic detection of seaweed also focuses on aerial imagery of seaweed [3]. Application of computer vision and machine learning techniques on underwater seaweed images hasn't been given much focus, to the best of our knowledge. Currently seaweed growth detection is in general being done manually in aquaculture farms and since the biomass is underwater, a diver must physically go under and take photos and samples from the seaweed to be analyzed and weighed later. For example, it is possible to scrape off some

part of the seaweed and calculate the mass of the whole biomass, but it requires a lot of manual labor to achieve [8] [9] (based on the method proposed by Can Bizsel, Dokuz Eylül University).

An approach developed and used in IMPAQT project uses a method in which seaweed leafs are marked with a staple to be checked again later, inferring the rate of growth, however, this method can be applied for only certain species of seaweed and it still mostly requires human labor since it uses manual measurement techniques. Therefore, it can be said that there is a need for automatic detection on this kind of imagery and this paper aims to fill the gap. The goal of this study is developing a novel technique of detecting the weight and growth rate of the biomass in aquaculture environments (mussel, fish, seaweed etc.). this technique can be employed in industrial facilities enabling the industry to save manual effort and related costs. Seaweed is chosen as our model organism, as images obtained from other options does not provide enough stability to make a proper segmentation.

2. Material and Methods

The novel approach is based on the embodiment of computer vision techniques applied to the seaweed growth problem combined with newly implemented data streaming architecture developed for and used within our projects. The method aims to achieve detection of the ratio of biomass in an underwater seaweed image with reasonable accuracy.

2.1. Seaweed samples and images

Seaweed samples images were taken using GoPro by SCUBA divers (courtesy of Şeyma Tarkan, Çamlı Yem ve Besicilik San ve Tic. A.Ş.). There are some difficulties in detecting the ratio of seaweed in the images such as:. 1) the samples were underwater images, which presented its unique set of challenges. 2) The contrast was variable and there wasn’t adequate amount of it in parts of the images. 3) The brightness was inconsistent and it varied based on factors as time of the day and depth. Initially a set of images was acquired from Camli aquaculture site, who is one of the partners in IMPAQT (Image 1). Our code was implemented following the results obtained from the samples from the image set using trial and correction. Samples were selected for clarity and usability. The presumption is, in realistic applications, images taken for detection should be similar in terms of angle and distance (proposed method is either manually or by using a preplaced camera, which will enable the user to take images from the same angle and distance).

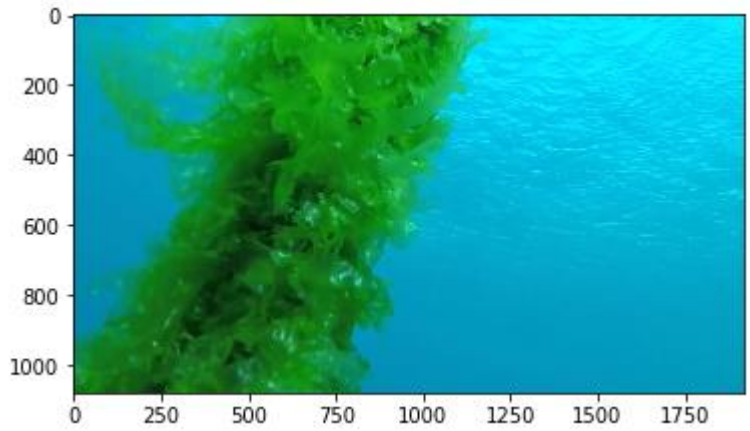

Image 1 - Sample image from the data set

The first step is to separate the seaweed and the background in the image to calculate the area of seaweed. This step is called ‘image segmentation’. Python’s scikit-image module was used for image segmentation operations. Scikit-image is an extensive library including a range of image processing methods using machine learning [4]. Simple Linear Iterative Clustering as also used, Simple Linear Iterative Clustering (SLIC) is one of the most excellent superpixel segmentation algorithms with the most comprehensive performance and is widely used in various scenes of production and living [5]. SLIC algorithm uses k-means under the hood, by which the image is segmented using the color value of the regions being truncated to the nearest mean. It takes in all the pixel values of the image and tries to separate them out into the given number of sub-regions [6] [7].

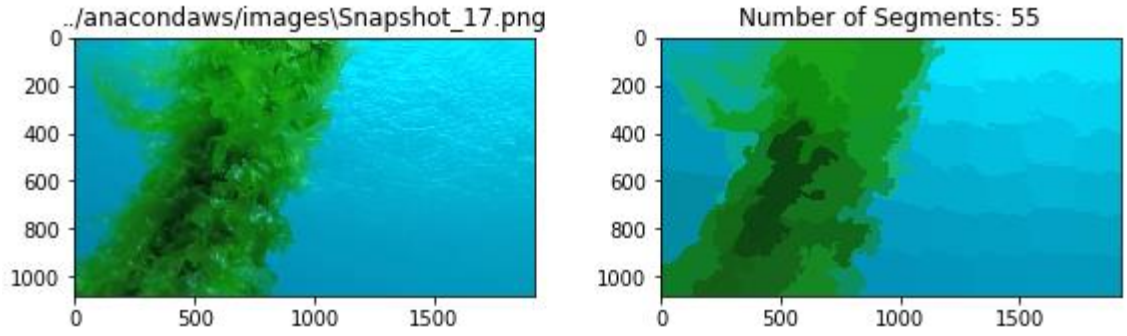

Image 2 - SLIC method applied with 55 segments

In the sample in Image 2, SLIC is applied and the image is segmented into 55 parts. Just after segmentation, each segment doesn’t necessarily have a realistic color value, just random colors denoting the difference in segments. To correct this, the segmented image is color mapped onto the original image using the average color values of each segmented area. This way, photorealistic image is obtained after segmentation. It will be used in the following steps as thresholding to convert it to a black and white image based on the color values. SLIC method can be counted as unsupervised segmentation as it does not take any threshold value from the user. The only

parameter is the number-K, which denotes the number of segments in the result. It is observed that the success of the segmentation shows a little variation between different numbers of segments and a value for number-K around '9' best suits this problem. Too high number of segments is not preferred as it starts to include parts of the background in the segmented object, nor too few, as some area from our target object is lost, which is seaweed in this case (Image 3).

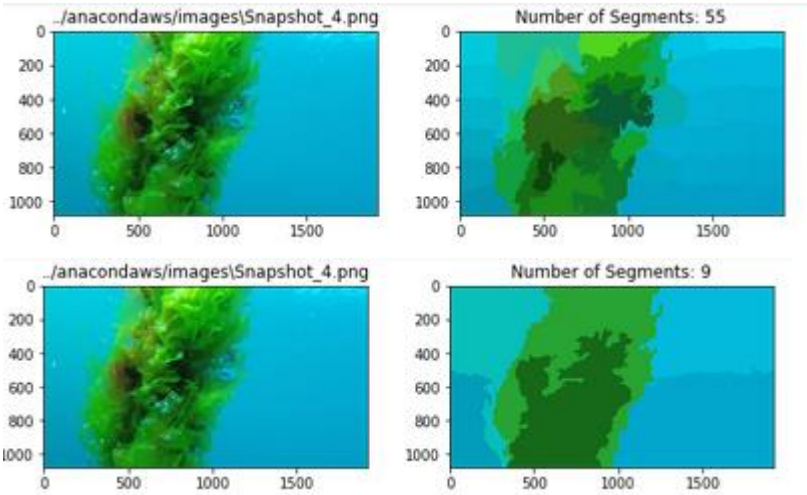

Image 3 - 55 segmentation compared to 9 segmentation

2.2. Simple color thresholding

Simple color thresholding is a technique used based on the comparison of the color values of each pixel in the image. It naturally requires some pixel operations on the image, in which each pixels green channel value is compared to the blue channel value in the RGB values of the pixel. This method is an heuristic solution developed for this study, step by step by evaluating the experimental results in each iteration to find out the solution which gave the best outcome.

2.3. The ratio of white pixels

As the final step, the ratio of white pixels in the final image will be calculated, as it will directly give us the desired result, which is the ratio that our target object (seaweed) takes space in the image (Image 4). The size of the image is known, therefore the total pixel value can be calculated: 1080 x 1920 = 2073600. The white pixels are counted by scanning the image by the code, running a 'for loop' through each pixel. From this point on, the ratio of white pixels can be obtained: 780004 / 2073600 = 0.3762 and 785239 / 2073600 = 0.3787 for the samples respectively.

```
Size of our image:
1080
1920

Number of white cells:
780004

Ratio:
0.3761593364197531
```

```
Size of our image:
1080
1920

Number of white cells:
785239

Ratio:
0.3786839313271605
```

Image4 - Final calculations for 7 and 9 segments. The code automatically detects the size of the image.

3. Results and discussion:

After trying various values for segmentation, the conclusion is; applying several values for number-K and taking the average is the best practice. Direct binary segmentation is not possible due to it losing too much area of the object because of truncating (Image 5).

Image 54 - Binary segmentation (2 segments)

Simple color thresholding method also worked well compared to other thresholding techniques based on histograms, as it does not need any input from the user, meaning it is unsupervised [6]. This technique alone is useful for truncating single pixels to black and white values but without segmentation, it introduces some error since there is a color variation in both the target object (seaweed) and the background (Image 6.1, Image 6.2).

Image 6.1 - Simple color thresholding without segmentation. The problem can be observed that some areas are falsely counted as background.

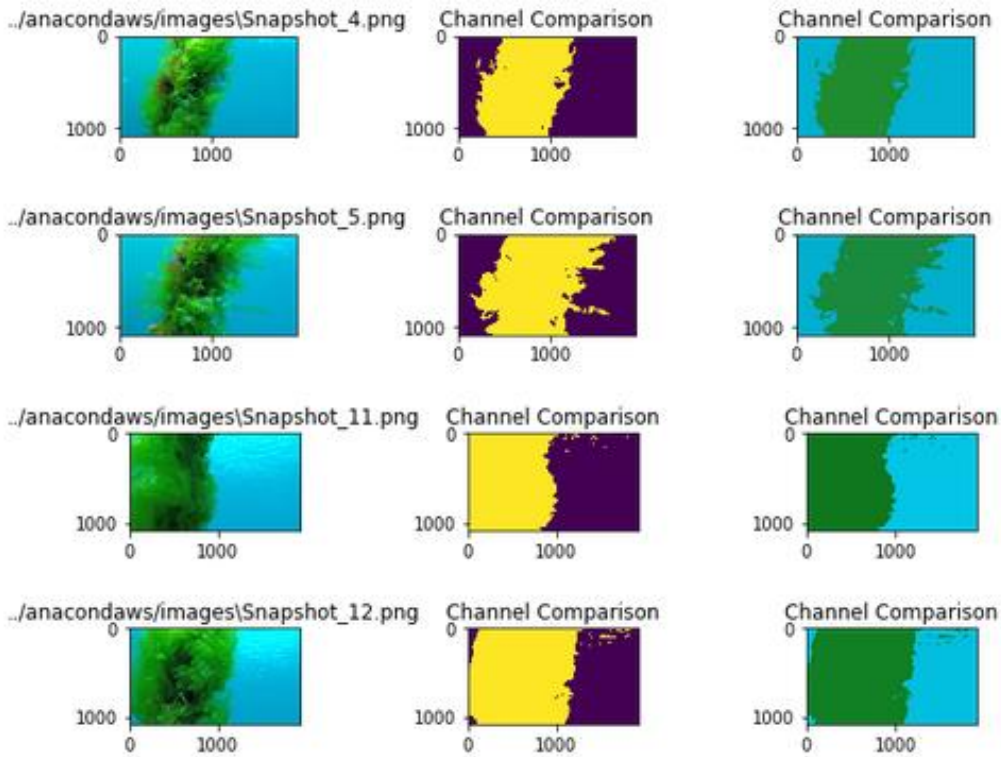

Image 6.2 - Various samples on simple color thresholding. Color mapping is applied.

After combining all three techniques, target results were achieved. A couple of successful examples of the application of thresholding onto an image with 7 and 9 segment SLIC method applied can be seen in Image 7. Color mapping is taken advantage of to create enough color variation for thresholding. This demonstrates that there is an optimum range for number of segments. Increasing the K value doesn't always result in new segments since some segments are truncated.

Image 7 – Resultant black and White image after applying all three techniques (SLIC, color mapping and thresholding). Number of segments is 7 for the first and 9 for the second image.

From image 7 it can be noticed the similarities in numbers for different K values of 7 and 9. It shows that for this image, segmentation is stable and successful. Taking the average value normalizes any variation in segmentation. The final output of the program is a single ratio for each image, as described for image 7. The K value is specifically set for this kind of binary segmentation as in our image set, which includes a color map (green & blue heavy) similar to what is widely seen in aquaculture environments. If needed, parameters can be tuned to work best in other kinds of images with little additional effort.

4. Conclusion

The system can be utilized in research or operational efforts where there is a need to decrease the manual work for segmentation where there is a large volume of an image set. Periodic control is very labor-intensive in aquaculture environments, so setting up a small camera will be useful to take stable images from the same distance and angle. It will help users obtain enough images to make an average calculation. When results obtained from image sets from different dates are compared, a growth rate and lifecycle estimation can be made, combined with interpretations of experts in the aquaculture field. After mapping the real weight of the biomass with the ratio values, it is possible to estimate the total mass of the seaweed using extrapolation. When used alongside conventional techniques, the developed technique can aid users with tools to make smart calculations based on a large set of images that cannot be manually inspected.

5. Acknowledgment:

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Great benefits of *Conocarpus erectus*

Wafaa Mohamed Masoud El Faras

Biotechnology laboratory-Faculty ofAgriculture

Alexandria University -Egypt

Email: welfaras@yahoo.com

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Abstract

Plants still an important source of omega oils that help to healthy for human and animals. Fresh leaves, stem, flower and fruits samples from Conocarpus erectus family Combretaceae has been analyzed using (GLC) Gas and liquid chromatography. The results indicated high levels of Omega oils which related to growth of nerve cells in brain. PH of fresh leaves determined and alkaloids with 8.2 which could help patients of diabetic type II

Key words: Conocarpus, Omega oils, diabetic type II

Introduction

The Conocarpus is composed of 2 species native to North America and Africa. Conocarpus scientific name is *Conocarpus erectus* and the common name: Buttonwood, family: Combretaceae. *Conocarpus erectus* can withstand elevated temperatures, air pollution, poor drainage, trampled soil and salty soil etc . This specie can grow almost in every type of soil as it can withstand air pollution, poor drainage, trampled soil and salt containing soil etc [1]. The species of this genus are native to shorelines in tropical and subtropical region of the earth [2, 3, and 4].

It can form dense, multi-trunked, shrubby thickets or grow as a tree up to 20m in height. Instead, the silver buttonwood has rounded flower heads covered in tiny, whitish florets that are said to

smell intensely of artificial grape. Cute as a button, the maroon-tinged, conical, button-like fruit clusters give the genus both their botanical name: Conocarpus means ‘cone-like fruit.[5]

Conocarpus has great medicinal importance like its leaves and fruits have been using traditionally as antipyretic, antidiabetic, antimalarial and for the treatment of conjunctivitis, syphilis, gonorrhea, orchitis, diarrhea, anemia, prickly heat and swellings.[6]

On the Other hand, it is reported that Omega-3 fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders, and cancer [7, 8, and 9]. Many studies on wild plants relative to the omega-3 fatty acids and antioxidant content are being carried out in various parts of the world. As expected, they show enormous variation in the content of both omega-3 fatty acids and antioxidants due to variation in climatic conditions and cultivars [10]. In present work studied the plant of Conocarpus as source of Omega fatty acids after propagating it under the Egyptian climatic conditions.

Material and methods

Plant material:

Green branches of Conocarpus plant were collect from faculty of Agriculture- Alexandria University.

Plant Propagation

The terminal buds with leaves of *Conocarpus erectus* were cultured in water for 2weeks then adaptation in pots with soil and compost 5weeks out door (Figure, 1).

Plant samples preparation:

Samples of leaves and fruits were collected. leaves and fruits washed and divided in fresh leaves dry leaves. Leaves were dried by two methods the first is using sun and the second in oven of 180 degrees for 20 minutes.

Figure 1: Terminal bud and cultivated plants of *Conocarpus erectus*

Fatty acids extraction

Folch method [11] was used to extract lipids which detected by gas liquid.

Estimation of fatty acids

Gas liquid chromatography (GLC), was used to detect and estimate fatty acids in lipid extract according to Radon, 1978 [12] using 10 fatty acids presented in table (1) as standard references.

Table 1: Fatty acids used as standard references in GLC

Symbol	Common name	Systematic name
12:0	Lauric	Decanoic
14,0	Myristic	Tetradecanoic
16:0	Palmitic	Hexadecanoic
18:0	Stearic	Octadecanoic
20:0	Arachidic	Eicosanoic
16:1	Palmitoleic	Hexadecenoic

18:1	Oleic	9, octadecanoic
18:2	Linoleic	9,12, octadecadienoic
18:3	linolenic	9,12,15 -Octadecatrienoic
20:4	Arachidonic	5,8, 11,14, eicosatetraenoic

Apparatus used for GLC was Gc Model Shimadzu Gc-4 CM(PFE), equipped with the following parts and conditions:

- PID detector
- glass column2,5m*3mmID
- Column:5%DEGSon 80/100chromo Q
- DetectorTemp:270C
- H2 flow rate:75ml/min
- Sensitivity:(16*10) 2
- Column Temp:180cISotherm
- N2 Flow rate:20 M.L./min
- Air flow rate:0,5ml/min
- Speed:2,5mm/min

Results and discussion

As shown in table (2) the group fresh leaves and fresh fruits have high content of saturated fatty acids (SFA). The highest SFA was Hexadecanoic acid (palmitic) (16:0) in fresh fruits (23.978 ug) while it was (16.772 ug) in fresh leaves. On the other hand, Tetradecanoic acid (Myristic) (14:0) was the lower acid in fresh fruit (0.904) ug and in leaves (0.740 ug). Generally, fresh leaves and fresh fruits were higher than dry leaves for saturated fatty acids content.

Regarding the unsaturated acids (omega acids):

Results in fresh leaves showed high content of Oleic (18.0) w9 octadecanoic (37.858) ug and low content in fruits (26.691) ug. While in the case of linoleic (18.1) w6 was high in fresh leaves (26.56) and in fruits fresh was (25.760) ug. Linolenic (18.3) w3octadecanoic trienoicw3 was high in fresh leaves (12.65), and also in fresh fruits (9.873) ug.

The content of arachidonic (20.4) octadecanoic tetraenoic w3 was high in fresh leaves (3.106) ug and low in fresh fruits (1.15) ug.

In the group of dry leaves:

Saturated fatty acids were high in dry leaves in Sun, where palmitic was (27.321) ug but in oven dried leaves was (25.866) ug. Unsaturated acid omega oil of dry leaves in sun was high in oleic acid (34.24 ug). On the other hand, linoleic acid w6 was high in dry in sun (21.359) ug. But in the case of dry leaves in oven was (16.166) ug, in linolenic w3 high in dry sun (36.607) ug but in dry in oven (15.273) ug, in arachidonic high in dry in oven. (1.155) ug, while in Sun (0.599) ug.

Table 2: Oil omega in leaves and fruits co carpus erectus

Symbol		Common name	Systematic name	Fresh leaves	Fresh fruits	Oven Dry leaves	Sun Dry leaves	S. D
12:00	Saturated Fatty Acids	Lauric	Decanoic	1.135	1.808	1.450	1.118	±0.3249
14,0		Myristic	Tetradecanoic	O.740	0.904	3.695	3.726	±1.6204
16:00		Palmitic	Hexadecanoic	16.772	23.978	25.866	27.321	±4.6794
18:00		Stearic	Octadecanoic	6.101	4.295	7.452	3.825	±1.6736
20:00		Arachidic	Eicosanoic	3.106	ND	1.155	0.559	±1.3322
16:01	Unsaturated Fatty Acids	Palmitoleic	Hexadecenoic	2.690	5.859	3.387	0.460	±2.2235
18:01		Oleic	9, octadecanoic	36.858	26.691	24	34.424	±6.6099
18:02		Linoleic	9,12, octadecadienoic	25.56	25.760	16.166	21.359	±4.5122
18:03		linolenic	9,12,15 - Octadecatrienoic	12.655	9.873	15.273	6.209	±5.3905

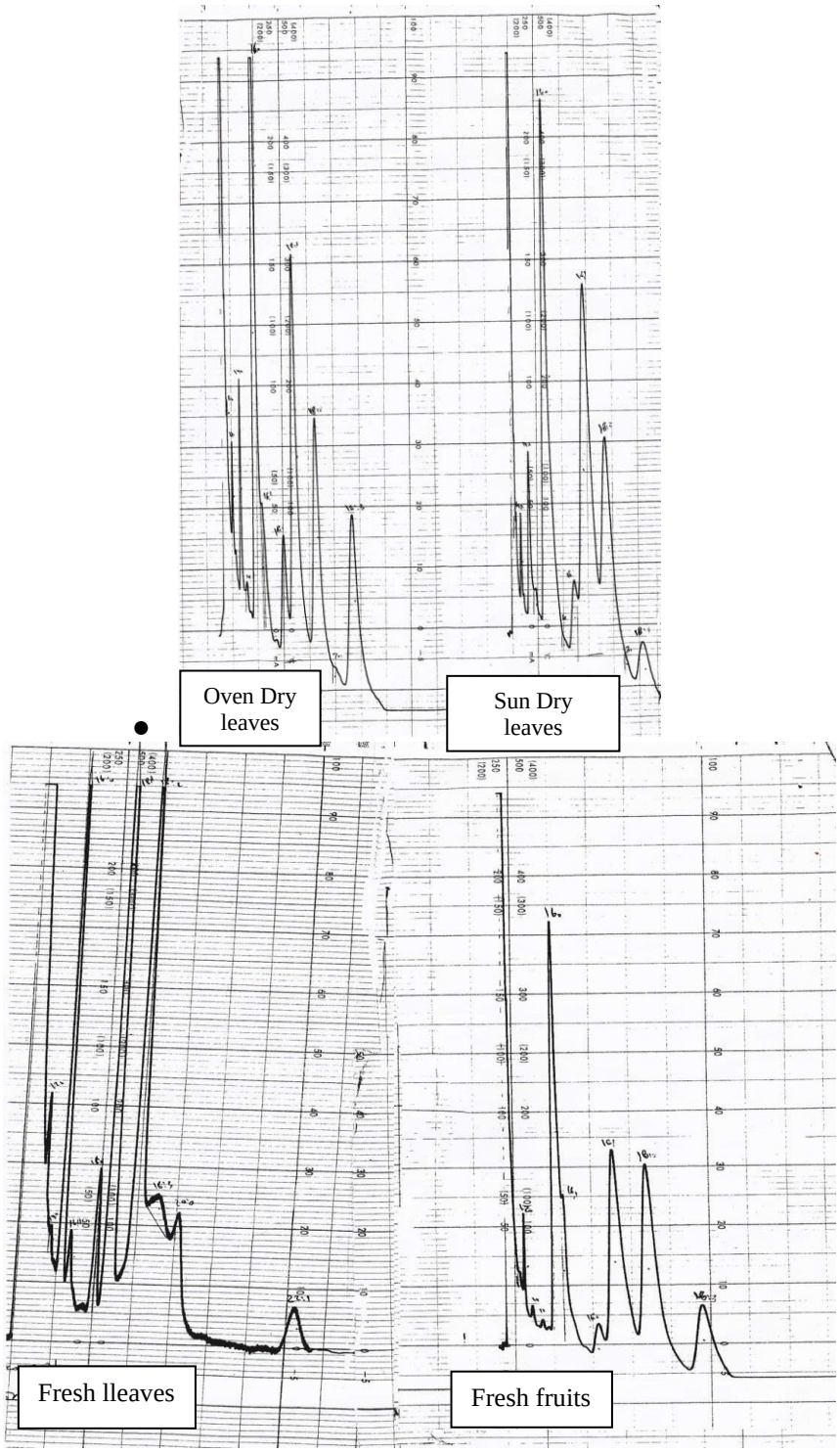

Figure 2: GLC curves for extracted fatty acids of *Conocarpus erectus*

As shown in the results of gas liquid chromatography fatty acids are different for quanties in fresh leaves and fresh fruits, omega oils are high in fresh leaves more than fresh fruits and also higher in oliec (18:1), linoleic (18:2), and linolenic (18:3) and in arachidonic (20:0). In dry leaves in oven

or dry in sun, results shown by gas liquid chromatography indicated that Omega oils in leaves dried in sun are higher than omega oils in leaves dried in oven.

Conclusion

Fresh leaves *Conocarpus erectus* content of Omega fatty acids is more 5 times than dried leaves in sun or oven and extract of leaves is great beneficial in health and medicine (Figure, 2).

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