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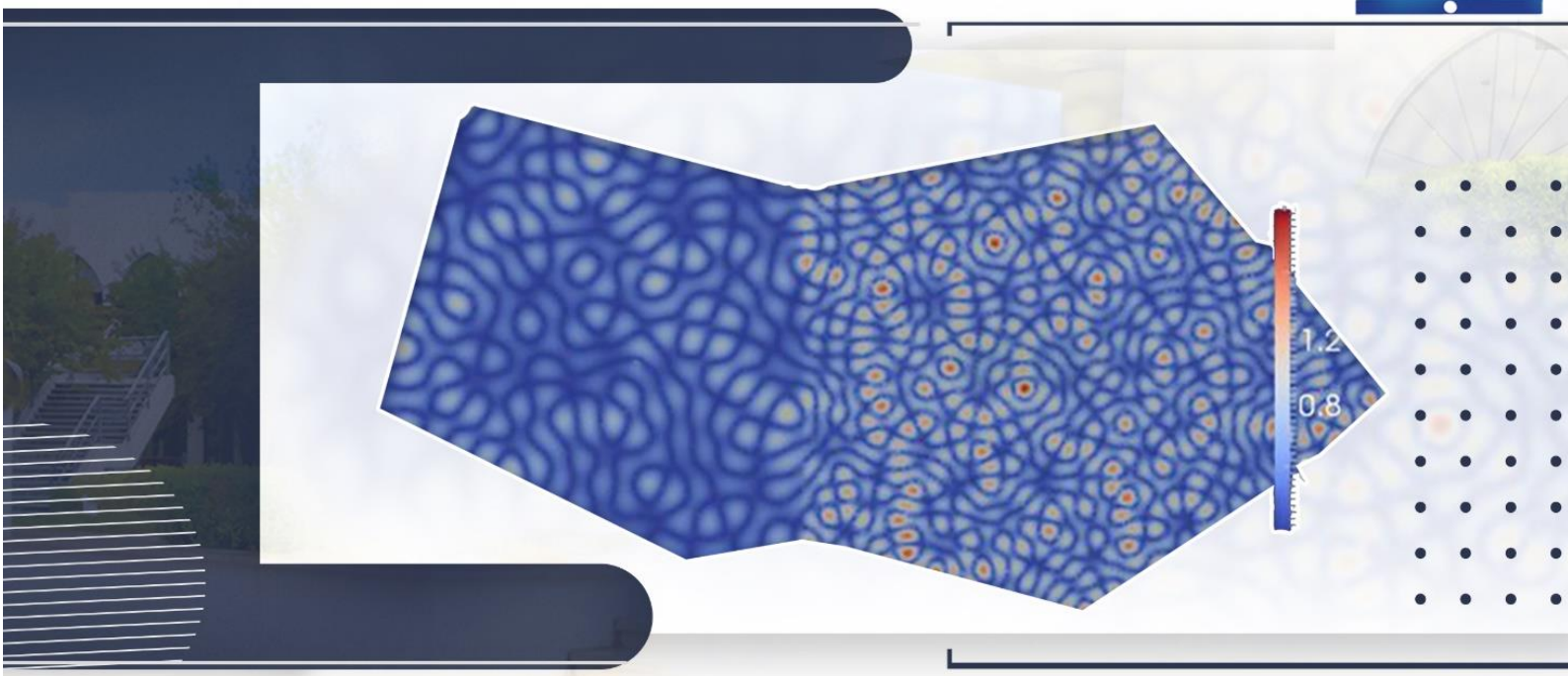

Volume 2 Issue 1 April 2022

Bi-annual, Peer-Reviewed, Indexed, and Open Accessed e-Journal

SJFSSU

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Scientific Journal for Faculty of Science-Sirte University (SJFSSU)

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Volume 2, Issue 1, April 2022

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Editor in-Chief's Word

In the name of ALLAH, the Most Gracious, the Most Merciful.

All praise is due to ALLAH, Lord of the worlds, and prayers and peace be upon the prophet Muhammad, and upon his followers and his companions.

At the outset, we are delighted to congratulate all members of the editorial board of the journal and all the teaching fraternity of the faculty of science at Sirte University on the launch of the first scientific journal of our faculty. Moreover, we would like to commend the efforts made by the Dean of the faculty, which bore fruit in establishing this scientific platform. Furthermore, we hope that this journal will be a scientific platform that musters academics from inside and outside the university in the field of basic sciences and their applications to share and exchange their experiences and scientific research. The editorial board of the journal will be keen to adhere to the common quality standards for scientific publications by following certain rules for writing scientific research, investigating honesty and scientific accuracy. We ask ALLAH to grant us success so that this journal contributes to raising the university's classification and progress at both the local and regional levels.

This journal will provide its services to the academic community in the vital sectors and will contribute effectively to the process of building the society.

This journal aims to enrich the culture of scientific research and encourages academics to engage in scientific research. We also aspire that this journal puts its unique mark to distinguish among other local and non-local scientific journals. With ALLAH's help, we will strive to ensure that the journal obtains the recognized quality standards, whether at the local or regional level.

My deep appreciation to the editorial board, reviewers and researchers who make our journal a vehicle for their research works.

Peace, mercy and blessings of Allah are upon you.

Prof. Dr. Abdalla Salem Radwan

Editor in chief

Editorial Manager's Word

Dear Readers and Researchers,

In the name of ALLAH, the Most Gracious, the Most Merciful.

All praise is due to ALLAH, Lord of the worlds, and prayers and peace be upon the prophet Muhammad, and upon his followers and his companions.

First and Foremost, I would like to congratulate my colleagues in the editorial board, the faculty members and all the administrative and technical staff in the faculty of science on the release of the second volume of the SJFSSU. The SJFSSU publishes an original scientific research that fulfils the academic integrity requirements and meets the common scientific standards.

The SJFSSU aims to consolidate scientific research and encourages everyone in the academic community to engage in the field of scientific research. The SJFSSU editorial board is keen to abide to the common quality standards by adhering to the ethics of scientific research and the investigation of accuracy and scientific integrity.

Our ultimate goal, as SJFSSU editorial board, is to become one of the well-known journals in the world. With Allah's help, we will strive to ensure that the SJFSSU reaches the standards that are recognized local, and regionally. Soon after the publication of the first Volume back in October 2021, the SJFSSU was registered in the Arab Impact Factor (AIF) database to obtain AIF=0.32 as well as in the Arab Citation & Impact Factor "Arcif" database. Moreover, the journal is indexed in the Crossref global platform and has an international Digital Object Identifier (DOI) for the whole journal and for the individual papers.

Finally, I would like to reiterate my gratitude and sincere thanks to all people who were involved, directly or indirectly, in the release of this issue. My special thanks go to my colleagues in the, editorial board, the peer reviewers and researchers who chose to publish their work in our journal. It is needless to say that any human work is not free of mistakes. This work is not exception despite the hard work that has been excreted in order to present it the best way possible. Therefore, should you have any comments /suggestions/ feedback, please feel free to contact us via email.

Peace, mercy and blessings of Allah are upon you.

Assoc.Prof. Haniyah A. S. Ben Hamdin

Editorial Manager

16-April-2022

About the Scientific Journal for the Faculty of Science-Sirte University (SJFSSU)

The Scientific Journal for the Faculty of Science-Sirte University (SJFSSU, henceforth) is a bi-annual peer-reviewed and open accessed journal issued electronically by the faculty of science at Sirte University. The SJFSSU aims to encourage research in the scientific community and publish papers reporting original work that are of high standards and contribute to the development of knowledge in all fields of applied and pure (theoretical) science, namely mathematics, statistics, physics, chemistry, zoology, botany, microbiology, astronomy, computer sciences, information technology, geology, environment sciences and oceanography.

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4. Affiliation with contact information including the (The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country). A clear indication and an active, official university email address of the corresponding author.
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Contents

Scientific Journal for the Faculty of Science-Sirte University Vol. 2, No. 1 April (2022) 1-8

Scientific Journal for the Faculty of Science-Sirte University

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Ecological Study of an Artificial Saline Lake Ecosystem in Wadi Al-Shatti, Libya

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A B S T R A C T

This study provided the first data related to the ecosystem of Ain Al-Mashashiya Lake. It is likely the new apperance of a saline artificial lake formed from agricultural drainage water from surounded local farms, small lakes provide an opportunity to understand intra-ecosystem connectivity and its dynamics. Water samples were collected representing two stations along edges and at the middle of the lake and other samples surrounded soil and vegetation. Lake depth was > 5 meters, the pH was found to be relatively high at edge of the lake (7.46 & 7.44) compared to at middle of the lake (7.38 & 7.32). Highly saline water, i.e., 129.79 & 125.48 dS/m & 126.82 & 133.74 dS/m at edge and middle of the lake respectively. The average level of PO_4 and NO_3 during winter is slightly lower than summer. The water cations abundances were typically Na > K > Ca > Mg, where were for anions, Cl > SO_4 > HCO_3 > PO_4 > NO_3. The lake is valuable for biodiversity and provide habitat for many species. Five phytoplankton types have been registered, i.e., Cocconeis, Notholca Pandorina, Oscillatoria, Chlamydomonas. The most common species were Pandorina & Oscillatoria. Soils of the study zone were classified as sandy loam, with a porosity of 44.53%. and saturated hydraulic conductivity (Ks) values was 4×10-3 cm/h and 6.4% of OM content. The soil reaction (pH) was 7.24 and its EC value was 19.42 dS/m that indicated high saline affected soil. The concentrations of Na, Ca & Mg, were as high as 193, 48 & 14.4 mg/kg, respectively, with low NO_3 content 0.293 mg/kg. Tamarix (Tamarix aphylla) commonly found along the lake edges. The dry desert climate of the area played a main role in imposing the lake ecosystems.

1 Introduction

Large areas of desert regions are characterized by endorheic drainage and consequently, waters collect in lower topographic areas creating lakes of different sizes and origins. If solute discharge increases, the lake becomes saline. Lakes also provide useful modern analogue environments to interpret fossil sedimentary records (Kumar and Abdullah, 2011). Hot deserts cover between 14 & 20% of the Earth's surface, approximately 19-25 million km^2. A defining characteristic of a hot desert is aridity. There are arid climates with > 500mm of annual rain that falls in intense events on hard soil or rock, and the water runs off horizontally or evaporates quickly (Rewald *et al*., 2012). According to the Koppen-Geiger climate classification, deserts are regions with an annual precipitation of less than 250 mm (Spinoni *et al*., 2015). However, the United Nations Environmental Programme's definition of desert is an annual moisture deficit under normal climatic conditions, where the potential evapotranspiration (PET) is over five times higher than actual precipitation (Huang *et al*, 2017). Because of these extreme conditions, deserts represent unique eco systems, which support significant plant and animal biodiversity. In fact, there are many different kinds of hot deserts with varying landforms, altitudes and

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life forms (Azizi *et al*., 2021). Sand dunes, instead rocky plateau, cover approximately 15-20% of deserts or mountain desert landscapes prevail (Rewald *et al*., 2012). Soil properties effect the degree of aridity and plant productivity. There are soils in so-called edaphic deserts, which are extremely porous and have such low water-holding capacity that the annual precipitation drains through them and rapidly is virtually unavailable for vegetation. Related to water, plant productivity in deserts can also be nutrient limited. For example, nitrogen is a key limiting nutrient in most deserts (Zhang *et al*., 2021), while phosphorous is the most limiting nutrient in deserts (Rewald *et al*., 2012). However, in the driest parts there is likely to be insufficient precipitation even for the development of any surface drainage system. Saline lake geochemistry varies over multiple timescales and is the product of a complex system involving precipitation, weathering, groundwater, evaporation, precipitation–dissolution reactions, and biological activity. These proximal controls are in turn controlled by the larger climatic, tectonic, and biological contexts in which saline basins form (Carroll and Bohacs, 1999). Small lakes provide an opportunity to understand intra-ecosystem connectivity and its dynamics. However, the information about the ecosystem is important even in the saline lakes that occur from time to time. The main factor that allows saline lakes to form is hydrological closure; that is, evaporation is the primary or only way for water to leave the basin. This condition occurs in many different settings around the world. The sources of water and solutes for saline lake brines are mainly direct precipitation, groundwater or associated surface flow. Groundwater can be derived from the local or regional meteoric system, interstitial water of sediments, or deep basin or hydrothermal fluids (Jones and Deocampo, 2003). In addition, the contributions of human activities, such as agricultural, domestic and industrial input are significant with regard to the physical characteristic and chemical composition of dissolved load of lakes and other water bodies (Khatri and Tyagi, 2015; Amnnah *et al*., 2020 Masood *et al*., 2021). The major problem of confined water bodies is usually the pollution from agriculture, industrial sewage and domestic wastewater (Barbosa *et al*., 2012). Confined water bodies may be regarded as an almost static system. The effects of the human activity on water resources can be divided into four factors: agricultural development; application of agricultural chemicals (nitrogen and pesticide); there are different types of monitoring programs according to their purpose, the most frequently tested parameters are: temperature, pH, turbidity, conductivity, dissolved oxygen, biological oxygen demand (organic matter), solids, nutrients, chlorophyll & coliforms; for in-depth research, metals, toxic substances and biological composition of the lake may also be studied (Bouderbala and Gharbi, 2017). Such information is crucial for developing strategies, programs or technical guidelines for the conservation and sustainable utilization of natural resources, particularly in the hyper-arid southwest region of the country. There is no published study on Ain Al-Mashashiya Lake, this is the first study conducted at three stations along the lake aiming to throw light on its ecosystem characteristics.

2 Materials and Methods

2.1. Site Description

Wadi Al-shatti is one of the districts of Libya in the south-west part of the country (27°44′6.53 N, 17.01′26°12 E). The area is mostly desert. Wadi Al-shatti is situated within the Murzuq Basin of south-west Libya. The Wadi Al-shatti area is mostly desert. The surface is generally covered by sand. Climatically, the study area is classified as an arid to extremely hyper-arid region. The climate of Wadi Al-Shatti is characterized by an annual mean temperature of 32.3°C, mean maximum temperature of 39.8 °C, mean minimum of 11.2 °C. The study zone has a wide range of temperatures between 0 ^{O}C in winter at night to 50 ^{O}C in summer at noon, Present day rainfall throughout the region is less than 20 mm per year on average, and exhibits high inter annual variability (Brooks *et al*., 2005). The potential evaporation rate is about 2000 mm, and the actual evaporation rate is negligibly less. The mean annual relative humidity ranges from 30 to 40%. The climate belongs to the type III semiarid of the African zonation (Griffiths, 1972).

Ain Al-Mashashiya Lake belongs to Wadi Al-Shatti, located at 27°25'04" N 13°52'49" E, about 10 km from Ain Al-Mashashiya village. According to information obtained from the local citizens, it is newly artificial lake, formed about 8-10 years ago. It is saline lake, oval shape 40 meters long & 10 meters wide. Lake level fluctuations depend upon water input and output balanceand mostly controlled by evaporation. Lake water volume depends on the relative altitude of the watershed boundaries in relationship to the lake bottom and the lake bathymetry, (Figure.1).

Figure (1). Lake View

2.2. Sampling

Water samples were collected at two stations along the lake edge and the middle of the lake. For plankton examination, upper layer water sampling was collected at the same three stations. At each station, one litter of water was preserved with 4% formalin. The samples were transported in the glass cylinders into the laboratory. Field measurements of some parameters were conducted according to Standard Methods (2000), such as water temperature, electrical conductivity using conductivity-meter; pH was measured using pH-meter.

2.3. Determination Methods

Titration methods were used for total alkalinity and total hardness and the results from both analyses were expressed as mg/L $CaCO_3$. Chloride (Cl) determination were done by titration method using Hg (NO_3) (Boyd and Tucker, 1992). Sulphates SO_4, Nitrate (NO_3) and phosphate (PO_4) measurements, which require photometric measurements were done according to the standard procedures (Standerd method ,2000). Calcium and magnesium ions were determined by titrimetric method using E.B.T and Murexid reagents. Na, K, were measured directly using the flame photometer (Franson *et al*., 1995). Each sample was examined and enumerated via a drop method (APHA, 2005). Various identification tools were used for phytoplankton species identification (Standard Methods 2000, Al-Yamani *et al*., 2011; Conway, 2012). Soil pH was measured using pH meter in solutions 1:1 ratio (Aishah and Elssaidi., 2019). Texture was analysed using standard pipette-analysis with gravimetric determination (Kettler *et al*., 2001). EC was measured using conductivity meter (Richards,1954). Total dissolved solids (TDS) were calculated as (**$TDS=EC_{25}\times0.064$**), The pycnometer method was used to measure soil particle density (Grossman and Reinsch, 2002). Particle density is expressed as ratio of total mass of soil to their volume ($g.cm^3$). Porosity of soil was calculated according to Anderson and Ingram (1993), using the following equation: **Porosity (%) = [1-(Bulk density/Particle density)] × 100**. Hydraulic conductivity (*Ks*) experiments were performed according to Darcy's Law calculation as described by Misra and Sivonghay (2009). Organic matter (OM) was determined using loss-on-ignition (LOI) method (ASTM, 2000), using the following equation: **%OM= Total C × 1.724**. Ca and Mg were extracted using ammonium acetate (pH 7.0). Calcium and magnesium ions were determined by titrimetric method using E.B.T and Murexid reagents (Franson *et al*., 1995). Cl analyses were done by titration method with Hg (NO_3) ((Standard method ,2000). Phosphorus was determined according to the procedure of Chang and Jackson (1958) and Aishah and Elssaidi, (2019). Sulphates SO_4 and Nitrate (NO_3) measurements, which require photometric measurements, were done according to the standard procedures (Standerd method ,2000). Calcium and magnesium ions were determined by titrimetric method using E.B.T and Murexid reagents. Na, K, were measured directly using the flame photometer (Franson *et al*., 1995). Flora of LIBYA and encyclopaedia of Libyan plants were used to identify the vegetation cover around the lake.

3 Results and Discussion

3.1. Lake Water Physicochemical Properties:

As the study provided the first data related to the ecosystem of Ain Al-Mashashiya Lake, there are no data on long-term changes in the lake. In as much as evaporation in excess of precipitation plays such an important role in the development of saline lakes, it is not at all surprising that this lake is to be found in the semiarid or arid regions of the world. However, in the driest parts of the true deserts of the globe, such as the Arabian or Atacama Deserts, there is likely to be insufficient precipitation even for the development of any surface drainage system. The pH was measured for all samples studied over two seasons summer and winter season at the middle and edge of the lake. (Table 1). At edge of the lake, pH found to be 7.46 & 7.44 in summer and winter season respectively, that was found relatively higher than middle of the lake, i.e., 7.38 & 7.32. Detailed analysis describing that the pH values were high in the summer season compared to the values recorder in winter season. However, the analysis also indicates the salinity of this lake was found to be very high, 129.79 & 125.48 dS/m at edge of the lake and 126.82 & 133.74 at middle of the lake in summer and winter season respectively, our result explained this changes in lake water proprieties. Saline inland lakes are often hypersaline (i.e., above the salinity of seawater). As noted, one of the first problems to be noticed in saline lakes studies is the origin of the salts. Due to water unbalance, salinity has gradually exponentially increased in this lake but at different rates. The degree of salinity of lake water indicated that it was brackish all over the lake according to 'Phocaides' classification (Phocaides, 2000). Climate plays a critical role in the water balance of all lakes, the water balance, must be closely controlled by evaporative loss in order to produce elevated salinities. An excess of precipitation will lead to a rise in lake surface area to enable evaporation to accommodate it, hence raising lake levels (Almendinger, 1990). In addition to the average precipitation, inflow has an important impacts on lake chemistry. the water balance highly seasonal or monsoonal systems experience dramatic hydrologic fluctuations, and these can play an important effects on the chemistry of saline lakes. The most important process in this area is agriculture. Currently one of the greatest threats to saline lakes is anthropogenic or secondary salinization as a result of human activity such as industry, agriculture, construction. Lakes from arid zones can dry out during long periods of time, although in most cases, they show a seasonal regime. Phosphorus is considered to be as one of the important nutrients. Dissolved reactive phosphate (PO_4) ranged from 2.16-3.22 mg/L at lake edge, the highest concentration was recorded during summer season. For the middle of the lake, the phosphate

ranged from 2.86-2.91 mg/L, the highest concentration was measured during winter season. Overall, average PO_4 levels during winter were slightly lower than summer and the variations during summer are apparently higher than winter. Also, the results showed that the average nitrate levels during summer was slightly higher than winter. NO_3 were ranged from 0.348-0.368 mg/L at edge of the lake, where the highest concentration was recorded in summer season. At middle of the lake, nitrate levels were ranged from 0.319-0.386 mg/L, the highest concentration was recorded during summer season. According to Downing and McCauley, (1992), nutrient sources of the lakes have divergent N:P ratios, ranging from 20 to >200 for precipitation, groundwater and for rural lands and soils were from 10 to <1 for sediments, sewage, urban runoff. According to the results (Table 1), CO_3 were not found, while different concentrations of bicarbonate were found in lake water, the HCO_3 concentrations were ranged from 28-36 mg/L at edge of the lake, and were from 20-24 mg/L at the middle of the lake, the highest concentration were measured during summer season. Lake water was found rich in sulphate 52.00-69.00 mg/L as a result of salt accumulation in studied lake basins. SO_4 reduction can occur in the center of the lake. Saline inland lake is often hypersaline. However, there was a clear seasonal pattern for Cl concentrations in the lake. Chloride concentration varied widely throughout the lake, the maximum recorded value was 481 mg/L at lake edge during summer season and the minimum level was 700 mg/L at lake edge during winter season. Increased chloride concentrations can negatively affect water quality. As well as impacting aquatic life, also it can increase the density of the water and affect thermal processes like stratification and mixing (Butcher *et al.,* 2015). Calcium concentrations were ranged between 49-52 mg/L in the edge of the lake and between 67-76 mg/L in the middle, the highest values were during summer season. The concentrations of Mg were high in the lake edges 29-36 mg/L compared to the middle 21.4-26.4 mg/L, the highest values were during summer season. The concentrations of K in the lake water varied considerably, where were high in the edge of the lake 248.49-372.49 mg/L compared to the middle 95.24-169 mg/L, the highest values were during summer season. Potassium is the key component of commonly used potash fertilizer, which is abundant in animal waste. The Na concentrations were varied widely all over the lake in a clear seasonal pattern. The maximum concentration was 557.5 mg/L at the lake middle during winter season and minimum amount was 118.55 mg/L was in the lake edge during summer season. The basic cations abundant concentrations were typically found, Na > K >Ca > Mg, For the concentrations of basic nations were typically $Cl>SO_4>HCO_3>PO_4>NO_3$. According to Tepe and Mutlu, (2004), the present of sodium and potassium ions may indicate to lake pollution caused by human activities. Sodium is often associated with chloride. It finds its way into lakes from road, fertilizers and human and animal waste. Sodium chloride dissolves readily and increase the solubility and mobility of salt. Calcium and Magnesium ions are both common in natural waters and both are essential elements for all organisms. Ca and Mg, when combined with bicarbonate, carbonate, sulphate and other species, contribute to the hardness of natural waters. The ions composition of lake water could be related to the geology of the area where the studied lake was surrounded by saline soil with a high EC. The salinity arises from differential ion precipitation as water evaporates from the lake. In general terms, $CaCO_3$ is precipitated first during evaporation and results in relative enrichment of Na, Mg, Cl, and SO_4 in remaining water

Table (1). Physicochemical properties of Lake water.

Parameters	Units	Lake Edge		Lake mid	
		Summer season	Winter season	Summer season	Winter season
pH	-	7.46	7.44	7.38	7.32
EC	dS/m	129.79	125.48	126.82	133.74
TDS	g/L	83.07	80.31	81.17	85.59
PO_4	mg/L	3.22	2.16	2.86	2.91
NO_3	“	0.368	0.348	0.386	0.319
CO_3	“	-	-	-	-
HCO_3	“	36.00	28.00	24.00	20.00
SO_4	“	68.10	69.00	59.40	52.00
Cl^-	“	1481.00	700.00	1168.00	1059.00
Ca^{++}	“	52.00	49.00	76.00	67.00
Mg^{++}	“	36.00	29.00	26.40	21.40
K^+	“	372.49	248.49	169.00	95.24
Na^+	mg/L	118.55	460.00	345.46	557.50

3.2. Microscopic Biodiversity of the Lake

Phytoplankton quantitative samples were collected by vertical net (55 mm mesh diameter). Samples were immediately preserved in 4% neutral formalin. The tabulated results are the mean of the three replicant values. The major groups of phytoplankton were subjected to detailed microscopic analysis and identification using the following identification tools used by (Al-Yamani *et al*., 2011; Conway, 2012). The results of this study (Table 2) showed low Algal biodiversity compared to other aquatic environments and were in line with (Oren, 2005). that could be related to high water salinity and anthropogenic impact. Five types have been registered, which are *Cocconeis , Notholca Pandorina, Oscillatoria,* Chlamydomonas, the most common species found were *Pandorina & Oscillatoria*. Lower phytoplankton taxa richness could be due to the lake's recent origin, small size, and high level of salinity. Weather conditions, physical factors, nutrient availability and also geographical location play important roles determining phytoplankton successions in the urban lakes. Higher pH average during summer compared to winter was due to the growth of aquatic organisms flourishes in worm weather, where favoured pH values ranging from 6.5-8.5 (Chapman, 1996). The phytoplankton succession might have specific patterns under weather conditions. The dry desert climate on the study area played a main role in imposing the lake ecosystems, given the high temperatures and little rainfall, this make the effects of human activities on biodiversity in this region much more dangerous. Saline lakes are important environmental features, with significant geochemical impacts on ecology, water resources, and deferent activity around the world. The lake is valuable for biodiversity conservation as they can provide habitat for many species and highly increasing local biodiversity of the surrounded area.

Table (2). Zooplankton in the Lake.

Cocconeis	
Kingdom	*Animalia*
Class	*Bacillariophyceae*
Order	*Achnanthales*
Family	*Cocconeidaceae*
Genus	*Cocconeis*

Notholca	
Kingdom	*Animalia*
Phylum	*Rotifera*
Class	*Monogononta*
Order	*Polima*
Family	*Brachionidae*
Genus	*Notholca*

Pandorina	
Kingdom	*Animalia*
Phylum	*Chlorophyta*
Class	*Chlorophyceae*
Order	*Chlamydomonadales*
Family	*Volvocaceae*
Genus	*Pandorina*

Oscillatoria	
Kingdom	*Animalia*
Phylum	*Cyanobacteria*
Class	*Cyanophyceae*
Order	*Oscillatoriales*
Family	*Oscillatoriaceae*
Genus	*Oscillatoria*

Chlamydomonas	
Kingdom	*Animalia*
Phylum	*Chlorophyta*
Class	*Chlorophyceae*
Order	*Chlamydomonadales*
Family	*Chlamydomonadaceae*
Genus	*Chlamydomonas*

3.3. Vegetation Cover

Tamarix (*Tamarix aphylla*) is found along watercourses in arid areas. It is a very resistant tree to saline and alkaline soils, distributed at the east and north side of the lake because it is tolerates very high salinity, drought and high temperatures. Saline tolerance is achieved in plants by various cellular and physiological mechanisms Tamarix tree is a sand dune stabilizer. It has the stamina, living and reproduction around highly saline water, temperature strikes and a dry desert climate. The present situation of biodiversity in this area is very critical and it is reflected by the certain natural ecosystems, However, detailed ecological and floristic accounts remain very scarce particularly for the study ears. However, saline lakes around the world are important nesting, feeding and staging areas for all types of water birds. *Tamarix aphylla* is found along watercourses in arid areas. It is very resistant to saline and alkaline soils.

3.4. Soil Physicochemical Properties

Soil properties influence the degree of aridity and thus plant productivity. Soil within the study zone, is classified as sandy loam soil and the most important characteristic is that it contains a small percentage of clay mineral 0.9%, and carbonite (CO_3) 0% (Table 3). The porosity of tested soil was 44.53%, which means that the soil is well aerated and permeable. The porosity is an important physical attribute in reproducing the field condition of soils in the laboratory. Saturated hydraulic conductivity (K_s) value of tested soil was 4×10^{-3} cm/h, it is within the usual values of such soils (Hussien and Fayyadh , 2014). The K_s determines the capacity of a soil

to transmit water and as such is an essential soil physical property that affects soil–plant–water relations and processes. It is one of the most variable soil properties because it is associated with soil texture and structure but is also affected by many other factors such as topography, vegetation, land use, climate and so on (Deb and Shukla, 2012). Accordingly, an accurate knowledge of the values of the *Ks* is a prerequisite for initiating an efficient water management scheme. Tested soil contains organic matter (OM) 6.4%. Desert soil in dry conditions often shows low levels of organic matter. The dry hot climate plays a vital role in determining the amount of organic material because the area is poor in vegetation cover. High temperatures affect all soil biological, physical and chemical reactions because high temperatures encourage the rapid disintegration and disappearance of organic residues from the soil. The overall soil pH tends to be slightly alkaline reaction 7.24. Moreover, due to a high degree of drought conditions and high evaporation rates the total dissolved salts in tested soil was 12.43 g/L and EC was 19.42 dS/m indicating high–saline soil. Due to high evaporation rates, desert soil is affected by accumulated several salt ions. The concentrations of Na and K were 193 & 38.6 mg/kg, respectively; while were for Ca and Mg were 48 & 14.4 mg/kg, respectively. However, the amount of Na, Ca and Mg in the surrounding soil were high. The NO_3 content of the tested soil was 0.293 mg/kg, which is considerably low. The primary purpose of NO_3 is to work as a source of nitrogen for the nutrition and growth of plants as well as soil microorganism. Next to nitrogen, phosphorus (P) is the second most important macronutrient as an essential plant nutrient (Srinivasan *et al*., 2012). The available phosphorus content of the desert soil was 39.65 mg/kg, and lake can sometimes be directly related to nearby agricultural activities as agricultural lands typically receive significant amounts of fertilizers and phosphorus) and plant protection products; high concentrations of specific chemicals in streams (Royer *et al*. 2006, Zobrist & Reichert, 2006). Soils with pH values between 6 and 7.5 are ideal for P-availability. Climatic and site conditions, such as rainfall and temperature, and moisture and soil aeration (oxygen levels), and salinity affect the rate of P mineralization from organic matter decomposition.

Table (3). Physicochemical properties of soil.

Soil Texture	Sandy loam	sand% 68.75 Silt% 30.35 clay% 0.9
Porosity	%	44.53%
Saturated hydraulic conductivity (K_s)	cm/h	4×10^{-3}
OM	%	6.4%
pH	-	7.24
EC	dS/m	19.42
TDS	g/L	12.43
PO_4	mg/kg	39.65
NO_3	"	0.293
CO_3	"	-
HCO_3	"	12.0
SO_4	"	56.6
Cl^-	"	149.0
Ca^{++}	"	48.0
Mg^{++}	"	14.4
K^+	"	38.6
Na^+	mg/kg	193.0

Finally, it could be concluded that irrigation with drainage water discharge from agricultural fields into depressions is a specific cause of artificial lake origin in the arid areas. The lake is mainly storage body. Unfortunately, saline lakes have been perceived as being unimportant, of less utility, and less abundant than fresh waters. Due to its geographical location and geological background, the water properties is deeply influenced by climate. The lake is unique ecosystem, and the seasonal differences were noted. The results indicate that the water quality of this desert lake could be affected by agricultural cultivation especially in the middle. The lake is mostly saline. The lake was characterized by the high salinity that results mainly from the small catchment area and high evaporation and temperature. The chemical composition of lake water in natural ecosystem is related to geological and lithological structure, topography, climatic conditions, vegetation and soil properties. However, soil properties play an important role in soil hydrology and influence the degree

of aridity and plant growth. We hope that our results can be used as the starting point for the long-time monitoring of changes in the lake ecosystem, which provide an important contribution to our knowledge of arid area lakes' response to climate variability.

4 Conclusions

Small lakes provide an opportunity to understand intra-ecosystem connectivity and its dynamics. This is the first data linked to the ecosystem of Ain Al-Mashashiya Lake. it is likely the new case of a saline artificial lake formed from agricultural drainage water from local farms. Lake depth was >5 meters. The chemical composition of lake water in natural ecosystem is related to geological and lithological structure, topography, climatic conditions, vegetation and soil properties. The average of PO_4 and NO_3 level during winter is slightly lower than summer. The water cations found abundance were typically Na > K > Ca > Mg, while were for basic anions, Cl > SO_4 > HCO_3 > PO_4 > NO_3. The lake is valuable for biodiversity and provide habitat for many species, five phytoplankton types have been registered, The most common species were *(Pandorina & Oscillatoria)*. Soils of the study zone is classified as sandy loam, with EC 19.42 dS/m indicating high –saline affected soil.. Tamarix (*Tamarix aphylla*) commonly found along the lake. The area dry desert climate played a main role in imposing the lake ecosystems.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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Building a 3D Form to Recognize Facial Images

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A B S T R A C T

There are several problems with the ability to recognize facial images, so the image was built in a 3D format, which allows users to evaluate and form the necessary properties of the entire object, while it is impossible to do this in two-dimensional form. Reprinting an object in an image form enables most of the properties of the target image by using layer-based neural networks that learn sequentially with discrete mathematical structures. A two-dimensional image is taken, and then the 3D shape is used in the image to spatially renovate it while different noise levels and different lighting levels are considered. This paper aims to show that the model for reconstructing the three-dimensional image reveals the dilemma of defining the basic characteristics of the image as a whole in all cases of interference, even if the viewpoint of taking the image changes. The processing has several stages. First, input the data processing result obtained in the previous level into the next level input to get the final result. After training, the first level sequences are represented as graphs, and then the input image data is sent to the first layer of the recognition model to calculate h. Consistent activation of learning validity for each subsequent level of the proposed model of reprinting an object in an image completely solves the problem of identifying a person from the front image as a whole under interference and regardless of the change in the perspective image.

1 Introduction

The precise problem of identification, namely the ratio of a particular person (class) to the original image, is not solved unless an enormous number of mechanisms related to the identification of facial images (Hemalatha *et al.*, 2014; Hasan *et al.*, 2012). The identification and recognition problems are inextricably linked. The following is the distinction: Identification is the correlation of the facial image, specifically found in the photograph or video material using recognition, with the facial image, stored in the identification system (Blanz *et al.*, 2003). Recognition of the facial image consists in finding signs in the photograph or video material faces, and identification is the correlation of the facial image, specifically found in the photograph or video material using recognition, with the facial image, stored in the identification system (Alexandre *et al.*, 2020; Albakri *et al.*, 2019).

The main challenge with the facial image identification method is that it must identify the person in the face of various "hindrances," external changes in the face (for example, plastic, cosmetics, etc.), and, most importantly, regardless of the change in viewpoint (Noyes *et al.*, 2018). To resolve this issue, this paper proposes a model for reconstructing an object in an image, which uses methods to restore an object's three-dimensional characteristics from its two-dimensional characteristics. This model can be used to rebuild the entire set of features. It is a series of reproducing levels L0, Ln made up of separate local sign and input data detectors. A multilayer neural network is used to train such a model. The training of a multilayer neural network must be done in a specific order. Related studies on how to recognize faces using techniques are presented in section 2 in this paper.

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A short synopsis of MATLAB in section 4, the majority of the target image's characteristics are specified in sections 5, image recognition and 3D shape of image is used for spatial renewal are described in sections 6 and 7. Layer-based neural networks that learn sequentially were used. A separate mathematical structure was used. The input takes 2D images in section 7. The results obtained and conclusion are shown in section 8 and 9.

2 Related Work

2.1 Emotion Recognition from Facial Images with Arbitrary Views

The emotional state of humans was studied using facial expression recognition. Almost no front view facial images are available in practice. As a result, the desired feature of facial expression recognition would allow the users to move their head in any direction. In order to recognize facial expressions in non-frontal facial images, some techniques for constructing a discriminatory subspace in specific viewpoints have recently been proposed. This method ignores the difference between samples from different classes with the same width label, as well as the proximity of samples within the same class with all width labels. Using discriminative adjacency, this paper proposed a new method for recognizing arbitrary vision facial expressions that preserves the concepts of implication and plurality of viewpoints. It begins by capturing the ability of samples to distinguish between classes. In addition, it explores how close the samples are within the layer with arbitrary widths in a low dimensional subspace. Experimental results on the BU-3DFE and Multi-PIE databases show that paper approach yields promising results for the recognition of facial expressions by arbitrary views.
Posture differences pose a challenge to recognizing facial expressions. Proposed a multi perspective discriminative neighbor method that maintains an embedding approach for recognizing facial expressions in different perspectives. The Proposed method is to exploit the intrinsic structure within the class and the punishment graph between classes to enhance the discriminative power of the inclusion preserving neighbor implication. In addition, offered to include this method in the multi display model, to make in class samples with distinct views that remain close together, MCCA is used to increase the correlation between them. Thus, these plots result in lower dimensional feature spaces that have an independent discriminative ability to display differences performance. The proposed method is tested on facial images with different views in the BU-3DFE and Multi-PIE databases. Experimental results show that the proposed method can achieve promising recognition (Huang *et al.,* 2013).

2.2 Face Model Construction Based on Kinect for Face Recognition

In human situations, these automatic expressions, poses, and intentional expressions are very common. These expressions are typically more durable than automatic expressions. Face movement and facial feature categories are classified using only visual information in expression recognition. Various applications in improving intelligence for the human computer interface, image decompression, and prosthetic face animation. To create an intelligent educational system, automatic face recognition is used. One method of detecting driver drowsiness and preventing a car accident is facial expression recognition. All existing facial expression analysis and recognition systems are currently based on either still photos or dynamic videos. Several technologies, including machine vision technologies, have been developed successfully using 2D still images or video sequences. Although some systems are successful, their performance deteriorates when handled. With different positions, expressions with a large head rotation, gentle skin movement, and/or lighting change. With the advancement of 3D imaging technology, quick and low cost 3D scanners have recently become available on the market. 3D scans, which extract features from faces, are expected to be more robust, resulting in more reliable final expression recognition. The susceptibility of 3D was assessed using 3D images in this study. Expression recognition rates for 3D images are over 90% in both cases. Otherwise, both classifiers are able to recognize about 80% of 2D images. 3D images were created. 2D images have a much lower discrimination rate than 3D images. This result backs up the paper's hypothesis that the 3D facial expression recognition system should outperform its 2D counterpart due to the advantages of 3D images. Without prior knowledge of a subject's neutral facial expression, an attempt was made to identify them. It is always more challenging. Recognizes absolute facial expressions without referring to a specific subject's neutral face. In several scenarios, it is possible to incorporate knowledge of the neutral expression algorithm, which is subject to change in order to improve performance (Hsieh *et al.,* 2015).

2.3 3D Face Recognition by ICP-based Shape Matching

Comparisons between forward facing images from the database and variable width point images Furthermore, compensating for expressions is a challenging task in the 2D approach, because it dramatically changes the facial texture profile. Facial recognition technology is rich in work aimed at solving problems related to this challenge.

The majority of this research uses density images of the face, called 2D model based techniques; for facial recognition.
Research-based on 3D modeling techniques, as well as compositional information, silhouette, and 3D face shape to reduce some differences. For 3D, faces and interests are saved to a library during an offline phase. During the online recognition stage, a 2.5D model captured from the face in the scene corresponds to the models in the library to find the identity and position of a person.
Similarities between layers and differences within a layer: the most recent 3D face recognition technology are presented, with proprietary techniques based on the model, and then on works developed for 2.5D and 3D face recognition saved in a performance database collection by work on recognition algorithms using 2.5D/3D face matching coupled with an area-based scale (Amor et al., 2008).

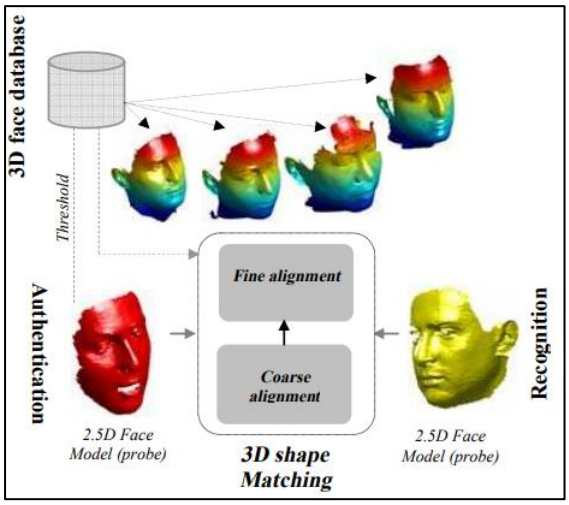

Figure (1). Approaches for face recognition (enrollment) and authentication based on 2.5D/3D face shape matching (Amor et al., 2008).

3 Proposed Methodology

Aims at developing the 3D face recognition method which is robust against illumination effect, changing expressions and varied poses of a face image.
This paper has proposed a new method to recognize the face, using methods to restore the three-dimensional characteristics of an object from its two-dimensional characteristics. Mathematical structure is also used. To train such a model, a multilayer neural network trained sequentially the entry takes two-dimensional images. This model can be used to form a rebuild of all features.

It is a sequence of reproducing levels L0 to Ln, consisting of separate local detectors of signs and input data. The aim of this research is the model for reconstructing the 3D image. The image is processed within MATLAB Biometric authentication.

4 MATLAB

MATLAB is high performance software for practical computing. It combines computation, visualization, and programming in a consumer interface, with problems and solutions written in standard mathematical notation (Tyagi, 2018). Some examples of typical applications are as follows: • Data analysis, exploration, and visualization • Math and computation • Development of algorithms • Modeling (Turk, 2019), simulation, and prototyping • Development of applications, including the creation of graphical user interfaces • Graphics for science and engineering.
MATLAB is a computer program that uses an array as its primary data element and does not require dimensioning. Many technical computing problems, especially those involving matrix and vector formulations, can be solved in moment while takes a long time by writing a program in a scalar of non-interactive language like C or python.
The term “MATLAB" stands for "matrix laboratory." MATLAB was created to make it easy to use the matrix software developed by the LINPACK and EISPACK projects, which together represent the state of the art in software development (Solomon *et al.,* 2011; Kaushik *et al.,* 2018).

5 LD Processing by Neural Networks

The approach is based on a photometric perspective that generates high quality models of faces from several images, but the recovery of regionally consistent shapes is accomplished by the collecting of discrete subsets of images. The route of neural networks is similar to that of the learning sequential based method. It modernized computer vision applications. For regression issues, these techniques work well (Cao *et al.,* 2021; Afzal *et al.,* 2020).
In the input data processing result obtained at the previous level, to the input of a subsequent level to obtain the final result, which wavelength selective switches (WSS) turn appears not only as a function of the activation values Learning Disabilities (LD) but also as a set of object parameters processed LD. Collectively it is called LD. Na input to the input level L0 is necessary to submit the data frame of local sites in sequences of images extracted from the video image.
After training the first level video sequences are represented as graphs (Fig. 2).

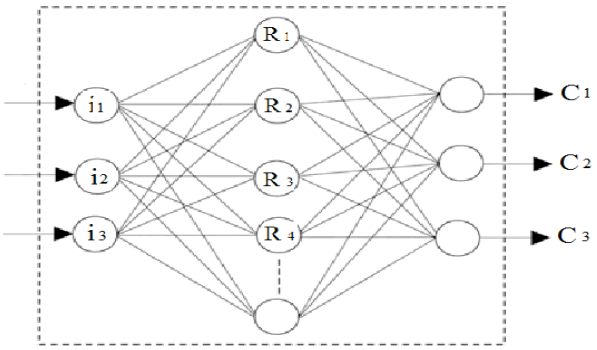

Figure (2). First level sequences are represented as graphs I - input image, R - set of frame by frame sequences, c - output result.

Trained at the first level local detectors are nodes of the graph. Each k^{th} level provides local data grouping. All other levels are trained. A trained model can present an image extracted from a video fragment as a limited number of LDs. Each local detector contains a representation of a complex object. The reprint model of the object in the image should contain:

- The number and local location of Li detectors.
- The number of Nc clusters for each level Lc.
- The number of reprint levels L0 to Ln.
- Internal parameters of the detectors.

For example, images in the image recognition function "m: i → j" are represented as a vector of length n attribute of this function. Many of the j classes used in this function are represented as the value of this function, which in in turn, it is mollified for a specific task. To test the recognition model of an object in the image, function used h: i → j for a subset of the set of pair attributes and values D = {(i-0, j-0),. , (i-n , y-n)}.

6 Image Recognition

For recognition function m in the entire domain of definition. The input image data must be submitted to the first layer of the recognition model to calculate h. Next, the consistent activation of the LD for each next level of the facial image recognition model is necessary. The binary value, represented as activation, is the result of processing the model and determines the ratio of a particular individual image to the corresponding class. This binary value has two states:

At the model output, a probabilistic estimate of the image parameters is calculated. To solve the classification problems, developer can use this model in its original form. The set of class's j consists of two elements. Function m = 1 if the image contains an object belonging to the required class.0 otherwise. The task of identifying an image of an object among many classes consists of 1) the relationship of the sequential verification of the image object to the model instance identification of facial images, i.e. function m = {m1, mj}. m ′ I ′swarm is the collection of classes J ′, for the i^{th} class J ′ = {Jw, z ≠ w jz};) for each particular class, model training takes place. The model diagram, consisting of reprint levels, is shown in Fig. 3.

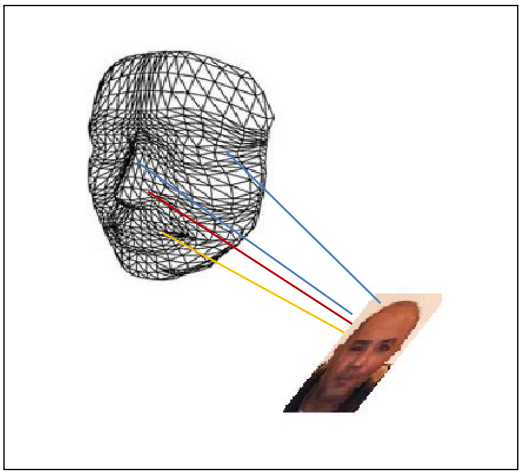

Figure (3). Model diagram, consisting of reprint levels.

7 3D Image Model

The projection of three-dimensional points on the plane can be described as Qs (Q) = CQ, where C is the matrix. Based o*n the definition of a local detector, the result of solving such a matrix will consist of an array of points b with coordinates (i, j). Such a definition LD clearly describes the structure, behavior, and result of the detector in the model of identification of facial images. (V, B) = V transformation operation in three-dimensional space. V is the matrix. The projection of the object is q0 = qs (Q).

Suppose there is some collection of some transformation matrices V1 to Vn and some set of projections q1 to qn, such that qj = Qs (vj, Q). Then the facial image identification model for such collections will use functions LI (qj) = q0; DV (qj) = vj, which in this case is a local detector. LD uses a set of representations of an object in three-dimensional form. For anyone's representation of the object, it is not only to determine the representation of the changed object but also to determine the initial shape of the object. The universality of the definition of LD will be allowed to get rid of the shape of the object when the use of the shape of the object is not necessary. For example, when qi = Tiq, qj = TjQ, qi, qj is the representation of an object. 1. Let Q0 to Qn, a collection of objects. 2. J0 to Jn is a set of classes. 3. J (Q) = J determination of a three-dimensional object belonging to a specific class. Such a definition of the local detector to identify facial models can accurately compare the three-dimensional (3D) object of a particular class.

The local detector must perform:
1) Object recognition,
2) The definition of a specific transformation applicable to the image.
To assess the effectiveness of the LD determines the concept of function error li transformation recovery:

$$J(Dt, q) = \frac{1}{9} \sum_{k=0}^{n} Dt(qi) \qquad (1)$$

The LD receives input in the form of two-dimensional images. Two-dimensional images, and, consequently, the local detector itself, have no data on the vector properties of the object, namely, on the additional coordinate Z. If the coordinate Z were known, then determining the transformation of the object would be a simple task not requiring specialized models using local detectors. For this feature of LD, the following symptoms exist take the required number of Transformed objects of facial images qi, qj corresponding to Ti, Tj. For the case when qi ≈ qj, but Ti ≠ Tj, the LD retroactively restores the changed object from the image. Thus, DT (qi) ≈ DT (qj). When 3D objects Q and Q1, belonging to different classes J1, j2, give similar projections q ≈ q1, the same result is obtained for the LD identification function, i.e., LI(q) ≈ LI(q1). There are such variations of the transformation (T) and the images of the object for which it is impossible to construct. Any possible deviations of 3D shapes of objects of facial images to various projections (facial images) are negatively affected; the variability of the facial image of the object increases the average error within one class. Possibly, there is a negative correlation between the accuracy of restoration of the changed image object, the size of the facial image, and the plurality of facial images. Reprint a complete facial image using only one LD is a complex task that surpasses the task of recognition in complexity. At the first level of the model, LD LI (0) is presented, whose ensembles and values are different personal for each specific object of the facial image that can react to different parts of the object of the facial image at different Perspectives. Data from the first level models of the local detector are necessary for teaching high level LDs that are representative for LD compositions, namely, a 3D feature map - first level LDs on which local detectors are placed by mi equivalent transformations. The belonging of an object to a category is determined by the identification function of the second level LD. Thus, the entire above are included in the MATLAB libraries for facial features, the MATLAB software employs the computer vision toolbox, which is then compared to the database. The system returns the final expected face matching result if the facial features match.

8 Results

The proposed model of reprinting an object in an image completely solves the problem of identifying a person from the front image as a whole under interference and regardless of the change in image perspective. Using layer-based neural networks that learn sequentially, allowable changes in viewpoint, different noise different lighting levels are considered.

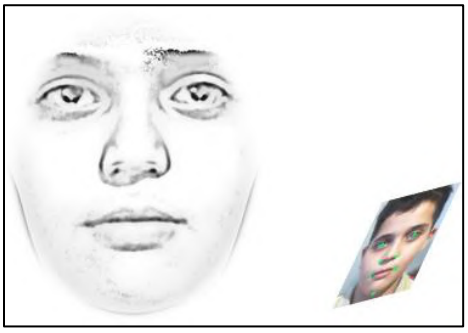

Figure (4). Model representing objects training 1 of 5.

Purposes the human body is based on the optimized serial neural network architecture through the behavior recognition algorithm, the network architecture, optimizing implicit neural network extraction of nonlinear features and complex images by building in 3D Form to facilitate the identification of the model image by humans. 5 training models have been worked on, and the results have been found to be reasonable. Ability to extract image feature. The Rectified Linear Unit activation function is used in the nesting layer. The model has both generalization performance and the ability to nonlinear fitting. Compared with the traditional methods, it has achieved good results except response time.

9 Conclusions

In previous studies, several methods were used, such as a novel face recognition method which is based on the Iterative Closest Point matching; others used multi view discriminative neighbor preserving embedding approach for recognizing facial expressions in different views. By looking at the result, a similar system can be used to generate 3D models. The basic idea of this work was to gain knowledge about different problem areas to generate 3D models from 2D images. However, with the current implementation, the output is too weak and slow to get high resolution. How important is the interchange between generation speed and output resolution when creating 3D models from a 2D image, using serial neural networks. The acquired accuracy increases exponentially with each level of detail, while the generation time is not linear. Since speed has a

correlation with the amount of mutation needed to move from one level of detail to another, the mutation

required is to have a lower percentage value by each increment of the level of detail. In conclusion, increasing the resolution also increases the effectiveness. However, the total number of mutations was still growing rapidly, high quality is more technically effective, it is still not possible to generate high resolution models, and the total generation time will be too much, however, the proposed method is cheaper than the previous ones, despite their lack of accuracy.

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Conflict of Interest: The author declares that there are no conflicts of interest.

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Identification of the Most Important Weed Species in A Barley Crops in Beir Bullerjam Region – Soloq, Libya

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A B S T R A C T

A field survey was carried out to highlight the most important and problematic weeds of barley crop at District Beir Bullerjam, Soloq Region, Libya for two seasons (2020 to 2021). The current study is based on field research where recorded most weed the grown species by survey followed by using quadrats and finally calculating the weed density and their frequency. samples from the fields of the barley crop using a quadrat of size 1 m × 1 m randomly thrown at different points in the fields. The plants were identified with the help of available literature and through comparison with the already identified plant species. Data inventory has been documented in the form of family, Botanical name, vernacular name and life cycle. A total of 67 weed species belonging to 54 genera and 21 families of angiosperms. The dominant families according to the number of species were Asteraceae (14 species), Fabaceae (11 species), Poaceae (9 species), and Brassicaceae (7 species). The most dominant life cycle was annuals having 57 followed by perennials having nine species and biennials represented by only two species.The results further revealed that the highest density (43.48 m^{-2}) and (30.36 m^{-2}) were recorded for *Lolium rigidum* and *Melilotus indicus* respectively, while the highest frequency were recorded for *Melilotus indicus* and *Eruca sativa* with 84% and 80 respectively.

1 Introduction

Hordeum vulgare L. As one of the earliest domestic crops, barley has been one of the most important staple crops in the old world Neolithic agriculture upon which early agriculture was built (Harlan and Zohary, 1966). Weeds are unwanted plants that provide a hard time to any particular crop in which it occurs. Its variety and distribution differ from crop to crop and generally have no aesthetic or economic value. Such vegetation is found abundant in cultivated fields of great financial and biological significance (Jabeen and Ahmed, 2009). In no other parts of the world does uncontrolled weed growth cause as a great a reduction in crop yields as it does in dry areas where soil moisture is the limiting factor and weeds compete significantly with the crop for available moisture (Robson,1992).

Weed infestation is one of the major hindrances to crop yield including pests, diseases, and climatic influences. Weeds and crops usually have the same requirements, but weeds make their living at the expense of the crop by competition for space, nutrients, moisture, light, and carbon dioxide (Abdul Ghafoor and Shad, 1995; Klingman *et al.*, 1975; Muzik, 1970). There are around 30,000 species of weeds in the world, of which 50 to 200

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usually cause considerable damage to the major food crops (Mahmood, 1992).

Weed problems in Libya have become very important not only in the coastal belt with its higher rainfall, but also in the newly established irrigation projects in the desert where it was very difficult to find a single weed in the past (Robson, 1992). In Libya however, the recognition of weed science as a discipline with the same position as other crop protection disciplines, such as entomology and plant pathology, has been neglected (Kukula and Ghanuni, 1992). There is a good number of studies on weeds of the crops that have been reported from different parts of the country i.e., Sirte (Alaib and Ihsaeen, 2008), Melytania (Al-Zerbi, 2004), Jardina- Soloq Region (Omar *et al*. 2020), Masiklo (Abu Khsheem, 2020).

The present study aims to identify different weed species and the most dominant weed species in the barley crop by calculating the density and frequency.

2 Materials and Methods

The study area is located about 50 Km South of Benghazi city adjacent to Soloq city at approximately 32º 08′ N latitude and 20º 32′ E longitudes. The area rises about 70 m above the sea level (figure 1). The total area is approximately 600 hectares. The study lasted two years and took place throughout the spring season (2020 to 2021). Field expeditions were more frequently done from January to April when the plants were in flowering and fruiting conditions. The crop-weed association was studied by quadrate of 1 meter diameter. Twenty five quadrates were randomly placed in the fields and the number of plants of each weed species falling in the quadrate were carefully counted and listed for density and frequency.

For drying, the presser containing the specimens was placed in the drying room. After that the specimens were examined individually, rearranged, transferred to fresh sheet and again tightly bonded in the presser. The specimens were changed to dry sheet every 24 or 48 hours, until they were completely dry. The data were recorded on the following parameters:

$$\text{Density} = \frac{\text{Total number of individuals per species in all quadrats}}{\text{Total number of quadrats studied}}$$

$$\text{Frequency} = \frac{\text{Total number of quadrats with the species}}{\text{Total number of quadrats studied}} \text{ X } 100$$

The genus and species was identified by the utilization of available taxonomic literature (Erteb, 1994; Ali and Jafri, 1976-1977; El-Gadi, 1988-1989; Keith, 1965; Jafri and El-Gadi, 1977-1986 and Tackholm, 1974).

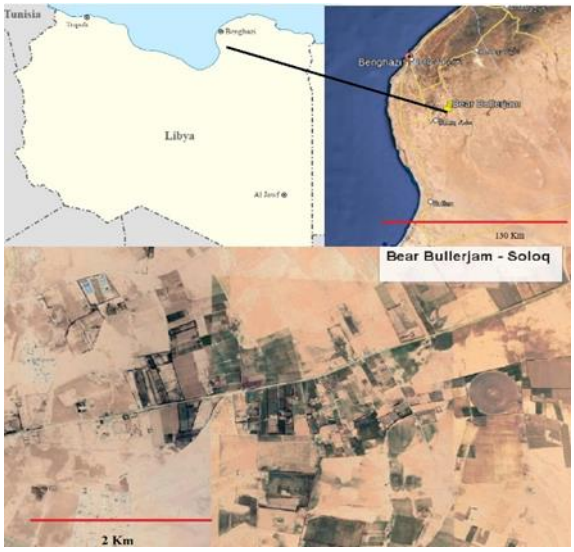

Figure (1): The study area and location (source: Bing Maps and Google Earth).

3 Results and Discussion

Weed flora of the study area is comprised of 67 species of flowering weed plants representing 54 genera and 21 families were record. A detailed list of weed species identified in barley crop in the study area along with their scientific names, families, vernacular names, and life form are shown in Table 1below.

Table (1): weed species, their vernacular names and life cycle of barley crop recorded in the study area.

Species	Vernacular name	Family	Life cycle
Allium nigrum L.	Thom Bary	Amaryllidaceae	Ann.
Allium erdelii Zucc.	Ghazool	Amaryllidaceae	Ann.
Amaranthus viridis L.	Buzinzir	Amaranthaceae	Ann.

Ammi majus L.	Sfinnari-Hameer, Khalla, Sfinnari el ma'iz	Apiaceae	Ann.
Deverra tortuosa (Desf.) DC.	Gazzah.	Apiaceae	Per.
Pseudorlaya pumila (L.) Gramde		Apiaceae	Ann.
Achillea santolina L.	Zefrah, El Batharan	Asteraceae	Ann.
Anthemis secundiramea Biv.		Asteraceae	Ann.
Calendula arvensis L.	Ain Al-Bugra	Asteraceae	Ann.
Carduus getulus Pomel		Asteraceae	Ann.
Centurea dimorpha Viv.	Bla ′ala	Asteraceae	Ann.
Chrysanthemum coronarium L.	Gahwan	Asteraceae	Ann.
Conyza bonariensis (L.) Cornq.	Ashbet Zamora	Asteraceae	Ann.
Echinops galalensis Schweinf	Shembet Elgatoos, Libid	Asteraceae	Per.
Launaea resedifolia (L.) O.Kuntze	Adeeda.	Asteraceae	Per.
Onopordum arenarium (Desf.) Pomel	Libid. Bairoff	Asteraceae	Bi.
Reichardia tingitana (L.) Roth.	Sahani.	Asteraceae	Ann.
Rhantterium suaveolens Desf.		Asteraceae	Per.
Senecio gallicus Chiax	Daraita , Mourare.	Asteraceae	Ann.
Sonchus oleraceus L.	Tefaf.	Asteraceae	Ann.
Brassica tournefortii Gouan	Al-Harra	Brassicaceae	Ann.
Capsella bursa-pastoris (L.) Medik.	Kais Al Rai	Brassicaceae	Ann.
Didesmus aegyptius (L.) Desv.	Lesless	Brassicaceae	Ann.
Diplotaxis muralis (L.) Dc.		Brassicaceae	Ann.
Enarthrocarpus clavatus Del.ex Goder.	Shultam	Brassicaceae	Ann.
Eruca sativa Mill.	Gargeer Barry	Brassicaceae	Ann.
Sinapis arvensis L.	Al-Khardal Al-Barry	Brassicaceae	Ann.
Echium angustifolium Mill.	Henna alagrab, abat elgula	Boraginaceae	Per.
Bassia muricata (L.) Asch.	Chouleta, Ghabbir	Chenopodiaceae	Ann.
Beta vulgaris L.	Selg, Selk	Chenopodiaceae	Ann.
Chenopodium murale L.	Effena	Chenopodiaceae	Ann.
Convolvulus althaeoides L.	Ullak	Convolvulaceae	Per.
Convolvulus arvensis L.	Ullak	Convolvulaceae	Per.
Astragalus asterias Stev ex Ledeb		Fabaceae	Ann.
Astragalus boeticus L.	Grambushia	Fabaceae	Ann.
Astragalus peregrinus Vahl		Fabaceae	Ann.
Hippocrepis multisiliquosa L.		Fabaceae	Ann.
Lathyrus aphaca L.		Fabaceae	Ann.
Medicago disciformis Dc.	Nafal	Fabaceae	Ann.
Medicago sativa L.	Gadb, safsafa, Berseem.	Fabaceae	Ann.
Medicago laciniata (L.) Mill.	Nafal	Fabaceae	Ann.
Melilotus indicus (L.) All	Handegog	Fabaceae	Ann.
Vicia sativa L.	Jilban.	Fabaceae	Ann.
Vicia villosa Roth	Jelbana –Hmam	Fabaceae	Ann.
Erodium cicutarium (L.) L' Herit	Dahmiyet el-ghazl.	Geraniaceae	Ann.
Erodium malacoides (L.) L' Herit		Geraniaceae	Ann.
Salvia lanigera Poir.	Sag en naga	Lamiaceae	Per.
Bellevalia mauritanica Pomel.		Liliaceae	Ann.
Malva parviflora L. var *parviflora*	Khobaiz	Malvaceae	Ann.
Malva sylvestris L.	Khobaiz, Hobbess	Malvaceae	Bi.
Orobanche schultzii Mutel.		Orobanchaceae	Ann.
Papaver hybridum L.	Bugraun, Garaun	Papaveraceae	Ann.
Papaver rhoeas L.	Bugraun	Papaveraceae	Ann.
Plantago crypsoides Boiss.	Aenm.	Plantaginaceae	Ann.
Avena fatua L.		Poaceae	Ann.
Avena sterilis L.		Poaceae	Ann.
Bromus rigidus Roth.		Poaceae	Ann.
Cutandia dichotoma (Forsk.) Trabut	Zewahn , bu ′rukba	Poaceae	Ann.
Cynodon dactylon (L.) Pers.	Najem , Najieel	Poaceae	Ann.

Hordeum murinm L.		Poaceae	Ann.
Lolium rigidum Gaud.	Bomanjor.	Poaceae	Ann.
Phalaris minor Retz.	Zewan	Poaceae	Ann.
Stipa capensis Thunb.	Behma	Poaceae	Ann.
Emex spinosus (L). Campd	Dors el-azouz & El-Enzab	Polygonaceae	Ann.
Polygonum equisetiforme Sibth. and Sm.	Gurdab	Polygonaceae	Per.
Anagallis arvensis var. *caerulea* (L.) Gouan	Ain Algatuus	Primulaceae	Ann.
Adonis dendata Delile	Zeghalil	Ranunculaceae	Ann.
Solanum nigrum L.	Anab ed. Deeb	Solanaceae	Ann.

***Abbreviations: Annual = Ann., Biennial = Bi., Perennial.**

Dicotyledons were represented by 18 families, 46 genera, 55 species whereas; Monocotyledons were represented by three families, ten genera and 12 species (Table 2). The ratio of Dicotyledons to Monocotyledons is 6: 1.

Table (2): Different taxonomic groups present in the study area.

Plant group	No. of families	No. of Genera	No. Species
Dicotyledons	18	46	55
Monocotyledons	3	10	12
Total	21	56	67

The dominant family contributing to weed flora was Asteraceae with 14 species, followed by Fabaceae with 11 species and Poaceae with 9 species. The next largest family was Brassicaceae with seven species, Apiaceae and Chenopodiaceae included three species each. The most previous studies on Libyan flora recorded that these families included the largest number of species, such as, (Ali and Jafri, 1976-1977; El-Gadi, 1988-1989; Alaib and Ihsaeen, 2008; Omar *et al*. 2020; and Ihsaeen, 2005). Five families namely, Amaryllidaceae, Convolvulaceae, Geraniaceae, Malvaceae and Polygonaceae included two species each. The remaining families were represented by one weed species each.

According to the number of species in each genus in the study area, *Astragalus* and *Medicago* were the only two genera represented by three weed species each. Seven genera, *Allium, Avena, Malva, Erodium, Convolvulus*, *Papaver* and *Vicia* were represented by two species each in the study area. The rest seventy four genera were represented by only one species each.

The study showed the majority of weed species 57 species associated with the barley crops were annuals, followed by perennials with nine species, whereas biennials represented by only two species (Figure 2).

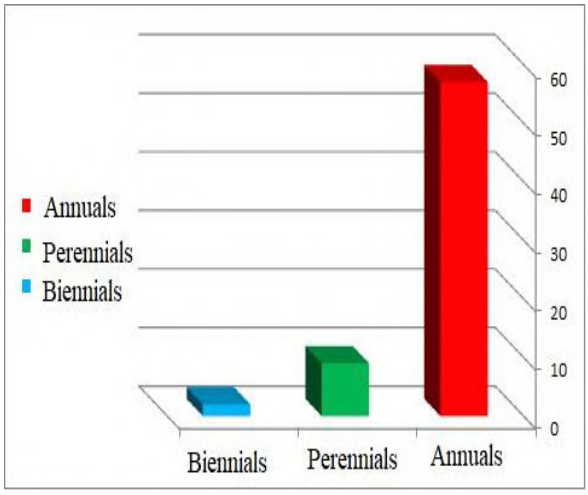

Figure (2): Life cycle of weed species in the study area**.**

The survey data showed that total 15 weed species fell into the quadrates studied. Species with a few densities were neglected (less than 0.5 plants in all quadrates). The results on weed density of a particular species are shown in Table 3. The data showed that the surveyed fields were mostly infested with *Lolium rigidum, Melilotus indicus*, *Hordeum murinm, Eruca sativa, Anthemis secundiramea*, *Vicia villosa*, *Chrysanthemum coronarium*, *Beta vulgaris, Medicago laciniata, Phalaris minor, Bromus rigidus, Malva parviflora, Avena fatua, Brassica tournefortii* and *Reichardia tingitana*. The study also showed that species of the field were a mixture of both broadleaf and grasses alike.

Table (3): Weed density (m^{-2}) and weed frequency (%) for the individual weed species of barley crop in the study area.

Species	Density (m^{-2})	Frequency (%)
Lolium rigidum	43.48	64
Melilotus indicus	30.36	84
Hordeum murinm	14.24	44
Eruca sativa	11.4	80
Anthemis secundiramea	5.12	44
Vicia villosa	3.68	52
Chrysanthemum coronarium	3.6	64
Beta vulgaris	2.36	32
Medicago laciniata	2.2	24
Phalaris minor	1.96	32
Bromus rigidus	1.92	12
Malva parviflora	1.92	44
Avena fatua	0.56	8
Brassica tournefortii	0.52	20
Reichardia tingitana	0.52	12

The weed density of dominated species (43.48 m^{-2}) was recorded for *Lolium rigidum* followed by *Melilotus indicus* with 30.36 plants m^{-2} and *Hordeum murinm* with 14.24 plants m^{-2}, whereas, the lowest weed density were calculated for *Brassica tournefortii* and *Reichardia tingitana* with 0.52 plants m^{-2} in the barley fields. In earlier study by Ihsaeen (2005) also reported that *Lolium rigidum* and *Melilotus indicus* were the highest density in barley fields in agricultural project of Sirte.

The weed frequency of weeds is the best way of indication for the prevalence of weed species in the studied area. The results of weed frequency (%) of a particular species are shown in Table 3. On the basis of the data provided the highest frequency was computed for *Melilotus indicus* with 84 % followed by *Eruca sativa* with 80 %, whereas, the lowest frequency recorded for *Avena fatua* with 8%. Most of the previous studies, such as Omar *et al.* (2020), Ihsaeen (2005) and Al-Zerbi (2004) stated that *Eruca sativa*, *Melilotus indicus*, *Lolium rigidum*, and *Bromus rigidus* have the highest frequency in different crops fields of Libya.

4 Conclusions

Among the different identified weeds *Lolium rigidum* and *Melilotus indicus* were the most problematic weed of the barley crop in Libya. This study contributed to the existing litetature by providing some knowledge about weed flora to design a solid integrated weed management plan for the different crops in the target areas.

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Supplementary material

Eruca sativa (Brassicaceae)

Calendula arvensis (Asteraceae)

Avena sterilis (Poaceae)

Melilotus indicus (Fabaceae)

Medicago disciformis (Fabaceae)

Malva parviflora (Malvaceae)

Phalaris minor (Poaceae)

Adonis dendata (Ranunculaceae)

Senecio gallicus (Asteraceae)

Achillea santolina (Asteracear)

Convolvulus althaeoides (Convolvulaceae)

Sinapis arvensis (Brassicaceae)

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A Study on the Relationship between Blood Group and Type of Cancer

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A B S T R A C T

The presence and lack of blood antigens in some blood groups induce blood membrane changes, morphologically and functionally, and it is related to some kind of disease such as cancer. Structure-dependent functions of blood types can relate the blood groups to health and diseases. The study aimed to determine the association of "ABO" and "Rhesus" blood groups with the frequency of cancer. A total of 576 cancer patients participated in this study, and they were diagnosed with different types of cancer. The results showed that blood types A and O have a high rate of occurrence of breast cancer and lung cancer, the positive Rhesus blood type has a high incidence of breast cancer. Meanwhile, the Rhesus negative blood type has a high incidence of stomach cancer. In conclusion, this study supports previous studies about the relationship between the blood group type and the probability of cancers occurring.

Introduction

Blood is the most significant body fluid, which is accountable for the circulation of nutrients, enzymes, and hormones all across the body, as well as the most critical substance, oxygen (Tesfaye *et al*., 2014; Stakisaitis, *et al*., 2018). There are two systems of blood groups classification, the Rh system, and the ABO system. In the Rh system, the blood is classified into Rh-positive and Rh-negative based on the presence or absence of inherited antigenic substances on the surface of the red blood cells. The antigens could be proteins, carbohydrates, glycoprotein, or glycolipid depending on the blood group system.

In the ABO system the blood is classified to type A, type B, type AB, and type O, which codes for glycosyltransferase that gives rise to the histo-blood group antigens of the ABO system, A and B glycosyltransferases produce A or B antigens respectively on cellular surfaces and secretions (El Jellas *et al*., 2017). Hypothesis comprises a dysregulation of the enzymatic activity of the ABO glycosyltransferases, which are specifically involved in the processes of intercellular adhesion and cellular membrane signaling, as well as in the immune response to the host. The alteration of these surface molecules may promote the process of malignancy, through a mechanism analogous to the well-known role played by the ABO glycosyltransferases in modulating the circulating plasma levels of von Willbrand factor. All of these alterations lead to consequent increased risk of venous thromboembolism, which gives the role of ABO blood types as prognostic biomarkers in different types of cancers (Franchini *et al*., 2016).

Cancer is an abnormal growth of cells that tend to proliferate in an uncontrolled way and in some cases spread, the frequency of particular cancer may depend on gender and the incidence of the most significant forms of the disease has increased.

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Excess of blood group O has been reported among patients with cancer of the breast (Newell *et al*., 1974), and blood group A was associated with increased breast cancer (Khalili *et al*., 2011). Pancreatic cancer carries an increase in risk for both blood types A and B, while blood type O confers a degree of protection (El Jellas *et al*., 2017; Wang, *et al*., 2017).

Furthermore, colon cancer is the most common gastrointestinal cancer, and it is one of the relatively few diseases with a significant association with an individual's Rh blood type (Wang *et al*., 2012). Patients with the Rh- blood group type had a more favorable stage distribution than patients with the Rh+ blood group type (Huang *et al*., 2017). In addition, gastric cancer is the second most common cause of cancer death worldwide, gastric cancer can be caused by the interaction between environmental factors and genetic variations. It has been consistently observed that blood type A is associated with an increased risk for stomach cancer and reduces the chance of survival. The presence of p53 mutations is associated with stomach cancer and blood type A, due to glucocorticoid receptors are found in high numbers on stomach cancer cells and increased risk of gastric cancer among individuals with blood group A (Urun *et al.*, 2013). On the other hand, peptic ulcer risk was instead highest among those with blood group O, that individuals with blood group O have a higher risk of peptic ulcers than those with other blood groups, blood group A is widely considered an established risk factor for gastric cancer (Edgren *et al*., 2010).

Previous studies suggest a possible association between the ABO blood group and the risk of certain malignancies including an increased risk of ovarian cancer for blood group A versus O. Furthermore, B antigen was positively associated with ovarian cancer incidence, while blood group A was not associated with risk (Kashfi *et al*., 2018). Blood type O was a risk factor for decreased ovarian reserve whereas the presence of the A antigen (blood types A or AB) was protective for ovarian reserve, women with blood type O and the presence of the B antigen (blood types B or AB) was a risk factor for decreased ovarian reserve (Edgren *et al*., 2010).

1 Materials and Methods

Blood samples were collected from 576 patients (inside the tumor hospital) and directly stored in a blood bank inside the hospital that determine the blood type for all samples.

Statistical analysis:

The data gathered were sorted, classified, tabulated, analyzed. Frequency distribution was used as a statistical tool and utilized used Microsoft Excel in processing the data, and SPSS software. The significance level was tested using a t-test A p-value <0.05 was considered Statistically Significant. The analysis of variance was also run to detect the significant influence of the type of cancer on the parameters of complete blood count.

2 Results

Our study in a Libyan hospital processed about 502 cases (60%) were females and (40%) males during the year of 2017and 77 cases (51%) were male and (49%) were female during the year 2018.

In terms of age groups identification, most of the cancer cases in age groups between 31 to70 years (77.7%), while lower cases in age groups were more than 70 years (17.7%) and less than 31 years (4.6%).

According to cancer types, this study was comprised of 502 and 77 cancer patients in 2017 and 2018, respectively, with 300 females and 202 males in the year of 2017 and 38 females and 39 males for the year of 2018. When all cancers were taken together, the highest cases is breast cancer (37%), followed by colon cancer (19%), and lung cancer (16%). The other types were considerably lower than those above, these are thyroid and uterus cancer (8%), followed by brain cancer (4%), stomach cancer (3%) liver and pancreas (2%), and the least noted cases are leukemia (1%).

The results of the year 2017, in assessing the different types of cancers according to blood group, the high incidence among patients of blood group A^+(60%), followed by those with blood group O^+(29%), while the lowest percentage in the other blood groups. Furthermore, the patients of blood group B^+ were (0.03%) and the patients of both blood groups AB^+ and O^+ were 0.01% collectively. In addition to, the results showed that lung cancer with the highest rate among patients of blood group A^+(58%) and the blood group O^+ (36%), while the rate among patients of the other blood groups O^-, B^+ and $A^-$was 13%. Colon cancer was highest among cases of the blood group A^+ (50%) and followed among the patients with the blood group O^+ cases (35%) and other the blood groups O^-, AB^+, B^+, A^-, collectively was 13%, meanwhile, the pancreatic cancer was associated with the patients of the blood group O^+ (75%), then those with blood group A^+ (25%). The results also showed that 50% of cases of uterine cancer were among patients of the blood group O^+ and 46% were among those with blood group A and 4% among those with the blood group A^+. The blood group A^+ was associated with thyroid cancer in 53% of the cases, 27% of which were from the blood group O^+ and 2% of the cases of the disease of B^+. Brain cancer was most common among patients of the group of blood O^+(36%),

followed by those with blood group A^+(31%), then those with blood group B^+(22%), while those with AB^+ blood group had the least rate (4%). Gastric cancer was most common in cases with blood group B^-(54%), blood group AB^+(26%), and collectively with blood groups O^+ and O^-(14%). Liver cancer was found among patients with blood group O^+ only (100%). Leukemia had the highest rate among the blood group A^+ patients (75%) and blood group O^+ patients (25%).

The results of 2018 showed that the most common occurrence of breast cancer was among the patients of blood group A^+(42%), followed by patients of blood group O^+ (38%), then those patients with blood group B^+ and AB (12% and 8%, respectively). In the cases of colorectal cancer, the patients of blood group A^+ were found with the highest rate (80%), then those with blood group O^+ (20%). Among lung cancer, the blood group A^+ patients have recorded the greatest rate (50%), then those with blood group O^+ (39%). The proportion of blood group A^+ patients among stomach cancer was (25%), while the proportion of patients with blood group B^- was (75%). Meanwhile the prostate cancer incidence was equal between the patient of the blood groups A^+ and B^+ 50% each.

Complete Blood Count (CBC) Test results

The analysis of variance revealed that the type of cancer was insignificantly (P>0.05) affected the amount of WBCs, HGB, RBCs and PLT, while the means separation showed that the prostate cancer patients had significantly higher HGB (10.33) than other cancer patients. On the other hand, the overall means of PLT, HGB, RBC, and WBC were found to be 223.58, 11.00, 3.98, and 6.78, respectively (Table 1).

Table (1). The complete Blood Count (CBC) parameters among cancer patients during year, 2018

Cancer type	No	PLT	HGB	RBC 10^6	WBC 10^3
Lung	18	215.78[a] ±120.89	10.68[a]± 2.34	3.77[a] ±0.82	6.93[a]± 3.66
Colon	15	219.07[a] ±58.21	11.26[a]± 1.79	3.99[a] ±1.01	7.89[a]± 3.68
Breast	24	236.29[a] ±121.48	11.02[a]± 2.41	4.00[a] ±0.56	6.88[a]± 2.64
Pancreas	5	275.80[a] ±80.40	10.54[a]± 0.60	4.31[a] ±0.99	5.54[a]± 1.92
Stomach	4	216.25[a] ±37.98	10.48[a]± 0.19	4.10[a] ±0.89	5.18[a]± 0.81
Prostate	3	150.00[a] ±111.32	14.33[b]± 0.90	4.53[a] ±0.93	6.30[a]± 1.15
Ovary	3	232.00[a] ±51.29	10.33[a]± 1.19	3.59[a] ±0.55	5.37[a]± 2.31
Cervix	2	157.00[a] ±26.87	9.95[a]±0. 64	4.23[a] ±1.51	5.10[a]± 1.41
Overall	74	223.58± 100.77	11.00±2 .09	3.98± 0.80	6.78±3 .00
Sign. Level		**NS**	**NS**	**NS**	**NS**

NS: Insignificant at P>0.05, a, b: Means with the same superscript were insignificantly different (P<0.05)

3 Discussion

Although, previous studies showed that many factors related to the occurrence of cancer, including external factors and inside body factors, still some doubt in the internal factors and their relationship to cancer and the type of cancer. In general, the results 2017 of this study suggested that there was a strong relationship between the blood group O^+ and most types of cancer, as well as a strong relationship between blood group A^+ and three types of cancers, namely colon, breast, and lung cancer. While medium relationship between blood group B^- with stomach cancer and blood group B^+ with breast cancer and prostate cancer and blood group AB^+ with breast cancer and blood group A^+ with lung cancer. It was noted that a weak relationship between B^+ blood type and prostate cancer, brain and uterine cancer, as well as a weak association with ovarian cancer and uterine cancer with the O^+ group, and also a weak association between AB^+ blood group with thyroid cancer and A^+ blood group with gastric cancer, prostate cancer, and ovarian cancer, A^- blood group with lung cancer.

According to the results of the present study, rate females are affected with cancers more than males. Another study shows similar results and that female have a high population than males, so this difference shows that men are more susceptible to colon cancer than women; most colon cancer cases in this study were married (72.2%) which can be related to problems that expose the married group to stresses and make them susceptible to many diseases including cancers (Ba *et al.*, 2017). The results of this study showed that colon cancer as the third type of cancer, the ratio is (21%) at blood group A^+ (80%) and O^+(20%) of analyses, showed relationship blood groups and different types of cancer, there was no statistical significance P>0.05, so no significant relationship between blood group and colon cancer. The highest frequency of colon cancer was observed in cases with blood group O^+ (47.8%) and B^+ (26.9%) (Kashfi *et al*. 2018).

Compared to the incidence in the general population, the analyses showed a relationship between blood group and colon cancer, the most patients with colon cancer (90.4%) had a positive Rh, which is almost in line with the Rh-positive frequency in the general population (92.3%). Although, the results of the study showed no important relationship between Rh and colon cancer ($P > 0.05$) (Sarafian *et al*., 1993a).

According to many studies, one of the genetic factors is blood group, the results of this study showed a higher frequency for cancer cases in blood group O^+ than in other groups and an important relationship between colon cancer and blood group of subjects (Sandlerand Mallory, 1995; Dabelsteen, 2002; Cooper, 2000).

The results of this study showed that the highest proportion of cancers in breast cancer spreading is a high rate in Libya and more exposed females (33%). The results of the year 2017 breast cancer is a high rate in blood group A^+ with rate of 60% and O^+ with rate of 29%, while the rate in blood groups B^+ is 3%, in blood groups $B^-$is 5%,$AB^+$is 1% and blood group O^- is 2%.

Furthermore, some previous studies have reported significant associations between the ABO blood group or Rh factor and breast cancer risk, overall the literature is inconsistent. The majority of the larger studies published to date observed no association with Rh factor and/or ABO blood group, while some studies tended to report significant associations (Lu *et al*., 2011; Hallouin *et al*., 1997; Sarafian *et al*. 1993b; Iwamoto *et al*., 1999). Blood type reported positive associations between type A and risk of breast carcinoma (Hallouin *et al*., 1997; Sarafian *et al*. 1993b). However, the other study reported a positive association between type O and breast cancer risk (Ba *et al*., 2017). the other hand, 48% of the patients with HELLP syndrome had a negative blood type O and Rh and they were at a greater risk for the syndrome (Cooper, 2000).

Blood group B has a correlation with heart disease in males and cancer in the upper third of the esophagus (Dean, 2005). Blood group A was more common in patients with pancreatic cancer and the blood group seems to be a protective agent against pancreatic cancer (Dabelsteen and Gao, 2005). As well as people with blood group O had higher survival compared to people with other blood groups.

Compared to other blood groups, the frequency of blood group A was higher in women with ovarian cancer. In addition, the relationship between ABO blood groups and the main risk factors for cardiovascular disease in the general population of Golestan province (Chihara *et al*., 2005), it was shown that, blood group O has the highest frequency and blood group A has a more family history of heart disease compared to other blood groups.

4 Conclusions

Cancer risks vary in people with different ABO blood types, with higher risks of breast cancer, lung cancer, and colon cancer associated with blood type A+ and pancreatic cancer associated with non-O blood types A^+,B^+ and AB^+, while the stomach cancer with blood type B^-. In conclusion of this study, there is a relationship between some blood groups and the type of cancer but is not clear that it is likely to increase the other surrounding factors of this relationship or reduce the strength of the link between the two variables and this depends on internal genetic and physiological changes.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Study of Principal Component Analysis (PCA) as a Face Recognition Method

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A B S T R A C T

Face recognition is a biometric technique that can be used for a variety of purposes, such as national security, access control, identity fraud, banking, and finding missing children. Faces are highly dynamic and facial features are not always easily extracted, which can lead to discarding textural information like the smoothness of faces, a hairstyle that, might contain strong identity information. In addition, brightness, scale, and facial expressions play a significant role in the face-recognizing process. Therefore, face recognition is considered as a difficult problem. To figure out this problem effective methods using databases techniques are needed. This paper describes face recognition methods and their structure. Based on Wen Yi Zhao and Rama Chellappa work the face recognition methods are divided into three groups: a holistic approach, feature-based approach, and hybrid approach, where Principal Component Analysis PCA, a holistic approach method, is presented as a mathematical technique that can assist the process of face recognition. Also, the paper shows how the PCA is used to extract facial features by removing the principal components of the available multidimensional data.

Introduction

Recently, face recognition has become a popular field of inquiry in computer vision, where a great deal of research is moving at this focal point to increase the optical ability of computing. Iris, face, fingerprints, and DNA are various human characteristics that are used in biometric systems. Face recognition can be a suitable alternative compared to other biometric techniques.

There are many approaches for face recognition in this field. Many researchers proposed algorithms to identify and recognize human beings' faces from a given database. The recent development in this field has facilitated the fast-processing capacity and high accuracy(Malakar et al., 2021)

The challenge facing of Face Recognition, is how to perform very well under hard conditions. For example, a personal variation processing system might need to process a low-quality face image which image, which has been acquired using a low-quality Personal computer camera and transferred over an IP address, or the image was captured in an uncontrolled environment with bad lighting.

In this paper, one of the most popular methods for face recognition will be presented, Principal Component Analysis (PCA), Facial recognition methods can be divided into three big groups, holistic approach, feature-based approach, and hybrid approach (Bansal et al., 2012) (Delac et al., 2005). These are described below:

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- Holistic approach or appearance-based approach (Bansal et al., 2012): which uses holistic texture features and is applied to either whole-face or specific regions in a face image uses (Delac et al., 2005). Where, each face is handled as a two-dimensional array of intensity values, which is compared to the intensity values of other facial arrays.
- Feature-based approach (A. Bansal et al., 2012): face verification is needed, which tries to verify a local feature on the face such as the nose and eyes from a given sample of that face and extracts descriptive information about them such as their widths and heights.
- Hybrid approach (Mwendwa, 2016): both face identification and face verification are used as the input to the face detection system. It can be similar to the human being's behavior to recognize the face (Mwendwa, 2016).

1 Methods of Face Recognition

To extract and recognize the human face, a variety of algorithms are used; each technique is unique, different in performance, accuracy, and effectiveness (Kumar &Kaur, 2012.) (Saini et al., 2014.)(Çarıkçı & Özen, 2012). The following are the most popular face recognition methods:

1. Principal Component Analysis (PCA).
2. The Hidden Markov method.
3. Linear discriminate analysis (LDA).
4. Neural Network method
5. Geometrical Feature Matching
6. Elastic Bunch Graph Matching (EBGM).
7. Independent Component Analysis (ICA).
8. Fisherfaces.

Most of these existing recognition methods are focused on the color-image-based (2D) appearance of faces and discard their video (3D). This leads to a poor discrimination power when dealing with variations such as illumination and makeup. Figure 1 shows the structure of general face recognition system.(Malakar et al., 2021)

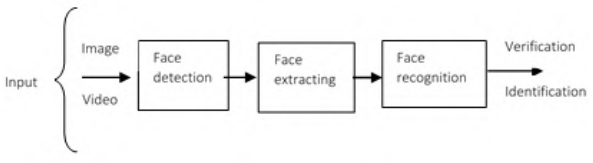

Figure (1). Structure of general face recognition system.

1.1 Principal Component Analysis Method (PCA).

PCA is also known as Karhunen-Loeve method or eigenface method. Recognition of human faces using the PCA was first done by Turk and Pentland (Batra&Goyal,2015), and many followed Kiry and Sirovich (Thakur et al., 2008). Principal Component Analysis (PCA) is a statistical method that has been used in image recognition. When there is a high correlation between observed variables, the PCA can be utilized as feature extraction in face recognition to reduce the big dimensionality of data space to lower dimensionality of feature space. The PCA is used for prediction, redundancy removal, feature extraction, and data compression (Tamimi et al., 2015). When dealing with large collections of data samples, it is a popular strategy in signal processing to lower the dimensionality of the image. By means of removing information that is not useful, and specifically decomposes the structure of the face into components, which are uncorrelated and are known as Eigenfaces (Turk & Pentland, 1991). PCA is a linear transformation that converts the data to a new coordinate system. These basis vectors represent eigenvectors of the covariance matrix of the data samples, and the coefficients for each data sample are the weights, or principal components of that data (the United States Patent Corcoran) Unlike other linear transformations, such as discrete cosine transforms (DCT) (Strang, 1999). Its basis vectors depend on the dataset. PCA can be used for reducing dimensionality in a dataset while retaining those characteristics of the dataset, that contribute most to its variance, by holding lower-order principal components and ignoring higher-order ones. Why the PCA? The principal component analysis (PCA) can be considered as one of the most important face recognition methods in the literature (Tamimi et al., 2015). Also, PCA is a very popular method. In Figure 2 below, the number of articles that have utilized the words facial recognition and PCA in their headers is shown. (Karamizadeh et al., 2013). Furthermore, the image size is unimportant for the PCA (Saini et al., 2014.).

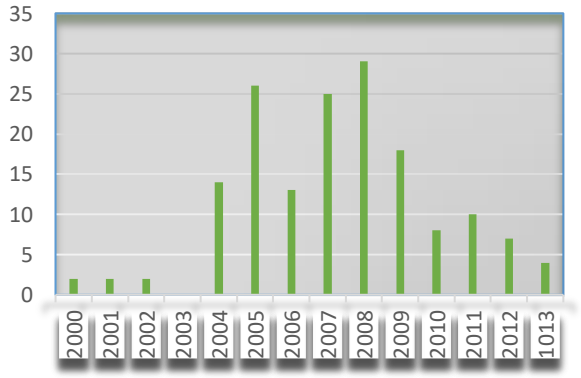

Figure (2). The number of publications utilized.

1.2 The PCA Methodology.

PCA is a mathematical technique, which acts as a dimensionality reduction technique by removing the principal components of the multidimensional data. The first principal component is the linear combination of the original dimensions, which has the highest variability. A s-dimensional vector, which is the representation of the weighted sum (feature vector) of the eigenfaces, could be used to represent each image of a face. In this method, a full front view of the face is required, otherwise, the output of recognition will not be accurate. The major benefit of this method is that it can decrease the data required to recognize the entity to 1/1000th of the existing data (Bansal & Chawla, 2013). First, all images of known faces are projected onto the face space to find sets of weights, that describe the contribution of each vector, to identify an unknown image. Then, that image is projected onto the face space as well to obtain its set of weights, by comparing a set of weights for the unknown face to sets of weights of known faces (Delac et al., 2005). Finally, the unknown face can be recognized. Figure 3 below shows the flow chart of the PCA methodology.

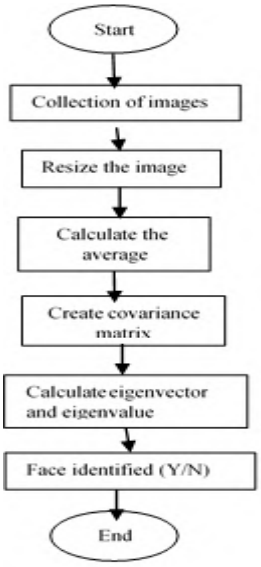

Figure (3). Flow chart of PCA methodology.

1.3 The PCA Matimatical Present.

PCA is a mathematical approach for reducing dimensionality in multi-dimensional data by extracting the primary components. The first principal component is the linear combination of the original dimensions that has the highest variability (Laltanpuia, 2018).

1. First, images with a two-dimensional N*N array must be trained. An image can be considered the basic vector of dimension N*N. Resize the image, so that a typical image of size 112x92 becomes a vector of dimension 10304 (Bansal & Chawla, 2013). If the training set of images {X1, X2, X3… XN} (Vyanza et al., 2017). The average face of the set is defined by equation (1) (Batra&Goyal, 2015):

$$\overline{X}=1\backslash N \sum_{i=1}^{n} Xi \quad (1)$$

2. Resize the images (Javed, 2013).

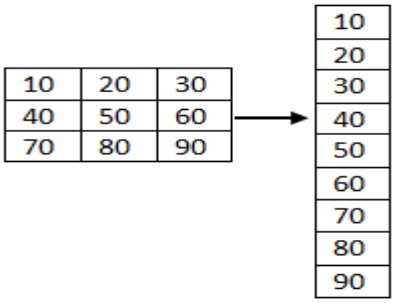

Figure 4. Resize the images

Where N is the number of sample images x is the average of the image, M is the number of images, I is the image vector.

3. The average face is calculated and subtracted from each face in the training set As given in equation (2) (Borade et al., 2016.).

$$\phi=\overline{Xi} - Xi \ , i=1,2,3,4,\ldots,N \quad (2)$$

The estimated covariance matrix (D) will be created using the results of the subtraction operation As given in equation (3) (Saha et al., 2014.) (Ejaz et al., 2019).

$$D=A^tA \quad (3)$$

Then a new matrix (B) is formed as B= [ϕ1, ϕ2, ϕ3... ϕn] (Batra&Goyal, 2015) (Abdullah, 2012).

4. The eigenvector and eigenvalue are calculated using the estimated covariance matrix. For the N-dimensional vector, there will be N eigenvalues and eigenvectors (Singh et al., 2003).
5. Finally, the eigenvalue is sorted out from high to low, then the first N eigenvectors that have large variances are chosen and removed the ones with low variance, so that could reduce the dimensionality. The eigenvector with the highest eigenvalue is the principal component of the dataset (Tamimi et al., 2015).
6. The unknown image will be compared with the training image in eigenspace and identify where the face recognition or not (Stephen et al., 2005) (Raut&Patil, 2012).

2 Principal Component Analysis' Benefits (PCA)

- Data compression is done using the low-dimensional subspace representation (Kumar &Kaur, 2012.).
- Intensity data are used directly for studying and recognizing without any type of level processing (Ameen et al., 2017) (Bansal & Chawla, 2013).
- It is very useful when you deal with a large number of variables (Deshpande & Ravishankar, 2017).
- Compared to other approaches recognition with the PCA method is simple and efficient (Kumar & Kaur, 2012.).
- Does not require any advanced knowledge of geometry or reflectance of the face (Phillips et al., 2005).
- The location and size of each face image remain similar (Singh et al., 2003).
- The method is powerful when dealing with expressions and glasses (these experiments were made only with frontal views) based on (Sharif et al., 2017).

3 Principal Component Analysis' Drawbacks (PCA).

- The size and location of each face image must remain similar (Singh et al., 2003).
- The approach is not robust when there is an extreme change in the pose as well as in the expression, disguise, and illumination.
- As it is an appearance-based method (A. Bansal et al., 2012), it is difficult to update the face databases. Therefore, learning is very time-consuming, which makes the covariance matrix difficult to solve.
- The method is very sensitive to scale (Kumar&Sehgal, 2016.).

4 Conclusions

The primary goal of this paper is to represent and review one of the popular face recognition techniques, Principal Component Analysis (PCA). The face recognition methods are derived into three groups. The paper presented the PCA functionality, method, and methodology, and reviewed its advantages and disadvantages. PCA can be used with a large dataset and it has been represented as a mathematical technique. Also, PCA is an essential approach that can be used to get clear face recognition. This is due to its technique where it passes on components where each face can be analyzed.

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Comparison between Alterations in some Hematological and Biochemical Parameters in COVID-19 and non-COVID-19 Patients Albaida, Libya

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A B S T R A C T

The COVID-19 outbreak has resulted in an unexpected health crisis all around the world. It was on March 11, 2020, when the WHO declared it as a pandemic. Ever since an exponential increase in the disease has been seen. The coronavirus disease 2019 or COVID-19 is a beta-coronavirus that has been closely related to the SARS-CoV-2 by means of genetic sequencing methods. This disease is associated with alterations in the complete blood picture and coagulopathies. In this present study, the role of hematological and biochemical parameters of blood in predicting COVID-19 infection w investigated, as these blood analyzes are considered a vital indicator for confirmation of infection. The present work was designed to diagnose COVID- 19 with five basic blood tests: white blood cells (WBC), lymphocytes (LYM), neutrophils (NEUT), platelets (PLT), and clotting factor D–dimer as well as the diagnosis of COVID-19 associated tests. Biochemical blood C-reactive protein (CRP), serum ferritin (FRR), and Lactic dehydrogenase (LDH). It was noted that the hematological and biochemical blood tests included in this study were capable of diagnosing COVID-19, due to their increase or decrease in the normal rates, according to what was shown in the results. N The diagnosis of COVID-19 using blood and blood biochemical tests in people who show some mild clinical symptoms, high and low levels of these parameters have been closely associated with COVID-19 infection and may also serve as biomarkers to predict infection.

1 Introduction

In December 2019, cases of pneumonia of unknown origin were discovered in Wuhan, the capital of Hubei province, China (Zhu *et al.*, 2020). The causative agent was named severe acute respiratory syndrome coronavirus (SARSCoV-2) (Gorbalenya *et al.*, 2020). The disease was named coronavirus disease (COVID-19). SARSCoV-2 is a highly contagious and moderately virulent virus, which was discovered soon after (Sanche *et al.*, 2020). SARS-CoV-2 spread around the world in the months after, and the World Health Organization (WHO) declared COVID-19 a pandemic on March 11, 2020 (World Health Organization, 2020). The COVID-19 diagnosis is crucial (Salathé *et al.*, 2020). The detection of RNA by reverse transcription polymerase chain reaction (RT-PCR) after upper respiratory tract sampling is used to diagnose COVID- 19) Although RT-PCR is critical in the event of a pandemic, it is a difficult test that requires large and sensitive infrastructure (Loeffelholz & Tang, 2020) Furthermore, even in completely symptomatic SARS- CoV-2 patients, the test is not always positive.

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The test's accuracy was only 30% to 60% (Li *et al*., 2020). In addition, there is a huge demand for RT-PCR testing, which is a barrier to managing the epidemic (Ai *et al*., 2020). In symptomatic COVID-19 illness cases (Lippi *et al*., 2020).

Recently, it has been reported that hematological and inflammatory parameters based on blood cell analysis have important value for predicting infection and susceptibility to many other diseases (Naess *et al*., 2017). High white blood cell count, neutrophil count, and low lymphocyte count are among the most relevant of these indications (Lagunas‐Rangel, 2020). There are simple basic criteria proposed for direct differentiation of COVID-19 patients (Ruan *et al*., 2020). T cells are vital in antiviral immunity. However, the reasons that promote T cell activation and reduction in COVID-19 patients are yet unknown (World Health Organization, 2020). Increased prothrombin time and D-dimer readings may also be markers of COVID-19 diagnosis (Azab *et al*., 2010), which is explained by COVID-19 patients' disordered coagulopathy. Inflammation-related biomarkers such as C-reactive protein (CRP) (Guan *et al*., 2020), serum iron and lactic dehydrogenase (LDH) appear to be useful prognostic indicators for determining whether COVID-19 cases are mild or severe (Assandri *et al*., 2020).

In this study, the role of hematological and biochemical blood parameters in predicting COVID-19 infection was investigated, as these blood tests are regarded a critical indicator for infection confirmation.

2 Materials and Methods

2.1 Sample Collection

The total number of patients was 75, and their blood samples were randomly collected from the laboratories of the Libyan city of Al-Bayda and were divided into two groups according to the COVID-19 antigen test. Biochemical and hematological examinations were performed on both groups.

2.2 Determination of Hematological Parameters

The hematological parameters, including white blood cell count (WBCs), lymphocyte (LYM), neutrophil (NEUT) and platelets (PLT) were estimated by Particle Counter (Sysmex, Europe. Model XP-300).

2.3 Determination of Coagulation

Determination of D-Dimer in human in whole blood\ plasma was determined according to the method of Bounameaux *et al*., (1994).

2.4 Determination of Biochemical Parameters

C-reactive protein (CRP) was assayed by the method of Shute and Maryon, (1966). Where, Lactate dehydrogenase (LDH) was measured as reported by Hochachka and Somero, (2002) method. While ferritin (FRR) of human in serum\ plasma was assayed by the method of Addison *et al*. (1972).

2.5 Statistical Analysis

Continuous variables were presented as mean ± standard deviation, median, or interquartile ranges based on the distribution of the data. Student t-test (0.01) is used for testing differences between the two study groups when relevant.

3 Results

The total number of samples included in this study is 75 samples. The results showed that 50 of these samples were from people infected with COVID-19, while 25 samples were from healthy people. Table (1) showed a significant ($P \leq 0.01$) increase in white blood cell count, neutrophils, D-dimer, C-reactive protein (CRP) levels, serum ferritin and lactic dehydrogenase (LDH) compared with normal for these parameters. However, there is a significant decrease ($P \leq 0.01$) in the number of lymphocytes and platelets compared to normal and healthy subjects.

Table (1). Hematological and biochemical parameters of blood for the diagnosis of patients with COVID-19 and non-patients COVID-19 compared to normal rates.

Paameter	Normal Value	Positive patients with COVID-19 N=50	*Negative patients with* COVID-19 *N*=25	*t*-Value	*p*-Value
WBC 10^3/ µL)	5-10	9.97±4.01	8.172±07	3.06	0.003
LYM (%)	20-40	18.59±8.60	27.06±6.27	-4.85	0.000
NEUT (%)	55-70	72.97±8.26	58.04±8.65	7.15	0.000
PLT 10^3/µL)	150-400	143±33.2	236±21.0	-14.69	0.000
D-Dimer (m cg/ml)	>4.0	1.03± 1.70	0.280± 0.07	3.11	0.003
CRP (mg/l)	Up to5.0	20.0 ±22.6	3.46 ±1.16	5.15	0.000
FRR (ng/ml)	13.0-40	299±218	93.4±21.4	6.60	0.000
LDH (U/L)	225-450	495± 121	284.8±24.0	11. 87	0.000

4 Discussion

Coronavirus disease 2019 is a dangerous illness caused by the Coronavirus 2 (SARS-CoV-2) of the severe acute respiratory syndrome (Huang *et al*., 2020). Respiratory distress syndrome is caused by a variety of processes in COVID-19 instances, including the activation of white blood cells, neutrophils (Ichikawa *et al*., 2013). The findings of routine blood and blood biochemistry analysis are presented in this study. The most important parameters in discriminating between COVID-19 patients and healthy people were coagulation activity, serum ferritin, and lactate dehydrogenase (Channappanavar & Perlman, 2017).

In this study, the results of those with COVID-19 showed that the number neutrophil significantly increase, whereas the levels of lymphocyte and platelet decreased in comparison to those who are not affected (Wu *et al*., 2020). It is then proposed as a suitable and potentially diagnostic marker for COVID-19. This is consistent with previous study (Wang *et al*., 2020).

The obtained results showed that, most investigations concentrating on abnormalities of immune inflammatory parameters in COVID-19 patients and documenting greater numbers of neutrophil in patients (Bo *et al*., 2020, Huang *et al*., 2020) this agreed with our findings. COVID-19 patients are more susceptible to bacterial and fungal infections as the disease progresses, due to diminished autoimmunity and greater neutrophil count, which represent higher levels of inflammation than mild patients (Li *et al*., 2020).

The rise in the number of neutrophil could be linked to viral invasion-induced cellular storms (Wang *et al*., 2020). Associated with decreased lymphocyte in COVID-19

Furthermore, the reasons for the platelet decrease in COVID-19 patients could be due to the followings: (1) Viral infection resulted in endothelial injury, platelet aggregation and pulmonary thrombogenesis (Yang *et al*., 2003). Megakaryocyte reduction, resulting in decreased platelet production and increased consumption; (2) the coronavirus directly invades hematopoietic cells or bone marrow stromal cells, inhibiting hematopoiesis (Eickmann *et al*., 2020). In addition, higher D-dimer levels have a strong link to the severity of COVID-19 infection. COVID-19 patients with acute respiratory distress syndrome will be treated according to our findings. Acute pulmonary inflammatory reaction may be linked to abnormal coagulation. Injuries to the lungs lining systemic endothelial injury with diffuse thrombosis of the small vessels or large veins coagulopathy with increased thrombi activity (Katneni *et al*., 2020). Deposits associated with inflammation within the alveolus Fibrin, systemic endothelial injury with diffuse thrombosis of the small vessels or large veins (Roncon *et al*., 2020) have coagulopathy with increased thrombi activity.

COVID-19 patients had higher levels of LDH, ferritin, and CRP, In addition to other cytokines, according to a previous study (García, 2020). Evidence suggests that ferritin can modulate the immune response by inducing anti- inflammatory cytokines and limiting free radical damage in the presence of chronic inflammation. Alternatively, new research reveals that ferritin may have a role in the inflammatory pathophysiology of illness (Kernan & Carcillo, 2017). In response to elevated levels of inflammatory cytokines, particularly IL-6, transcriptional activation of the CRP gene occurs mostly in hepatocytes. CRP, like ferritin, appears to be a crucial regulator of inflammatory processes rather than just a marker (Sproston & Ashworth, 2018). Additionally, Huang *et al*. (2020) and Wang *et al*. (2020), infections that produce lung tissue damage mediated by cytokine and LDH enzyme production had a significantly higher level of LDH (Martinez-Outschoorn *et al*., 2011). LDH-3 (LDH-3) is a coenzyme that is found in lung tissue.

5 Conclusions

Because high and low levels of hematological and biochemical parameters have been closely associated with COVID-19 as well as serving as biomarkers to predict infection, this pilot study highlights the potentials of the diagnosis of COVID-19 using blood and blood biochemistry tests in people who show some mild clinical symptoms.

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Using Anuj Transform to Solve Ordinary Differential Equations with Variable Coefficients

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A B S T R A C T

In this paper, a new integral transform was applied to find the solution of ordinary differential equations with variable coefficients because of its importance in various fields of science, especially mathematics. This integral transform is called Anuj transform. The Anuj transform is one of the modern integral transforms that is effective in solving ordinary differential equations in general and ordinary differential equations with variable coefficients in particular. In order to achieve this; and based on a set of relations that have been included in this paper, we have implemented a number of methods to reach important formulas and results. These results have been applied to different set of problems of ordinary differential equations with variable coefficients. The solutions of those problems were reached in a smooth manner, based on some basics of ordinary differential equations which give a simple and clear idea of using the Anuj transform to solve ordinary differential equations with variable coefficients.

1 Introduction

Ordinary differential equations (ODE) are one of the most important areas of mathematics science in general, applied mathematics in particular. Ordinary differential equations have many types and one of them, not limited to, is ordinary differential equations with variable coefficients. These days, ordinary differential equations with variable coefficients are widely used in astronomy, physics and engineering mathematics (Aggarwal et al., 2018). Occasionally, to solve like these equations, the calculations may be very complicated and ultimately frustrating. Integral transforms play a big role in solving such equations (M. & Mahgoub, 2019, 2017 & 2016), (Elzaki et al., 2012). Many papers relevant have been recently published carried out to solve ordinary differential equations with variable coefficients using integral transforms, for instance, Sumudu transform, Kamal transform and Mohand transform, to name a few. In general, integral transforms techniques are not useful for most problems. However, integral transforms have become an important tool to deal with problems in applied mathematics, theoretical mechanics, statistics, mathematical physics and pharmacokinetics (M. & Mahgoub, 2017), (Devi et al., 2017). "Integral transform method is widely used to solve the several differential equations with the initial values or boundary conditions" (Devi et al., 2017). The great importance of such transforms appears in solving this type of ordinary differential equations without much complexity. Latterly, a new transform has appeared which named Anuj transform. The definition of Anuj transform as mentioned by Kumar et al. (2021) of a piecewise continuous exponential order function $\psi(t); t > 0$ is defined as the following integral equation:

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$$\Lambda\{\psi(t)\} = \nu^2 \int_0^\infty \psi(t) e^{-\frac{t}{\nu}} dt = \Psi(\nu)\,, \nu > 0 \qquad (\mathbf{1})$$

where ν is real parameter and Λ is the Anuj transform operator, This paper will deduce Anuj transform formulas for some functions such as $t\psi(t), t^2\psi(t)$ & $t\psi'(t)$, which enable us to extract any formulas of these kinds.

Therefore, the purpose of this paper will show the applicability and efficiency of Anuj transform to solve some ordinary differential equations with variable coefficients.

1 Anuj Transform and Inverse Anuj Transform of Some Functions

The following Anuj transform and inverse Anuj transform of some functions are summarized in tables (1) & (2) (Kumar et al., 2021). Furthermore, these formulas are adapted from the same reference.

Table (1). Anuj transform of some functions.

S. No.	$\psi(t)$	$\Lambda\{\psi(t)\} = \Psi(\nu)$
1	1	ν^3
2	t	ν^4
3	t^2	$2!\,\nu^5$
4	$t^m\,; m \in \mathbb{N}$	$m!\,\nu^{m+3}$
5	$t^m\,;$ $m > -1$	$\Gamma(m+1)\nu^{m+3}$
6	e^{at}	$\frac{\nu^3}{1-a\nu}$
7	$\sin\beta t$	$\frac{\beta\nu^4}{1+\beta^2\nu^2}$
8	$\cos\beta t$	$\frac{\nu^3}{1+\beta^2\nu^2}$
9	$\sinh\beta t$	$\frac{\beta\nu^4}{1-\beta^2\nu^2}$
10	$\cosh\beta t$	$\frac{\nu^3}{1-\beta^2\nu^2}$
11	$e^{at}\psi(t)$	$(1-ap)^2\Psi\left(\frac{\nu}{1-a\nu}\right)$

Table (2). Inverse Anuj transform of some functions.

S. No.	$\Psi(\nu)$	$\psi(t) = \Lambda^{-1}\{\Psi(\nu)\}$
1	ν^3	1
2	ν^4	t
3	ν^5	$\frac{t^2}{2!}$
4	$\nu^m\,;\, m \in \mathbb{N}$	$\frac{t^{m-3}}{(m-3)!}$
5	$\nu^{m+3};$ $m > -1$	$\frac{t^m}{\Gamma(m+1)}$
6	$\frac{\nu^3}{1-a\nu}$	e^{at}
7	$\frac{\beta\nu^4}{1+\beta^2\nu^2}$	$\frac{\sin\beta t}{\beta}$
8	$\frac{\nu^3}{1+\beta^2\nu^2}$	$\cos\beta t$
9	$\frac{\nu^4}{1-\beta^2\nu^2}$	$\frac{\sinh\beta t}{\beta}$
10	$\frac{\nu^3}{1-\beta^2\nu^2}$	$\cosh\beta t$

2 Anuj Transform of Derivatives of Some Functions (Kumar et al., 2021)

$$\Lambda\{\psi'(t)\} = \frac{1}{\nu}\Psi(\nu) - \nu^2\psi(0) \qquad (\mathbf{2})$$

$$\Lambda\{\psi''(t)\} = \frac{1}{\nu^2}\Psi(\nu) - \nu\psi(0) - \nu^2\frac{d}{d\nu}\psi(0) \qquad (\mathbf{3})$$

$$\Lambda\{\psi'''(t)\} = \frac{1}{\nu^3}\Psi(\nu) - \psi(0) - \nu\frac{d}{d\nu}\psi(0) - \nu^2\frac{d^2}{d\nu^2}\psi(0) \quad (\mathbf{4})$$

3 Anuj Transform of $t\psi(t)$ & $t^2\psi(t)$:

If $\boldsymbol{\Lambda\{\psi(t)\} = \Psi(\nu)}$, then:

i. $\Lambda\{t\psi(t)\} = \nu^2\frac{d}{d\nu}\Psi(\nu) - 2\nu\Psi(\nu)$.

Proof. Since, $\Lambda\{\psi(t)\} = \nu^2\int_0^\infty \psi(t)e^{-\frac{t}{\nu}}dt = \Psi(\nu)$

$$\therefore \frac{d}{d\nu}\Psi(\nu) = \frac{d}{d\nu}\left(\nu^2\int_0^\infty \psi(t)e^{-\frac{t}{\nu}}dt\right)$$

$$= \nu^2\int_0^\infty \frac{t}{\nu^2}\psi(t)e^{-\frac{t}{\nu}}dt + 2\nu\int_0^\infty \psi(t)e^{-\frac{t}{\nu}}dt$$

$$= \frac{1}{\nu^2}\Lambda\{t\psi(t)\} + \frac{2}{\nu}\Psi(\nu)$$

$$\Rightarrow \Lambda\{t\psi(t)\} = \nu^2\frac{d}{d\nu}\Psi(\nu) - 2\nu\Psi(\nu). \quad (\mathbf{5})$$

ii. $\Lambda\{t^2\psi(t)\} = \nu^2\left[\nu^2\frac{d^2}{d\nu^2}\Psi(\nu) - 2\nu\frac{d}{dp}\Psi(\nu) + 2\Psi(\nu)\right]$

Proof. Since, $\Lambda\{t\psi(t)\} = \nu^2\frac{d}{d\nu}\Psi(\nu) - 2\nu\Psi(\nu)$, so putting $t\psi(t)$ instead of $\psi(t)$ yields:

$\Lambda\{t^2\psi(t)\} = \nu^2\frac{d}{d\nu}\Lambda\{t\psi(t)\} - 2\nu\Lambda\{t\psi(t)\}$
$= \nu^2\frac{d}{d\nu}\left[\nu^2\frac{d}{d\nu}\Psi(\nu) - 2\nu\Psi(\nu)\right] - 2\nu\left[\nu^2\frac{d}{d\nu}\Psi(\nu) - 2\nu\Psi(\nu)\right]$
$= \nu^4\frac{d^2}{d\nu^2}\Psi(\nu) + 2\nu^3\frac{d}{d\nu}\Psi(\nu) - 2\nu^3\frac{d}{d\nu}\Psi(\nu) - 2\nu^2\Psi(\nu) - 2\nu^3\frac{d}{d\nu}\Psi(\nu) + 4\nu^2\Psi(\nu)$
$\therefore\ \Lambda\{t^2\psi(t)\} = \nu^2\left[\nu^2\frac{d^2}{d\nu^2}\Psi(\nu) - 2\nu\frac{d}{d\nu}\Psi(\nu) + 2\Psi(\nu)\right]. (\mathbf{6})$

4 Anuj Transform of $t\psi'(t)$ & $t^2\psi'(t)$:

If $\Lambda\{\psi(t)\} = \Psi(\nu)$, then:

i. $\Lambda\{t\psi'(t)\} = \nu\frac{d}{d\nu}\Psi(\nu) - 3\Psi(\nu) - \nu^4\frac{d}{d\nu}\psi(0)$

Proof. Since, $\Lambda\{\psi'(t)\} = \frac{1}{\nu}\Psi(\nu) - \nu^2\psi(0)$, so in (**5**) put $\psi'(t)$ instead of $\psi(t)$. Therefore:

$\Lambda\{t\psi'(t)\} = \nu^2\frac{d}{d\nu}\Lambda\{\psi'(t)\} - 2\nu\Lambda\{\psi'(t)\}$
$= \nu^2\frac{d}{d\nu}\left[\frac{1}{\nu}\Psi(\nu) - \nu^2\psi(0)\right] - 2\nu\left[\frac{1}{\nu}\Psi(\nu) - \nu^2\psi(0)\right]$
$= \nu\frac{d}{d\nu}\Psi(\nu) - \Psi(\nu) - \nu^4\frac{d}{d\nu}\psi(0) - 2\nu^3\psi(0) - 2\Psi(\nu) + 2\nu^3\psi(0)$
$\therefore\ \Lambda\{t\psi'(t)\} = \nu\frac{d}{d\nu}\Psi(\nu) - 3\Psi(\nu) - \nu^4\frac{d}{d\nu}\psi(0)\ (\mathbf{7})$

ii. $\Lambda\{t^2\psi'(t)\} = \nu\left[\nu^2\frac{d^2}{d\nu^2}\Psi(\nu) - 4\nu\frac{d}{d\nu}\Psi(\nu) + 6\Psi(\nu)\right] - \nu^4\frac{d}{d\nu}\left[\nu^2\frac{d}{d\nu}\psi(0)\right]$

Proof. As before, we get:

$\Lambda\{t^2\psi'(t)\} = \nu^2\frac{d}{d\nu}\Lambda\{t\psi'(t)\} - 2\nu\Lambda\{t\psi'(t)\}$
$= \nu^2\frac{d}{d\nu}\left[\nu\frac{d}{d\nu}\Psi(\nu) - 3\Psi(\nu) - \nu^4\frac{d}{d\nu}\psi(0)\right] - 2\nu\left[\nu\frac{d}{d\nu}\Psi(\nu) - 3\Psi(\nu) - \nu^4\frac{d}{d\nu}\psi(0)\right]$
$= \nu^3\frac{d^2}{d\nu^2}\Psi(\nu) + \nu^2\frac{d}{d\nu}\Psi(\nu) - 3\nu^2\frac{d}{d\nu}\Psi(\nu) - \nu^6\frac{d^2}{d\nu^2}\psi(0) - 4\nu^5\frac{d}{d\nu}\psi(0) - 2\nu^2\frac{d}{d\nu}\Psi(\nu) + 6\nu\Psi(\nu) + 2\nu^5\frac{d}{d\nu}\psi(0)$
$= \nu^3\frac{d^2}{d\nu^2}\Psi(\nu) - 4\nu^2\frac{d}{d\nu}\Psi(\nu) + 6\nu\Psi(\nu) - \nu^6\frac{d^2}{d\nu^2}\psi(0) - 2\nu^5\frac{d}{d\nu}\psi(0)$
$\therefore\ \Lambda\{t^2\psi'(t)\} = \nu\left[\nu^2\frac{d^2}{d\nu^2}\Psi(\nu) - 4\nu\frac{d}{d\nu}\Psi(\nu) + 6\Psi(\nu)\right] - \nu^4\frac{d}{d\nu}\left[\nu^2\frac{d}{d\nu}\psi(0)\right]\ (\mathbf{8})$

Thus, we can deduce the following relations:

$\Lambda\{t\psi''(t)\} = \frac{d}{d\nu}\Psi(\nu) - \frac{4}{\nu}\Psi(\nu) + \nu^2\psi(0) - \nu^3\frac{d}{d\nu}\left[\nu\frac{d}{d\nu}\psi(0)\right]. (\mathbf{9})$

$\Lambda\{t^2\psi''(t)\} = \nu^2\frac{d^2}{d\nu^2}\Psi(\nu) - 6\nu\frac{d}{d\nu}\Psi(\nu) + 12\Psi(\nu) - \nu^3\frac{d}{d\nu}\left[\nu^3\frac{d^2}{d\nu^2}\psi(0)\right]. (\mathbf{10})$

Notice: Anuj Transform of functions $\{t^n\psi^{(n)}(t)\}(;\ n \in \mathbb{N})$ can be calculated as the way posed in the last paragraphs.

5 APPLICATIONS

In this section, we will provide some different examples:

Example (1): Solve the differential equation:

$t^2y'' - ty' + y = 5$, with $[y(0) = 5\ \&\ y'(0) = 3]$.

Solution: Applying the Anuj transform to both sides of the given equation, we get:

$\Lambda\{t^2y''\} - \Lambda\{ty'\} + 6\Lambda\{y\} = \Lambda\{5\}.$

$\nu^2\frac{d^2}{d\nu^2}\Lambda\{y\} - 6\nu\frac{d}{d\nu}\Lambda\{y\} + 12\Lambda\{y\} - \nu^3\frac{d}{d\nu}\left[\nu^3\frac{d^2}{d\nu^2}y(0)\right] - \nu\frac{d}{d\nu}\Lambda\{y\} + 3\Lambda\{y\} + \nu^4\frac{d}{d\nu}y(0) + \Lambda\{y\} = \Lambda\{5\}.$

$\nu^2\frac{d^2}{d\nu^2}\Lambda\{y\} - 7\nu\frac{d}{d\nu}\Lambda\{y\} + 16\Lambda\{y\} = 5\nu^3.$

Let $M = \Lambda\{y\}$

$\Rightarrow \nu^2\frac{d^2M}{d\nu^2} - 7\nu\frac{dM}{d\nu} + 16M = 5\nu^3$

which is the Cauchy-Euler differential equation. So, we will consider:

$\nu = e^x \Rightarrow x = \ln\nu\ \Rightarrow \frac{d^2M}{d\nu^2} = e^{-2x}\left(\frac{d^2M}{dx^2} - \frac{dM}{dx}\right), \frac{dM}{d\nu} = e^{-x}\frac{dM}{dx}.$

Thus, by substituting above work into the last equation, we get

$$e^{2x}e^{-2x}\left(\frac{d^2M}{dx^2}-\frac{dM}{dx}\right)-7e^{x}e^{-x}\frac{dM}{dx}+16M=5e^{3x}$$

$$\Rightarrow M''-8M'+16M=5e^{3x}$$

$$\Rightarrow M_c=C_1e^{4x}+C_2xe^{4x}\Rightarrow M_c=C_1v^4+C_2v^4\ln v$$

$$\&\ M_p=\frac{5}{D^2-8D+16}e^{3x}\ \Rightarrow M_p=5e^{3x}\Rightarrow M_p=5v^3$$

$$\therefore\ M_g=C_1v^4+C_2v^4\ln v+5v^3.$$

Here, note that if we require $y(0)$ to be finite, we are forced to conclude that $C_2=0$.

$$\therefore\ \Lambda\{y\}=C_1v^4+5v^3\Rightarrow y=\Lambda^{-1}\{C_1v^4\}+\Lambda^{-1}\{5v^3\}\Rightarrow y=C_1t+5$$

$$\because y'(0)=3\Rightarrow\ y'=C_1\Rightarrow C_1=3.$$

$$\therefore\ y(t)=3t+5.$$

Example (2): Solve the differential equation:

$$ty''-ty'-y=0$$

with the initial condition $y(0)=0$ & $y'(0)=2$.

Solution: Taking Anuj transform to both sides of given equation to give us:

$$\Lambda\{ty''\}-\Lambda\{ty'\}-\Lambda\{y\}=0.$$

$$\Rightarrow\frac{d}{dv}\Lambda\{y\}-\frac{4}{v}\Lambda\{y\}+v^2y(0)-v^3\frac{d}{dv}\left[v\frac{d}{dv}y(0)\right]-v\frac{d}{dv}\Lambda\{y\}+3\Lambda\{y\}+v^4\frac{d}{dv}y(0)-\Lambda\{y\}=0.$$

$$(1-v)\frac{d}{dv}\Lambda\{y\}-\left(\frac{4}{v}+4\right)\Lambda\{y\}=0.$$

$$\Rightarrow\int\frac{d\Lambda\{y\}}{\Lambda\{y\}}=\int\frac{4(1+v)}{v(1-v)}dv\Rightarrow\ln(\Lambda\{y\})=4\ln v-2\ln(1-v)+\ln C$$

$$\Rightarrow\frac{(1-v)^2}{v^4}\Lambda\{y\}=C\ \Rightarrow\ \Lambda\{y\}=C\frac{v^4}{(1-v)^2}.$$

Now, by applying inverse Anuj transform, we get

$$y=\Lambda^{-1}\left\{C\frac{v^4}{(1-v)^2}\right\}\Rightarrow y=Cte^t.$$

$$\because\ y'(0)=2\ \Rightarrow 2=C\ \Rightarrow C=2$$

$$\Rightarrow\ y(t)=2te^t.$$

Example (3): (Attaweel & Almassry 2020)

Solve the differential equation:

$$ty''-y'=t^2$$

with the initial condition $y(0)=0$ & $y'(0)=0$.

Solution: Applying Anuj transform to both sides of given equation which give us:

$$\Lambda\{ty''\}-\Lambda\{y'\}=\Lambda\{t^2\}.$$

$$\Rightarrow\frac{d}{dv}\Lambda\{y\}-\frac{4}{v}\Lambda\{y\}+v^2y(0)-v^3\frac{d}{dv}\left[v\frac{d}{dv}y(0)\right]-\frac{1}{v}\Lambda\{y\}+v^2y(0)=\Lambda\{t^2\}$$

$$\frac{d}{dv}\Lambda\{y\}-\frac{5}{v}\Lambda\{y\}=2v^5$$

which is a linear differential equation and it has the integrative factor: $\lambda=\frac{1}{v^5}$.

$$\Rightarrow\frac{1}{v^5}\Lambda\{y\}=2v+C\Rightarrow\Lambda\{y\}=2v^6+Cv^5$$

Now applying inverse Anuj transform, we get

$$y(t)=\frac{t^3}{3}+\frac{Ct^2}{2}.$$

Example (4): (Aggarwal et al., 2018)

Solve the differential equation:

$ty'-2y=0$, with $y(0)=0$.

Solution: Applying the Anuj transform to both sides of the given equation, we get:

$$\Lambda\{ty'\}-2\Lambda\{y\}=0.$$

$$v\frac{d}{dv}\Lambda\{y\}-3\Lambda\{y\}-v^4\frac{d}{dv}y(0)-2\Lambda\{y\}=0.$$

$$\Rightarrow\frac{d}{dv}\Lambda\{y\}=\frac{5}{v}\Lambda\{y\}.\ \text{Let}\ M=\Lambda\{y\}$$

$$\Rightarrow\ \frac{dM}{dv}=\frac{5}{v}M\ \Rightarrow\ \frac{dM}{M}=5\frac{dv}{v}$$

$$\Rightarrow\ M=Cv^5\Rightarrow\Lambda\{y\}=Cv^5$$

$$\Rightarrow\ y=\Lambda^{-1}\{Cv^5\}$$

$$\therefore\ y(t)=C\frac{t^2}{2}.$$

6 Conclusions

In this paper, the researcher has discussed the application of Anuj transform to solve ODE's with variable coefficients by obtain Anuj transform formulas of functions:
$t\psi(t), t^2\psi(t), t\psi'(t), t^2\psi'(t), t\psi''(t)$ & $t^2\psi''(t)$, and then apply those formulas in some problems. The results of the present study show that the Anuj transform is very useful integral transform for solving such equations. In addition, all the obtained solutions of the indicated problems are satisfied by putting them back in the corresponding equations. In future, Anuj transform can be used to solve differential equations more broadly.

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The Phylogeography of Rhizophora in Peninsular Malaysia: High Genetic Variation between West and East

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Geographic history of *Rhizophora* species and hybrid is effect on their genetic structure. In addition, determining population genetic variation is essential for phylogeography research. For this phylogeography investigation, Four natural population of *Rhizophora* were collected from five different regions in peninsular Malaysia for this phylogeography study. This study aims to evaluate the genetic diversity of *Rhizophora* species *namely Rhizophora apiculata* , *Rhizophora mucronata* , *Rhizophora stylosa* and hybrid *R.* × *lamarcki* and clarify their genetic structure of its populations using Nucleotide polymorphism in chloroplast intergenic spacer between trnL and trnF genes was studied.The populations of *Rhizophora* species and hybrids were divided into two groups: the east coast populations, which were represented by samples collected from Mersing, Tanjang Piai, and Sedili; and the west coast populations, which were represented by samples collected from Mersing, Tanjang Piai, and Sedili. The population of the west coast was the second cluster. This result was supported by UPGMA analysis using MEGA5 software.

1 Introduction

Rhizophora is a genus of tropical and subtropical coastal plants native to tropical and subtropical coastal areas from Africa's east coast through Asia, Australia, and most of the eastern Pacific Ocean's islands. East Pacific mangroves whose range naturally overlap only in small number of southern Pacific islands (Duke 2006). According to Tomlinson (1986) *Rhizophora mucronata* ranges from east Africa to the western Pacific further eastward in the Indo-Malayan region where it grows with *Rhizophora apiculata* and *Rhizophora stylosa*. But it becomes progressively less conspicuous element of mangrove floras as one moves eastward In Peninsular Malaysia; the most widely distributed species are *Rhizophora apiculata* and *Rhizophora mucronata* throughout mangrove forest areas. In the forestry and fishing industries, both species are economically important. Apart from these two economic species, Malaysian foresters are less familiar with *Rhizophora stylosa*. There is little information about *R. stylosa* in Malaysia. *R. stylosa* does not grow in all mangrove habitats in Peninsular Malaysia, and it can only be found in a few places. *Rhizophora stylosa* has a wide distribution in the Indo-Pacific region. However, its distribution is very localized and restricted in very specific sites in Peninsular Malaysia. *R. stylosa* is only found in Pulau Langkawi, Melaka and Johor (Kochummen, 1989). Others than these areas, Nasir & Yusmah. (2007) investigated the distribution of Rhizophora stylosa in Peninsular Malaysia and discovered it in Sg. Kurung Tengar, Perlis, Bagan Lalang, Sepang, Selangor, Pulau Besar, Melaka, Pulau Burung, a small rocky island off the coast of Port Dickson, and two sites in Sg. Mawar, Endau, Johor, Pulau Sibu and Pulau Tinggi, Both islands are located off the coast of Johor's Mersing..There are about 20 million hectares of mangroves in Asia, Oceania, Africa, the Americas and the Middle East (Saenger *et al*. 1983). Malaysia has about 6400 km2 of mangrove forest, of

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which 5000 km2 are found in Sabah and Sarawak and in Peninsular Malaysia about 1400 km2 (Mansor *et al*. 2005). Of the total mangrove areas in Malaysia, Sabah covers 57%, followed by Sarawak 26% and Peninsular Malaysia has 17%. Peninsular Malaysia has about 86,545 ha of mangrove areas gazetted as forest reserves, whereas Perak has the largest area (47.8%), followed by Johor (20.6%), Selangor (17.3%) and Kedah (9.2%) (Wan Juliana et al. 2007).

Nucleic acid variation can be described most directly by using the nucleotides themselves as discrete characters, each with four possible states. Sequencing can identify very modest levels of variation since nucleotides are the basic units of genetic information in all organisms. Virtually any taxon's nuclear and organelle genomes can be sequenced; just little amounts of DNA or RNA are required, and fresh or stored DNA can be used (Avise 1994).The universal primers of the *trn*L–*trn*F spacer were designed by Taberlet *et al*. (1991), and are now widely used to survey the intraspecific genetic variation and to construct phylogenetic trees for various plant species (Fujii et al. 1995;Wang *et al*. 1999).

2 Materials and Methods

2.1 Plant Materials

Leaves were obtained from 20 trees of *R. apiculata*, *R. mucronata*, *R. stylosa and R.* × *lamarcki* from different localities in peninsular Malaysia. The locations for each site are listed in table 1 and showed in Figure1.

2.2 Genomic DNA Extraction

Genomic DNA was isolated using DNeasy Plant Mini Kit method protocol. Approximately 40 mg of leaf tissue was ground to a powder in liquid nitrogen with a mortar and pestle. The powder was combined with 400 ml RNase stock solution and incubated for 10 minutes at 65 C°. the mixture was centrifuged at 10000 rpm. The lysate was transferred into QIA Shredder Mini spin column placed in a 2 ml collection tube and centrifuged for 4 min at 10000 rpm. Without disrupting the cell-debris pellet, the flow through fraction was transferred to a new tube. 1.5 volume of buffer AP3 ⁄E was added and mixed. 650 ml of the previous step's mixture, including any precipitate that may have formed, was placed in a 2 ml collection tube with a DNeasy Mini spin column. Centrifuged for 1 min at 8000 rpm and discarded the through reuse the collection tube in next step. The last step was repeated with the remaining sample and discarded the flow-through and collection tube. The DNeasy Mini spin column was placed into a new 2ml collection tube. 500 µl Buffer AW was added centrifuged for 1 min at 8000 rpm and discarded the flow-through and reuse the collection tube in next step. 500 µl Buffer AW was added to DNeasy Mini spin column and centrifuged for 4 min at 10000 rpm. The flow-through was discarded and then centrifuged for 1 min at 10000 rpm. The DNeasy Mini spin column was transferred to 1.5 ml micro centrifuge tube. And then 100 µl of Buffer AE was added directly onto DNeasy membrane and incubated for 20 min and then centrifuged for 1 min at 8000 rpm. The Buffer AE was added onto DNeasy membrane and then incubated for one night.

2.3 Polymerase Chain Reaction (PCR)

The region was amplified by the Polymerase Chain Reaction (PCR). The universal primers for amplification of the *trn*L-*trn*F spacer were those of Taberelt et al. (1991). PCR reaction mixtures (25 µl) contained approximately 1 µl of total DNA, 0.5 µl of each primer, 3 µl $Mgcl_2$, 1µL DNTp and 0.2 µl of Taq polymerase. Amplifications were carried out using 30 cycles for 1 min at 94 Cº, 1 min at 51 Cº or 48 Cº, and 2 min at 72 Cº the PCR products were excised from agarose gel under the long wave UV light. The PCR products were purified with GF-1(PCR clean- up kit)

2.4 Alignment of DNA Sequences and Data Analysis.

For aligned the DNA sequences, we used MEGA5 program. Clustering was done following unweighted pair group with arithmetic mean average (UPGMA) method MEGA5 software.

Table (1). List of specimens and collection sites of each species.

Species		Specimen
R.apiculata	AJ1	Johor, Mersing
	AL1	Kedah, Pulau Langkawi
	AS1	Johor, Sedili
	AM1	Melaka, Sungai linggi
	AT1	Melaka, Port Dickson, Cape Rachado Beach
R. mucronata	ML2	Kedah, Pulau Langkawi.
	MS2	Johor, Sedili,
	MO	Johor, Mersing
	MM2	Melaka, Sungai Linggi
	MP2	Selangor, Morib
R. stylosa	SL3	Kedah, Pulau Langkawi.
	SM3	Melaka, Port Dickson, Cape Rachado Beach
	SP3	N. Sembilan, Port Dickson, Teluk Tanjung
	SJ3	Johor, Mersing
	ST3	Johor, Tanjung Piai
R. lamarckii	LL4	Kedah, Pulau Langkawi
	LS4	N. Sembilan, Port Dickson, Teluk Tanjung Pelandok
	LM4	Melaka, Port Dickson, Cape Rachado Beach Resort
	LT4	Johor, Tanjung Piai
	LJ4	Johor, Mersing

Figure (1). The locality of collections sites.

3 Results

In *Rhizophora apiculata*, clustering based on genetic distances showed that the populations of *Rhizophora apiculata* are grouped into two distinct clusters. The three populations of *R. apiculata*, Sungai linggi (AM1), Cape Rachado Beach Resort, (AT1) and Mersing (AJ1) formed the first cluster wherein AM1 (Sungai linggi) and AT1 (CapeRachado Beach Resort population) was clustered to AJ1 (distance 0.017) and formed the second cluster a long with AS1 (Sedili population) at 0.021 and formed a node with AL1 (Langkawi population). There were clear relationship between AM1 (Sungai linggi population) and AT1 (Cape Rachado Beach Resort population). Whereas, Sungai linggi and Cape Rachado Beach Resort population had shorter genetic distance (Figure 2).

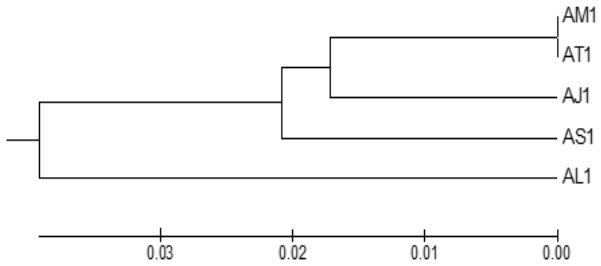

Figure (2). Dissimilarity coefficient UPGMA dendrogram of *R. apiculata* based on the G_{ST} of the *trnL-trnF* spacer of *cpDNA.*

Rhizophora mucronata populations are grouped into three distinct clusters. MS2 (Sedili population), MO2 (Mersing population) formed a first cluster wherein MS2 was clustered to MO2 (distance of 0.014) and formed second cluster along with ML2 (Langkawi population) at 0.019 and MM2 (Sungai linggi population) formed a third cluster with MP2 (Morib population) at 0.014. The two groups of populations formed a node at 0.026. Sungai Linggi and Morib population with Sedili and Mersing population had a same and a shorter genetic distance (Figure 3).

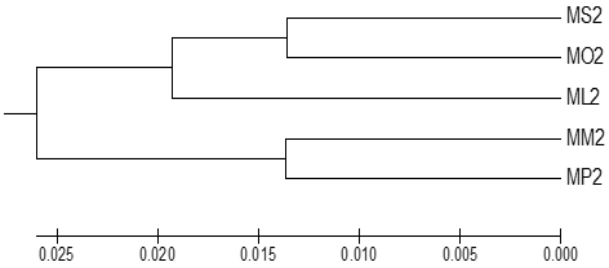

Figure (3). Dissimilarity coefficient UPGMA dendrogram of R. *mucronata* based on the G_{ST} of the trnL-trnF spacer of cpDNA.

R. stylosa populations are grouped into three distinct clusters. That of, SP3 (Cape Rachado Beach Resort) population and SM3 (Teluk Tanjung Pelandok) population formed a first cluster wherein SP3 was clustered to SM3 (distance of 0.025) and SJ3 (Mersing population) was clustered with ST3 (Tanjung Piai population) at 0.005. The two groups of populations formed a node at 0.031 and formed a third cluster along with SL3 (Langkawi population). Tanjung Piai and Mersing populations had shorter genetic distance than those of the Cape Rachado Beach Resort and Teluk Tanjung Pelandok populations (Figure 4).

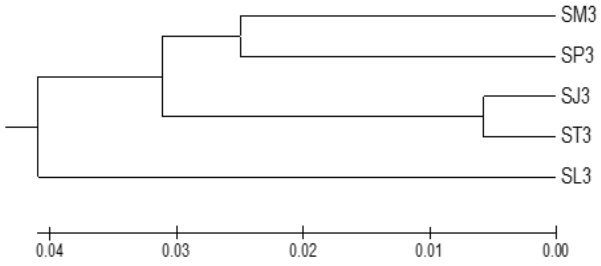

Figure (4). Dissimilarity coefficient UPGMA dendrogram of *R. stylosa* based on the G_{ST} of the trnL-trnF spacer of cpDNA.

The population of natural hybrid (*R.* × *Lamarckii*) grouped into two distinct clusters. LS4 (Teluk Tanjung Pelandok population) and LM4 (Cape Rachado Beach Resort population) formed a first cluster with LL4 (Langkawi population) and formed second cluster a long with LT4 (Tanjung Piai population) and LJ4 (Mersing population) (Figure 5).

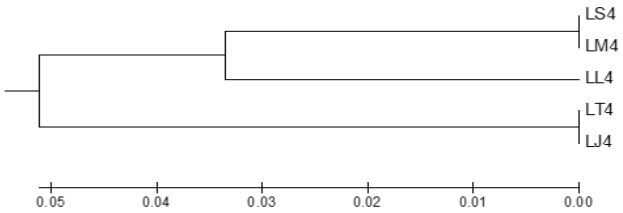

Figure (5). Dissimilarity coefficient UPGMA dendrogram of *R* × *lamarckii* based on the G_{ST} of the *trn*L-*trn*F spacer of cpDNA.

4 Discussion

For population studies, samples were collected from different geographically locations from east and west coast of Peninsular Malaysia. There were high genetic variation in east and west coasts of Peninsular Malaysia for population for all *Rhizophora* species and hybrid. The east coast population is represented by samples collected from Mersing, Tanjang Piai and Sedili while the west coast populations are represented by Cape Rachado Beach Resort, Teluk Tanjung Pelandok, Sungai linggi and Morib. This result is consistent with Huang et al. (1999) who had found a relatively high genetic variation in both populations of east and west coasts of Malay Peninsula of *Rhizophoraceae* using isozyme analysis.

In this study, it was noticed that the UPGMA analysis based on genetic distances could reveal in all *Rhizophora* taxa, there are high genetic variation between both west and east coasts population of *Rhizophora*.

5 Conclusions

The geographic history is another factor that may have influenced genetic differentiation in this area. In this study, from the result of data analysis the geographical haplotype distribution reveals significant discrimination between eastern and western Malay Peninsula populations.

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Control of Apple Fruit Rot Caused by *Alternaria porri* and *Alternaria mali* by Using Hot Water Treatment and Some Inorganic Salts

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Apple rot is one of the most important apple diseases worldwide. The disease causes significant losses in both the quantity and quality of apple fruits. In this study, the antifungal activity of hot water treatment and salts were investigated against apple fruit rots caused by *Alternaria porri* and *Alternaria mali*. Linear growth of tested fungi was inhibited at 5ºC, however, growth was increased by increasing storage temperature degree from 15ºC to 25ºC and decreased at 35ºC. On the other hand, hot water treatment at 55ºC significantly reduced the decay index and severity of infection. When salts such as potassium bicarbonate (KCO_3), calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$) and ammonium bicarbonate (NH_4CO_3) was applied, a significant reduction in linear growth and fruit rot incidence was noticed using potassium bicarbonate. Calcium chloride, ammonium bicarbonate and sodium bicarbonate greatly inhibited growth of *A. porri* and *A. mali*. The most effective inhibitor of fruit decay was potassium bicarbonate and calcium chloride.

1 Introduction

Apple fruit (*Malus domestica*) is considered an important fruit worldwide. It is a fruit belonging to the family Rosaceae. The apple fruit has the ability to store long-term and due to this characteristic it exists in markets all year round (Petres, *et al.* 2020), furthermore it is transported from localities of production to far off places for marketing and consumption. Succulent apple fruits can be damaged and degraded with improper handling during harvest, marketing, storage, transportation and consumption. (Rotondo *et al.* 2012, Llyas et *al*. 2007 and Harteveid et al. 2014). During the transfer process and storage, apple fruit quality can be affected by several factors including plant pathogenic fungi, which can cause major postharvest losses during storage. The most common fungal species that cause storage losses apple are *Botrytis cinerea* Pers., *Monilinia fructigena* Honey, *Penicillium expansum* Link, as well as *Alternaria* spp., *Mucor* spp., *Rhizopus* spp. and *Botryosphaeria* spp., etc. (Grahovac *et al*. 2011, 2012; Petres *et al*. 2017).

Therefore, monitoring of these species in stored apple fruits is of high importance. Keeping in mind that postharvest use of synthetic pesticides are not allowed in many countries, there is a need for finding alternative strategies for apple fruit rot control.

The fungal infestation of fruit and vegetables in post-harvest storage severely limits their economic value due to degradation. Although fungicide treatments have been the primary method of monitoring post-harvest diseases, public concerns about fungicide residues in food and the developing fungicide resistance by pathogens has rising the search for suitable means of disease control agents (Tian *et al.* 2001). Non-fungicidal treatments has become a most desirable strategy for disease control (Lieperuma *et al*. 2000, Yacoub 2005, Abd-Allah ,2007

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and Nikolov *et al*. 2013). Hot water treatments might be a very successful option process to control rot, especially in organic production (Maxin *et al*. 2006; *Gasser et al.* 2015).

Heat treatment has been extensively studied as an effective method of disinfesting fruit with microorganisms (Couey 1989). Water is a more efficient medium than air, and the cost of treating hot water is much cheaper than that of treating hot air, hot water treatment is gaining commercial acceptance (Fallik 2004). According to Maxin et al. (2012) and Maxim (2012), there are three modes of action of hot water treatments: washing off the inoculum from the fruit surface, heat inactivation of spores and activation of the defense response in fruits (stress-induced transcription of heat shock proteins HSP). It is necessary to find a suitable combination of temperature and exposure time that will successfully suppress fruit rot while not damaging the fruit. Some inorganic salts used in the food industry as antimicrobial agents and preservatives have proven to be viable alternatives to synthetic fungicides in controlling plant diseases (Russell and Gould, 1991).

These compounds have demonstrated broad antimicrobial activity with little mammalian toxicity (Olivier et al., 1999), are biocompatible (Horst et al., 1992) and have been shown to be safe. Furthermore, they are less sensitive to ecological conditions than other options, for example biological control agents, which may make them suitable for controlling plant infections or suppressing mycotoxin production (Roinestad *et al*. 1993; Singh and Chand 1993).

Bicarbonates are regularly used in the food industry (Lindsay 1985) and have been found to suppress several fungal diseases in cucumber plants (Ziv and Zitter 1992). Kuepper *et al*. (2001) reviewed several research papers on the benefits of sodium bicarbonate as a safe fungicide to treat various plant diseases. Palmer et al. (1997) found that ammonium, potassium, and sodium bicarbonates can inhibit colony growth of *Botrytis cinerea* at concentrations as low as 20 mM.

The aim of the study aims to identify the best degrees of temperature, hot water treatments and salts compounds that would improve the control of pathogens caused by apple fruit rots.

2 Materials and Methods

2.1 Collection of Disease Samples

Diseased golden apple fruits were collected from the market, kept in sterilized polythene bags and transported to the Botany laboratory, Sirte University, Libya for the isolation of the pathogen.

2.2 Isolation, Purification and Identification of Pathogens

For isolation and purification of the pathogen, diseased portions from fruits were cut with a sterilized blade into small pieces (4-6 mm). The pieces were surface sterilized with sodium hypochlorite NaClO (1%) for approximately 2 min. The surface sterilized pieces of apple fruit were washed with sterilized distilled water, then placed in Petri dishes containing 15 ml Potato Dextrose Agar (PDA) media and incubated at 30ºC ± 2ºC. Petri dishes were monitored and observed for growth of fungi daily. After five days, the young growing hyphal tips were transferred to freshly prepared PDA media plates (Figures 2 and 3). The purified fungal isolates were identified according to the procedures described by Ellis (1971) and O`Donnell (1979).

2.3 Effect of Temperature Degree on Linear Growth of The Tested Pathogenic Fungi

The effect of temperature on the growth of *A. porri* and *A. mali* was carried out in accordance with the methods of Malik and Singh (2004) The influence of temperature on the growth of *A. porri* and *A. mali* was carried out according to the methods of Malik and Singh (2004).. The PDA medium was used to investigate the effect of different degrees of temperature on linear growth of the tested pathogenic fungi. Inoculated plates were incubated at **5**˚C, 15˚C, 2**5**˚C, **35**˚C for **7** days and results were recorded at the 7th day. There were three replicate panels per temperature treatment

2.4 Hot Water Treatment

For hot water treatment, healthy golden apple fruits, of uniform size, were divided into two groups. (Tohamy et al. 2004). First group was sterilized by dipping in 70% ethanol for one minute, air dried and inoculated with mycelial discs (4mm. in diameter) by tested pathogenic fungi through scratch in the surface of each fruit and stored at room temperature and served as control. The second group of fruits were inoculated with mycelial discs (4mm. in diameter) from tested pathogenic fungi through a scratch in the surface of each fruit, then stored at room temperature for 72 hrs. After that inoculated fruits, were dipped in hot water at 35ºC, 45ºC and 55ºC for 2, 4 and 6 min. per each degree and stored at room temperature for 2 weeks.

2.5 Salts

Four inorganic salts namely calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), potassium bicarbonate (KCO_3) and ammonium bicarbonate (NH_4CO_3) were used for their antifungal activity against mycelial growth and control of apple fruit decay caused by *A.porri* and *A. mali* (Zaker 2014), Moreover, they are less sensitive to

environmental conditions. Several documents are available proving this fact. (Hervieux *et al*. 2002, Jamar *et al*. 2007, Nahal and Mokhtar 2009, Turkkan 2013.).

2.5.1 Effect of Salts on Mycelial Growth

Methods used in evaluating control of mycelial growth properties of the selected salts: calcium chloride, sodium bicarbonate, potassium bicarbonate and ammonium bicarbonate followed the protocol detailed by Schmitz (1930). Pure isolates of selected fungi were grown in petri dishes on PDA with different salt concentrations 2%, 3% and 4% at 28 ± 2ºC. PDA discs (4mm. in diameter) of actively growing mycelia of tested fungi were used to inoculate the plates. For each plate, diameter of colony was determined after 7 days of the inoculation period. Inhibition of mycelial growth was calculated as follows:

[(control radial growth - salt amended radial growth) / control radial growth] × 100.

2.5.2 Effect of Salts on Apple Fruits Decay

Surface of apple fruits were disinfected with 2.5% sodium hypochlorite for 3 minutes, rinsed with sterilized water and air-dried, then wounded using 1 mm in diameter needle at one marked point and dipped for 3 minutes, into the solution of calcium chloride, sodium bicarbonate, potassium bicarbonate and ammonium bicarbonate at 4% concentration of each of the salts, then picked up and left to air dry on filter paper. After 1 hr all treated fruits were inoculated by fungi (2 mm in diameter). Control treatments consisted of apple fruits inoculated with sterilized distilled water. Thereafter, all treated apple fruits were air-dried, placed into nylon bags with 3 fruits capacity and stored in a cold room at 10ºC ± 2ºC for four weeks. Each treatment had replicates.

3 Statistical Analysis

All the experiments were repeated at least twice. The results are averages from treatments within each experiment. Data were analysed by ANOVA using SPSS v 20. Different letters above the bars on graphs or after figures in tables indicate a significant difference in means from post hoc Tukey test.

4 Results

4.1 Effect of Storage Temperature on Linear Growth

The results from Figure 1 indicate that linear growth incited by *A. porri* and *A. mali* was significantly affected by storage temperature ($P > 0.05$), with the largest linear growth at 25°C for both species at 96 mm and 85 mm in *A. porri and A. mali* respectively. The lowest linear growth was at 5 °C for both species compared to other temperatures. In general, the line of growth in *A. mali* was higher than in *A. porri*, except at 5 °C where linear growth was 19 mm for *A. mali* and 24 mm for *A. porri*. On the other hand, mycelial growth increased with increase in storage temperature degree from 5ºC to 25ºC then decreased at 35ºC.

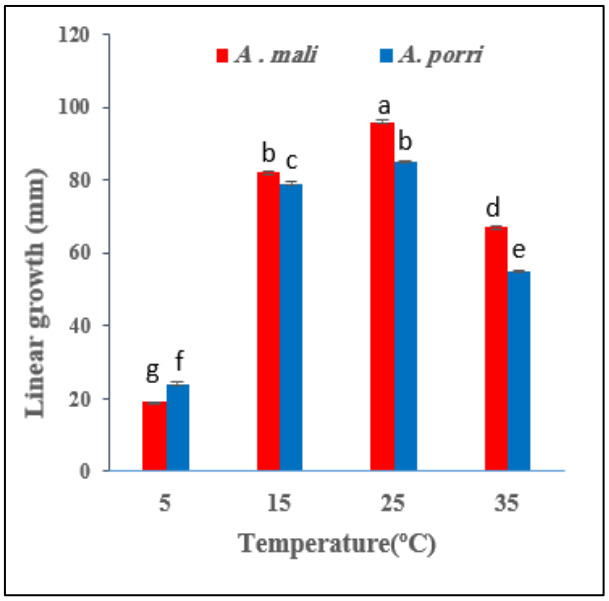

Figure (1). The effect of different degrees of temperature (5ºC, 15ºC, 25ºC and 35°C) on mycelial growth (linear growth) of *A. mail and A. porri* grown on potato dextrose agar (PDA) after 7 days. Letters above error bars (n = 3) indicate significant difference in means from post hoc Tukey tests ($P < 0.05$).

4.2 Effect of Storage Temperature on Decay Index and Severity of Infection

According to data illustrated in Figure 2A, decay index was significantly affected by storage temperatures (P > 0.001). Results showed that *A. porri* had a low decay index of 0.8% at 5ºC, followed by 1.02% by *A. mali*. It was clear that disease peak was at 25ºC and decreased at storage temperature of 35ºC.

The present investigation showed that the optimum storage temperature suitable for the development of the tested fungi was 25ºC. Severity of infection showed a similar pattern to decay index in response to different degrees of storage temperatures (Figure 2B).
Furthermore, severity of infection was lower at 5ºC than other treatments in both species.

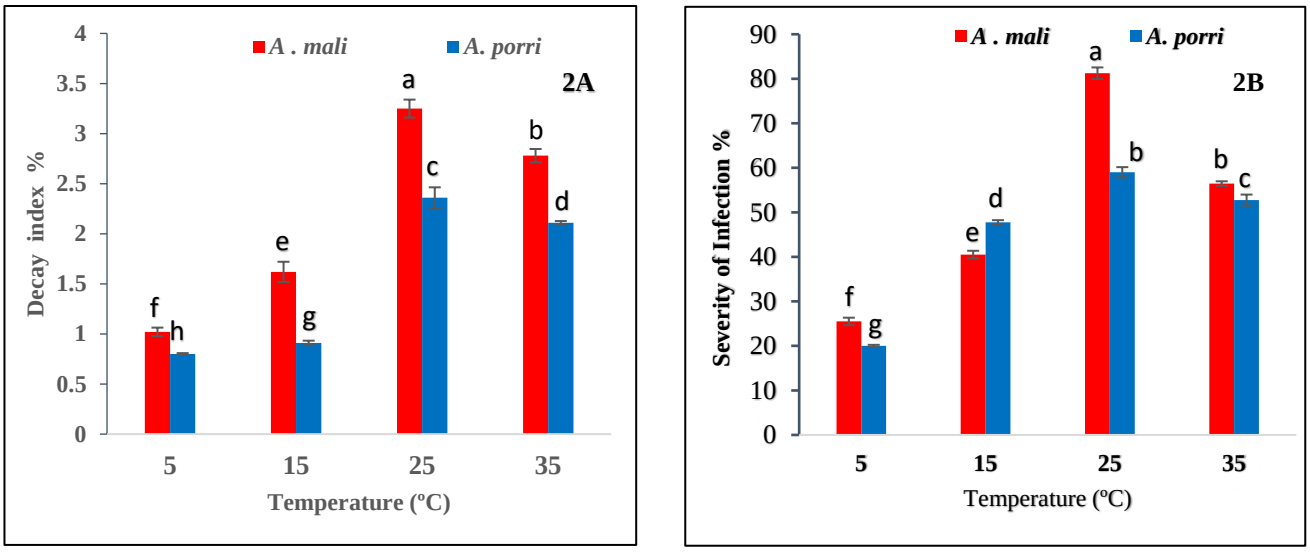

Figure (2). The effect of different degrees of temperatures (5°C, 15°C, 25°C and 35°C) on (2A) decay index and (2B) severity of infection of *A. mail and A. porri* grown on potato dextrose agar (PDA) after 7 days. Letters above error bars (n = 3) indicate significant difference in means from post hoc Tukey tests ($P < 0.05$).

4.3 Effect of Hot Water on Apple Fruit Rot Diseases

The results from (Table. 1) indicated that the three (35○C, 45○C and 55○C) temperature degrees of hot water at three different dipping times (2, 4 and 6 minutes) had significant effect on fruit rot disease development (Severity of Infection and Decay Index (SIDI)) ($P \leq 0.01$). The SIDI was significantly reduced by increasing hot water treatments compared with the control. However, the SIDI was not stable with increasing dipping time at 35○C and 45○C, while at 55○C it decreased with increasing dipping time. Fruit rot disease was completely inhibited when apple fruits were exposed to hot water at 55○C for 4 and 6 minutes in *A. porri* and 55 ○C for 6 minutes in *A. mali*. Decay index was totally inhibited at 55 degrees at 6 minutes in *A. porri* and at 4 and 6 minutes for *A. mali*.

Table (1). Effects of different hot water treatments and durations on apple fruits infected by tested pathogenic fungi. *A. porri* and *A mali* stored at room temperature for 2 weeks.

Treatment	Severity of Infection %		Decay index %	
	A. porri	*A. mali*	*A. porri*	*A. mali*
Control- not treated	89 ± 1.5^{a}	95 ± 1.7^{a}	4.2 ± 0.2^{a}	3.4 ± 0.4^{a}
35 °C, 2 min	68 ± 1.3^{b}	58 ± 2.9^{c}	2.7 ± 0.3^{b}	2.3 ± 0.3^{b}
35 °C, 4 min	37.5 ± 0.6^{c}	16.8 ± 0.6^{g}	1.5 ± 0.06^{c}	1.2 ± 0.1^{d}
35 °C, 6 min	25 ± 0.7^{h}	60 ± 1.2^{b}	1.01 ± 0.06^{ef}	2.4 ± 0.2^{b}
45 °C, 2 min	28.3 ± 1.3^{f}	$55.8\pm0.4_{d}$	1.13 ± 0.1^{e}	2.32 ± 0.2^{b}
45 °C, 4 min	31.3 ± 0.7^{e}	31.5 ± 0.6^{f}	1.25 ± 0.09^{d}	1.26 ± 0.1^{d}
45 °C, 6 min	33 ± 1.2^{d}	33.5 ± 1.6^{e}	1.32 ± 0.05^{d}	1.34 ± 0.05^{c}
55 °C, 2 min	22.5 ± 0.9^{i}	17.5 ± 0.9^{g}	0.90 ± 0.06^{f}	$0.70\pm.1^{e}$
55 °C, 4 min	0.00 ± 0.0^{j}	2.5 ± 0.3^{h}	0.10 ± 0.01^{g}	0.0 ± 0.0^{f}
55 °C, 6 min	0.0 ± 0.0^{j}	0.0 ± 0.0^{i}	0.0 ± 0.0^{h}	0.0 ± 0.0^{f}

Superscripts within results indicate significant difference in means (n = 3) from post hoc Tukey tests ($P < 0.05$).

4.4 Effect of Salts on Disease Incidence.

4.4.1 Effect of Salts on Mycelial Growth.

Data from Table 2 shows that, salt concentration had a significant effect on linear growth ($P \leq 0.05$) of mycelial fungi. Linear growth decreased with increasing salt concentrations in all treatments for both species. Potassium bicarbonate had the highest antifungal activity against tested pathogenic fungi, followed by calcium chloride, ammonium bicarbonate and sodium bicarbonate. Compared with *A. mali*, ammonium bicarbonate had the highest significant effect on antifungal activity on linear growth of *A. porri* however,

linear growth for both species for sodium bicarbonate at 2 % was 88 mm and 86 mm respectively, while potassium bicarbonate and calcium chloride had highest effect on linear growth of *A. mali* compared with *A. porri.*

Table (2). Effects of different salt concentrations on the mycelial growth (mm) of tested pathogenic fungi.

Salts	Concentration %	Linear growth of fungi (mm.) ±SE	
		A. porri	*A. mali*
Potassium bicarbonate	2	76±1.2^{c}	65±2.32^{d}
	3	54±0.3^{f}	42±1.2^{h}
	4	33±0.9^{g}	23±1.6^{i}
Calcium chloride	2	82±1.8^{b}	75±1.1^{c}
	3	64±2.3^{e}	56±1.7^{f}
	4	34±0.5^{g}	22±0.8^{i}
Ammonium bicarbonate	2	81±2^{b}	89±3^{a}
	3	52±1.2^{f}	65±2^{d}
	4	35±1^{g}	47±0.6^{g}
Sodium bicarbonate	2	88±0.6^{a}	86±1^{b}
	3	67±1^{d}	72±2^{c}
	4	31±1^{h}	59±1^{e}

Values marked with different letters (n = 3) indicate significant different in means from post hoc Tukey tests ($P < 0.05$).

4.4.2 Effect of Salts on Fruit Rot Development

Data in Table (3) revealed that potassium bicarbonate was the most effective inorganic salt for controlling the causal organisms of apple fruit rots followed by calcium chloride for both species, while ammonium bicarbonate and sodium bicarbonate had the least effect on controlling mycelial growth within both species. It was also noticed that *A. mali* was more sensitive to potassium bicarbonate and calcium chloride salts than *A. porri*. The best inhibitory effect of fruit rot was potassium bicarbonate followed by calcium chloride, while the least inhibitory effect in this experiment was observed by ammonium bicarbonate. Furthermore, the same pattern was recorded in Severity of Infection.

Table (3**)**. Efficacy of inorganic salts on apple fruit rots development stored in cold room at 10±2ºc for four weeks

Salts	Concentration %	Decay Index %		Severity of Infection %	
		A. porri	*A. mali*	*A. porri*	*A. mali*
Potassium bicarbonate	4	0.91±0.01^{c}	0.67±0.01^{d}	25.5±.4^{c}	16.7±0.4^{d}
Calcium chloride	4	0.98±0.01^{c}	0.86±0.01^{c}	24.5±0.3^{c}	21.5±0.5^{c}
Sodium bicarbonate	4	1.14±0.03^{b}	1.52±0.05^{b}	28.5±0.5^{b}	38±0.6^{b}
Ammonium bicarbonate	4	1.23±0.07^{a}	2.42±0.06^{a}	30.7±0.8^{a}	60.5±1.4^{a}

Values marked with different letters (n = 3) indicate significant different in means from post hoc Tukey tests ($P < 0.05$).

5 Discussion

Effect of Storage Temperature on Linear Growth and Decay Index and Severity of Infection

One of the most important environmental factors affecting mycelial growth and growth is temperature, which occurs over a diverse, varying temperature range. In order to evaluate the effect of storage temperature on the linear growth of *A. porri* and *A. mali* fungi, it was necessary to expose both species to different degrees of temperature (5ºC, 15ºC, 25ºC and 35ºC). Results in Figures 1, 2A and 2B showed the highest liner growth for (2A) Decay Index and (2B) Severity of Infection was recorded at 25 ºC and the lowest was at 5 ºC. These results were similar to Neelam *et al*. (2013) who reported optimum temperature for growth of *Pleurotus ostreatus* in a variety of 25°C to 30°C. Also, Farooq *et al.* (2005) observed that growth of *Fusarium oxysporium* achieved its maximum after 7 days of incubation at 30°C and growth was drastically decreased at temperatures under 15°C and above 35°C. Similar results were obtained by Ibrahim *et al* (2011) who observed that maximum growth of *Helminthosporium fulvum* was obtained at 25˚C and 30˚C temperatures. Furthermore, Mishra and Thawani (2016) discussed poor growth of *Alternaria*

alternate at temperatures under 20°C compared to its great growth at 27°C. The same authors noted temperature of 5°C as a growth limit for *A. alternate*, which is in agreement with the results obtained for our study on apple fruits stored at 5°C. Grzegorzewska *et al* (2022) also reported that a temperature of up to 5◦C markedly reduced fungal development.

Effect of Hot Water on Apple Fruit Rot Disease

The recent research has provided evidence of a fundamental efficacy of hot water treatment against *A. porri* and *A. mali* fungus in apples that have been infected artificially. Apples inoculated with *A. porri* and *A. mali* were subjected to hot water treatment at different temperatures (35◦C, 45◦C and 55◦C) and duration (2, 4 and 6 minutes), followed by ambient conditions for 2 weeks. Hot water at 55ºC was the best temperature, which gave the lowest fruit rot disease and completely prevented the development of fruit rot at 4 and 6 minutes for *A. porri* and for 6 minutes in *A. mali* (Table 1). This result was similar to that of Petres *et al* (2020) who reported that *Fusarium avenaceum* and *Fusarium graminearum* were exposed to hot water treatments ranging from 45°C to 90°C at different durations ranging from 30 to 20 minutes, their results showed that the treatments that significantly inhibited mycelial growth were temperatures of 53°C and 57°C for 3 and 5 minutes. Also, Di Francesco et al. (2018) found that defense response in apple fruit against pathogens can be stimulated by hot water treatments. Grzegorzewska *et al* (2022) reported that heat water treatment at temperatures of 53 °C for 3 seconds and 55 °C for 3 seconds substantially inhibited deterioration during short-term storage. According to Loayza *et al.* (2012), hot water treatment at 52°C for 5 min improved the sensory profiles of intact tomato fruits of two varieties. Trierweiler *et al*. (2003) observed that stored apple fruits previously treated with 53°C for 2 minutes in hot water significantly reduced the occurrence of *Gloeosporium* fruit rot compared to untreated fruits. Many authors such as (Fallik *et al.*1995, Lieperuma *et al.* 2000, Fallik 2004 and Tohamy *et al.* 2004 reported similar results. However, Maxin *et al.* (2014) stated that a temperature of 53◦C or higher increased the incidence of fruit rot was noticed probably since this is the point where physiological damages occurs in apple fruit. These claims are opposite to results achieved in this study. In our study no visible damage was discovered on treated fruits, this is likely because each variety reacts differently to the same water temperature. Treatment 55°C for 5 minutes showed the strongest necrosis inhibition without detrimental effects on fruits stored at at ambient temperature. Our results indicated that the most promising hot water treatment was 55 °C with an exposure time of 4 to 6 minutes. This can be explained by the antifungal influence of the applied temperature, as well as by the activation of the defense reaction in apple fruits. Maxine *et al*. (2012) concluded that the main effects of hot water immersion against this fruit rot is mediated through heat-induced acquired resistance of the fruit and not heat-induced spore mortality. Amongst different solutions, hot water treatment seems to be an encouraging means to decline the physiological aging process, prevent the development of physiological disorders and minimize microbial growth in freshly cut products (Fallik and Ilic, 2017). Koukounaras *et al*. (2008), Lurie (2006) and Siddiq et al. (2013) demonstrated that hot water treatment has been illustrated to have profound special effects on tissue metabolism and maintaining the quality of fresh-cut products.

Effect of Inorganic Salts on Disease Incidence

Potassium bicarbonate, Calcium chloride, sodium bicarbonate, and ammonium bicarbonate have been shown to prevent fungal pathogens in vegetables, fruit, field crops, and ornamental plants (Ziv and Zitter 1992; Palmer *et al*. 1997). Four inorganic salts namely calcium chloride, sodium bicarbonate, potassium bicarbonate and ammonium bicarbonate, have been used for their antifungal activity against mycelial growth and control of apple fruit decay caused by *A.porri* and *A. mali*. In all salt treatments, linear growth of fungi decreased with increasing salt concentrations (Table 2) and these results agree with Nahal *et al* (2009) who stated that the application of calcium chloride or sodium bicarbonate considerably reduced early blight and its severity by increasing their concentrations. Previous research showed that raising sodium bicarbonate concentrations caused in a constant improvement in efficacy (Mlikota and Smilanick 1998, 2001). The inhibitory impact of bicarbonate salts on microorganisms may be due to a decrease in cell turgor, which causes hyphae and spores to collapse and shrink, resulting in fungistasis (Fallik *et al*. 1997).

This finding supports the results of Wisniewski *et al.* (1995) who discovered that calcium chloride can minimize fungal infections by directly inhibiting growth and spore germination. Maouni *et al*. (2007) recorded that calcium chloride considerably decreased pear fruit decay induced by *Penicillium expansum* and *A. alternata* and when used at 4% and 6%. The exact mechanism by which calcium reduces fungal infection is unknown, but it may work by interfering with the activity of pectolytic enzymes (Conway et al. 1992) and may be due in part to a decrease in cell wall maceration by polygalacturonase (PG) due to improved structural integrity caused by increased calcium content (Conway *et al*. 1998). Calcium applications have been shown to improve

membrane functionality and maintaining integrity,which could explain why calcium treated fruits possess lower linear growth(Shirzadeh *et al.* 2011).

Bicarbonate salts have an inhibitory effect on fungi due to a decrease in fungal cell turgor pressure, which caused hyphae and spores to breakdown and shrink of preventing fungi from sporulating (Fallik et al. 1997).

In addition to controlling nutritional disorders, increasing the calcium content of fruits and vegetables increases their shelf life. It is believed that this effect is mainly due to the role of calcium in alleviating physiological disorders and thereby indirectly reducing pathogen activity (Bateman and Lumsden 1965, Conway et al. 1992). Much of the research with apples to improve storage quality and reduce decay with calcium supplementation has been done in the post-harvest environment. Alan R. Biggs (1999) reported that the results of this study show that calcium salts directly suppress the bitter rot pathogens *Colletotrichium gloeosporioides* and *Colletotrichium acutatum* Suppressive effects include reduced germ tube growth, reduced in-vitro mycelial growth, and reduced severity of infection of calcium pretreated host tissues. Zaker (2014) reported that among the four inorganic salts, potassium bicarbonate achieved greatest antifungal activity against *Fusarium oxysporum*, *Alternaria alternata* and *B. cinerea*. Zaker (2014) reported that potassium bicarbonate had the highest antifungal activity against *Fusarium oxysporum*, *Alternaria alternata* and *B. cinerea* among the four inorganic salts

Calcium as a component of the cell wall plays an essential role in Cross-bridge formation that impact cell wall strength and is considered regarded the last barrier before cell separation (Fry, 2004) Exogenously supplied calcium stabilizes the plant cell wall and protects it from cell wall-degrading enzymes (White and Broadley, 2003). Post-harvest calcium treatment considerably reduced decay in peaches by *Monilinia fructicola* (Conway *et al*., 1987 a,b) and in apples by *Botrytis cinerea* (Klein *et al*., 1997). Tian *et al*. 2001, found that the biocontrol efficacy of yeast (*Trichosporon* spp.) used to control gray and blue mold apple fruit was enhanced in the presence of 2% calcium chloride. Tian *et al* (2001) reported that calcium chloride (2% w/v) significantly inhibited the growth of the pathogen *Rhizopus stolonifer*.

6 Conclusions

Different degrees of temperature, hot water treatment and inorganic salts were investigated for their efficacy in reducing the incidence and severity of infection of apple fruits by *A. porri* and *A. mali*. It is apparent from the study that a low temperature of 5°C and exposure of apple fruits to hot water at 55°C for 4 to 6 minutes using potassium bicarbonate and calcium chloride improved the sensory profiles of intact apple fruits against two cultivars of fungi.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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The Effect of Decreased Voice Quality in a VoIP Network

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A B S T R A C T

Voice over Internet Protocol (VOIP) is a telephone system telecommunication technology that uses an IP (Internet Protocol) network. VoIP offers numerous advantages for customers and communication, including cost savings, phone and service portability, mobility, and integration with other applications. Because voice is sensitive to delay and jitter, bandwidth must be guaranteed while it is being transported. There are some issues with Quality of Service (QoS), the most significant of which is latency in VOIP. The purpose of this paper is to investigate the effect of delay on speech quality in Voice Over Internet Protocol (VOIP). One source of delay is the buffer that already exists in the original multiplexing'-multicast node. There will be no buffering to store packets in the proposed multiplexing'-multicast algorithm, so any packet received will be sent immediately unless the packet arrives after a specific time or in a different order, where OPNET IT Guru Academic Edition simulation was used to analyze network performance.

1 Introduction

Voice over Internet Protocol (VoIP) is a method of transmitting audio and video over a network (Alhayajneh, A. et al.,2018) VOIP allows users to make and receive voice calls over the Internet. IP telephony is the transmission of voice, fax, and other services over packet switched IP-based networks (Odii, N. 2017) The VOIP infrastructure consist of telephone, control node, gateway node and the IP based network , the goal of VOIP is providing voice transmission over network, Reducing the latency is of prime importance to voice data services as it directly affects the acceptance trend of VoIP among mass consumers (Karim, K. 2020) VoIP converts analogue voice signals into digital data packets and supports real-time communication, including packet generation of digital singles using the Transport Control Protocol (TCP), User Datagram Protocol (UDP), and Internet Protocol (IP), as well as packet reception and analogue single reconstruction at the destination. The IP networks used the transmission control protocol (TCP) for transmission of the packet.

1.1 Multiplexing Algorithm

A multiplexer is a device that allows digital information from several sources to be routed onto a single line for transmission to a single destination (Vijayakumar. E et al, 2019) Multiplexes have the advantages of being able to transmit a high number of signals over a single channel, increasing management conversation visibility, and allowing users to utilize the entire bandwidth of the channel. Multiplexer has only one output which is connected to the single communication channel (Singh, C. 2018).

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1.2 Multicast Algorithm

Multicast is one of the mechanisms by which the power of the Internet can be further harnessed in an efficient manner (Pati, M. 2020) The benefit of multicast technology is that it supports dispersed applications and allows for greater bandwidth consumption. However, the disadvantages are that the drops are to be expected, there is no congestion avoidance, and data is duplicated, multicasting deals with forwarding packets to a set of destinations (Farhan, K. A et al., 2019).

2 Materials and Methods

The main goal of proposed algorithm is reducing the delay as most as we can. When we use the original algorithm, which combines multiple channels into one, the multiplexer sends all packets at the same time, and if one packet is delayed, all packets will wait for it. The proposed algorithm that will be used is sending the received packet immediately after receiving, will not be buffering to store the packet in it.

2.1 Original Multiplexing Algorithm

The transmission medium transports the signal from the sender to the receiver. To make the best use of that medium, we must ensure that the channel's bandwidth is used to its full potential. Multiplexing is a method of dividing the available bandwidth of a single transmission medium into multiple channels. (Liew, H et al 2002) This algorithm will discuss a number of variables that explain how multiplexing works.

Steps:

- ❖ Received: This variable refers to the number of packets received in order.
- ❖ Played: This variable indicates the sequence number of the packet being played out.
  - Over-run: This variable informs the play-out buffer that it is full.
  - The Time Division Multiplexer (TDM) which operates asynchronously to the packet arrival processing and is not defined here, empties the play-out buffer.
- ❖ Under-run: This variable indicates to that the play-out buffer been Expected.
- ❖ D: This variable indicates to the difference between expected and received.
- ❖ L: This variable indicates to difference between sequence numbers of packet

```
Upon receipt of a packet
if received = expected  { treat packet as in-order }
if not over-run then
        place packet contents into play out buffer
else
        discard packet contents
        set expected = (received + 1) mod 2^16
else
        calculate D = ( (expected-received) mod 2^16 ) - 2^15
if D > 0 then  { packets expected, expected+1, ... received-1 are lost }
while not over-run
place filler (all-ones or interpolation) into play out buffer
if not over-run then
     place packet contents into play out buffer
else
     discard packet contents
    set expected = (received + 1) mod 2^16
else { late packet arrived }
        declare "received" to be a late packet
        do not update "expected"
        either
                discard packet
calculate L = ( (played-received) mod 2^16 ) - 2^15
        if 0 < L <= R then
replace data from packet previously marked as lost
else
        discard packet
```

Figure (1). Original Multiplexing Algorithm (Liew, H et al 2002).

2.2 The Multicast Algorithm

The data in multiplexing divides a communication channel into several number of logical channels where in broadcast, the sender will send a copy to all the member of network, but sometime, there is no need to send to all the members for many reasons, such as, some data is not supposed to reach some employees and other reason, the overload will make an effect on the performance of the network. In multicast transmission, the sender transmits only one copy of data that is delivered to multiple receivers. VoIP's request is based on its capability to make possible for voice and data convergence at an application layer. The big challenge in multicasting is to minimize the amount of network resources utilized to compute and setup multicast trees. When the packets are small and consist of a small amount of data, the loss of packet will have less impact of the quality.

There are many variables that will be mentioned in this algorithm that explain the way that Multicast algorithm works.

Steps:

Socket: through the socket, adding the member to the group or remove the member from the group will be done through the socket.

Port: every data transferred through a network use a port number to distinguish it from other data.

IP address range: in this algorithm, we are going to use IP address included in this range 224.0.0.100.

The Server Side
Open the connection // through the socket
Chose the group that will receive the data
if sender = expected
receive the sender packet
set expected = (received + 1) mod 2^16
else
calculate D = ((expected-received) mod 2^16) - 2^15
Chose the type of socket
Chose the protocol // in our algorithm UDP will be used.
Put the IP multicast range // such as 244.100.0.1 that mentioned before
Put the port number //such as 9050 that mentioned before
IP End Point = new IP Endpoint (range, port)
Data=" this variable will include the data"
Server.send (IPEndPoint, Data)

Figure (2). Multicast Algorithm.

2.3 The Proposed multiplexer Algorithm

The problem can be solved using multiplexer – multicast algorithm that combines the data which comes from several streams into a single large packet, where the packet that comes first is sent over multiplexer for multicast. The technique that will be used is combines the data which comes from several streams into a single large packet, where the packet that comes first is sent over multiplexer for multicast**.** The differences between the two algorithms, the source of latency in the original algorithm is a buffer, and any packet that comes, the algorithm will check the buffer to see whether there is a space in the buffer or not, so, the elements in the buffer will be counted, then the decision will be making. This process will time consuming will result in increasing the latency algorithm, but in the proposed algorithm, there is no such a processing for the packet to be put in the buffer. The Proposed algorithm is show in Figure (3).

Upon receipt of a packet
if received = expected
send the received packet
set expected = (received + 1) mod 2^16
else
calculate D = ((expected-received) mod 2^16) - 2^15
if D > 0 then
{ packets expected, expected+1, ... received-1 are lost
Send the received packet
set expected = (received + 1) mod 2^16}
else { late packet arrived }
discard packet

Figure (3). Proposed Algorithm.

2.4 VoIP Deployment

Flowchart below gives an 8-step methodology for a successful VoIP deployment. The first four steps can be performed in parallel. Before embarking on the simulation in step 7, step 5 must be carried out which requires upfront modification to the existing network. Both step 6 and 7 can be done sequentially. The final step is the pilot deployment.

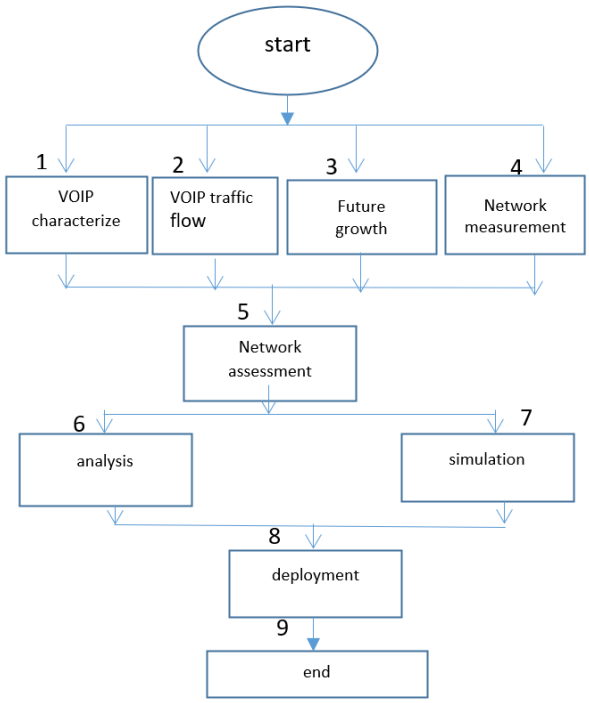

Figure (4). Flowchart Illustrating VoIP Deployment

2.4.1 VoIP Traffic Characteristics, Requirements and Assumptions

The configuration of the workstations will be as the default configuration except that we need to select the supported profile in the workstations in subnet1 which will send the packets, and also, we need to configure the supported services in the workstation in subnet2 in order to receive the packets

2.4.2 VoIP Traffic

Using the predefined voice application as a model for VoIP traffic in OPNET becomes a approach. In OPNET, an application is a collection of tasks, each of which is defined as a set of phases, each of which occurs between two endpoints and has configurable traffic behavior. Applications can be defined and configured using the Application Definition, the application are already defined usually give some flexibility to the user to configure their attributes such as, the configurable parameters.

2.4.3 Growth Capacity

The growth expected of the network has to be all taken into consideration to extrapolate the required growth capacity. To achieve the desired output, The OPNET simulation has been configured to be 20 minutes. Network resources of the router, Workstation, VoIP Application and Profile Settings. This percentage in practice can be variable for each network resource and may depend on the current utilization and the required growth capacity.

2.4.4 Network Measurements

In this step, the existing network based on the existing traffic and the requirements of the new service to be deployed. Immediate modifications to the network may include adding and placing new servers or devices, upgrading PCs, and utilized links.

2.4.5 Network Assessment /Modification

We need to change the default configurations of some network components in order to develop a realistic model. The following Figure (5) describes the configuration changes applied to each of the components of the network.

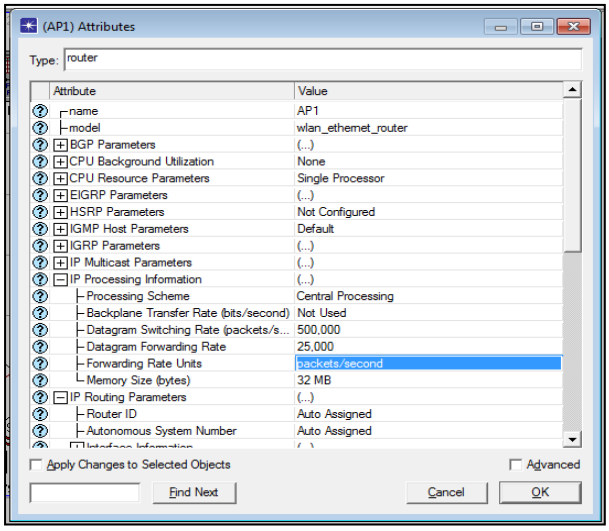

Figure (5). The configuration changes to components the network

2.4.6 Analysis

In order to gain accuracy of the simulation results, five simulation replications were run. Simulation replications were sufficient. Each simulation replication produced very similar graphical results.

Figure (7 and 8) show that the proposed algorithm has a shorter end-to-end delay than the original algorithm. The original algorithm's source of delay is the buffer that already exists in the multiplexing node, as well as the process that can be done in the packets before sending them to the multicast node.

Bandwidth Bottleneck Analysis

A bandwidth constraint is a phenomenon in which the performance of a network is limited because not enough bandwidth is available to ensure that all data packets in the network reach their destination. A network bottleneck occurs when a system working on a network delivers a higher volume of data than what is supported by the network's existing capacity.

Delay Analysis

This delay limits the amount of time that can be sustained. It should be noted that the primary goal is to determine the VoIP network capacity, that is, the maximum number of calls that the existing network can support while maintaining VoIP QoS. This can be accomplished by gradually adding calls to the network while monitoring on the VoIP delay threshold or bound. When the end-to-end delay, including network delay, exceeds 150ms, the maximum number of calls can be determined.

Comparison between Original and proposed algorithm

For the proposed algorithm, the latency will be reduced since the received packet will be transmitted directly to the next receiver as we will clarify it in Table (1).

Source	Original Algorithm	Proposed Algorithm
Source of Latency	Existing in the following: If received = expected If not over-run then Place packet contents into play out buffer - There is a buffer to be checked for a space and the elements in the buffer will be counted. - Consuming Time. -Increase Latency, packet stay in buffer for a *T* time.	If received = expected Send the received packet - No process to keep packet in buffer. - There is no buffer

Table (1). A comparison between Original and proposed algorithm

The source of delay in the original algorithm existing in the following:

if received = expected

if not over-run then
insert packet contents into playout buffer

there is a buffer, and any packet that comes, the algorithm will check the buffer to see whether there is a space in the buffer or not, this process will consume a time which as result will increase the latency.

In the proposed algorithm, there is no such a processing for the packet

if received = expected
send the received packet

2.4.7 Simulation

Models were simulated using OPNET on a Toshiba laptop running Windows 7.
The simulation approach uses the popular OPNET IT Guru Academic Edition simulation. There are many features of OPNET such as comprehensive library of network protocols and models, GU I interface, graphical results and statistics.

Modeling the Network

The popular OPNET IT Guru Academic Edition simulation, Release: 9.1, is used in the simulation approach. OPNET Modeler already has a large number of models of available system components, as well as various network simulator configuration capabilities. As a result, the simulation of a real-world network environment is very close to reality. OPNET also includes a comprehensive library of network protocols and models, a GU I interface, graphical results and statistics, source code for all models, and many other features. The fact that OPNET is offered free of charge to academic Universities and institutions is even more significant for its popularity in academia. This is why OPNET has an academic advantage over DES NS2.

Simulation Procedure

Subnet1 has been modeled as a subnet that encloses a router and five Ethernet workstation used to model the LAN users activities. AS it shown in the subnet 1, the five workstations will generate the traffic, that means the workstations that labeled with SUB1_WS1, SUB1_WS2, SUB1_WS3, SUB1_WS4, and SUB1_WS5 is a source for sending VoIP calls. AS it shown in the subnet 2, the five workstations will received the traffic, that means the workstations that labeled with SUB2_WS1, SUB2_WS2, SUB2_WS3, SUB2_WS4, and SUB2_WS5 is a sink that will receive VoIP calls**.** The simulation run can take up to 20 minutes. The simulation approach, as it will be demonstrated, is automated.

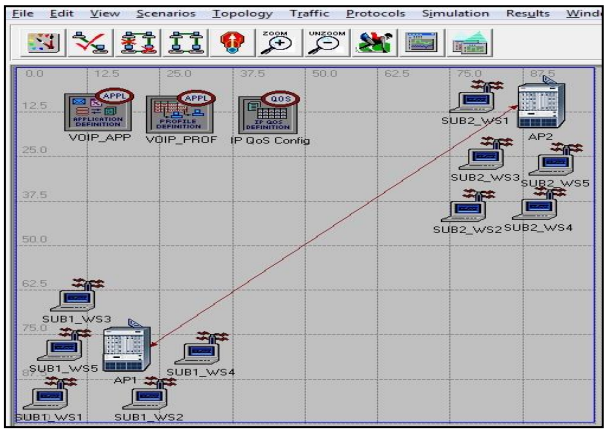

Figure (6). Shows the described topology

2.4.8 Pilot Deployment

A pilot deployment is the place for the network engineers, support and maintenance team to get firsthand experience with VoIP systems and their behavior. During the pilot deployment, the new VoIP devices and equipment are evaluated, configured, tuned, tested, managed and monitored, During the pilot deployment, the new VoIP devices and equipment are evaluated, configured, tuned, tested, managed and monitored.

3 Simulation Results

Using the predefined voice application as a model for VoIP traffic in OPNET becomes an approach. In OPNET, an application is a collection of tasks, each of which is defined as a set of phases, occurs between two endpoints and has configurable traffic behavior.
The graph (7) appears that the maximum point of the delay after running the simulation for 20 minutes is approximately 1.65 second in the original algorithm.

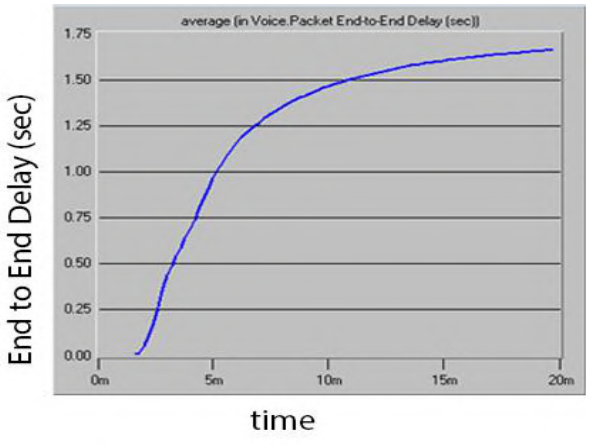

Figure (7). End-to-End delay in the original algorithm

Figure (8) shows how the End – to – End delay increases as the number of calls increases over the network's lifetime. The maximum point of the End to End time is approximately 0.5 second. The processing that will be done in the multiplexer node is the cause of this minor delay.

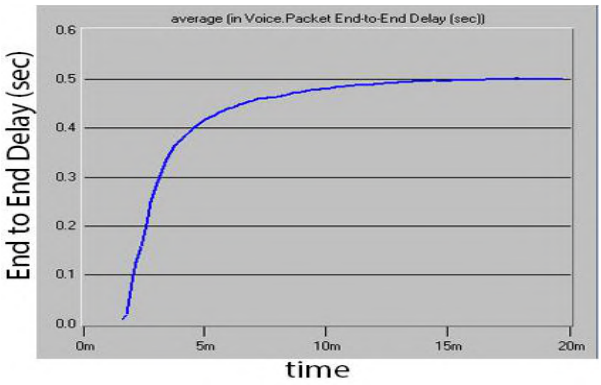

Figure (8). End-to-End delay in proposed algorithm.

4 Conclusions

The simulation route takes about 20 minutes and produced 46975482 events. According to Figure (7 and 8), the proposed algorithm has a shorter end-to-end delay than the original algorithm. The buffer that already exists in the multiplexing-multicast node is the source of the delay in the original algorithm.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Expression of Deubiquitinating Enzymes in Lung Cancer Cells with Different REST Status

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A B S T R A C T

The aims of this study were: (1) to screen a panel of lung cell lines for expression of the proteins UCHL1, USP49, USP15, USP4, and USP11. (2) To investigate the correlation of transcript and protein expression level of these DUBs. (3) To find any proteins that discriminate non-small cell lung cancer (NSCLC) from neuroendocrine (NE) SCLC or benign lung carcinoid. (4) To investigate any correlation of DUB proteins expression with lung cancer sub-types that has different amounts of REST protein.

Method: Western blots were performed to detect the expression of DUB expression (USP4, USP11, USP15, USP49, and UCHL1) and REST protein in extracts from normal lung cell lines, SCLC, carcinoid, and NSCLC

Results: we found that the transcript level was in general not a good indicator of DUB protein level. There was a significant positive correlation of REST with DUB proteins from whole-cell extracts for USP15, USP11, USP4, and USP49 in normal lung cell lines. The P values were 0.016, 0.002, 0 .018 and 0 .034 respectively. Also in both normal and NSCLC cell lines total USP15, USP11 and USP49 showed a positive correlation with REST. P values were 0.007, 0.029 and 0.001 respectively. However USP11 protein was highly abundant in REST-deficient SCLC. In contrast, both USP11 and USP15 showed a positive correlation with REST in SCLC and NSCLC nuclear extracts. P values were 0.001 and 0.005 respectively. A positive correlation of DUBs with REST would be consistent with their putative role in stabilizing REST.

1 Introduction

Ubiquitin (Ub) is a highly conserved protein composed of 76 amino acid and plays an important role in protein-degradation. Ubiquitin is essential in many cell-mechanisms and biological processes, including the cell cycle, growth control and prevention of neurodegeneration (Amerik & Hochstrasser, 2004). It is also involved in the organizing and stability of many proteins. Ubiquitin does not degrade proteins by itself, but it works just as a mark of proteins that will then be degraded by the 26S proteasome. The ubiquitin- protein conjugation requires three types of enzymes, ubiquitin activating enzymes (E1) which is activated the C-terminal glycine in ATP, then the activated ubiquitin transmitted to cysteine residue of ubiquitin conjugating enzymes (E2) and finally catalyzed by ubiquitin ligase (E3) enzymes to which the substrate protein is specifically bound (Haglund & Dikic, 2005).

Similar to phosphorylation, ubiquitination is a reversible process. Hydrolysis of ubiquitin or ubiquitin chains from proteins are catalyzed by deubiquitinating enzymes (DUBs) in cells (Komander, Clague, & Urbe, 2009).

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In mammals there are about one hundred DUBs, which can be divided into five sub-classes based on their ubiquitin-protease domains. These are ubiquitin-specific proteases (USPs), ubiquitin C-terminal hydrolases (UCHs), ovarian tumour proteases (OTUs), Josephins and JAB1/MPN/MOV34 metalloenzymes (JAMMs). DUBs can play an important role in regulating many tumour suppressors and oncoproteins (Sacco, 2010). There are a large number of DUBs which are linked to tumors such as USP4 which was identified as oncoprotein related to lung cancer (Gray *et al*., 1995).

Ubiquitin is becoming an interesting target to find biomarkers or drugs for a variety of diseases. These drugs might prevent protein-degradation or could activate the system to damage proteins which we do not need.

Lung cancer is the leading cause of cancer death between both women and men in the worldwide and has poor prognosis. It is divided in small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC). SCLC accounts for approximately 20%, while NSCLC accounts for 75% of all lung cancers. Early detection and diagnosis are the most important factors in determining the outcome in lung cancer patients (Bührens, Amelung, Reymond, & Beshay, 2009). Both SCLC and lung Carcinioid express neuroendocrine markers, whereas SCLC is the most aggressive neuroendocrine lung tumors, Carcinioid lung cancer is a benign cancer (Reports-Mortality., February 2007). Although NSCLC don't have neuroendocrine gene, they express repressor element 1-silencing transcription factor (REST) at different levels (Coulson, Ocejo-Garcia, & Woll, 2003).

The repressor element-1 silencing transcription factor, which is also known as neuron restrictive silencer factor (REST/NRSF) is a transcriptional suppression factor that acts as repressor. REST plays an essential role in many biological processes in human medulloblastomas REST is overexpressed (Coulson, 2005). REST is an interactor of the F-box protein β-TrCP and is degraded by the ubiquitin ligase SCF β-TrCP through the G2 phase of the cell cycle to allow transcriptional derepression of Mad (Guardavaccaro, 2008; Westbrook, Martin, & Schlabach, 2005). It shows interaction with a number of proteins, many of which are required for its repressor function, including histone deacetylases HDAC1 and HDAC2 There is a splice variant of REST that has associated with SCLC (Coulson & *et al*, 2003).

We, therefore, thought there might be a DUB that can revere SCFβTRCP mediated REST ubiquitation and this may be changed REST-deficient in neuroendocrine lung cancer. Specific DUBs may have a role in the REST - stabilization. The specificity of DUB in stabilization of proteins dysregulated in cancer could make them potential drug targets (Daviet & *et al*., 2008). Therefore, we here will focus on five DUBs. The ubiquitin Carboxyl-terminal Hydrolase-L1 gene (UCHL1) and the ubiquitin-specific protease USP49 and USP15 are three candidates that arose from library a siRNA screen for DUBs that may reverse REST ubiquitination. USP4 and USP11 DUBs are closely related to USP15 (Westbrook & *et a*l., 2008).

The aims of this project were (1) to screen a panel of lung cell lines for expression of the proteins UCHL1, USP49, USP15, USP4 and USP11. (2) To investigate the correlation of transcript and protein expression level of these DUBs. (3) To find any proteins that discriminate non-small cell lung cancer (NSCLC) from neuroendocrine (NE) SCLC or benign lung carcinoid by compare the results which is might be identified a potential protein biomarkers. (4) To investigate any correlation of DUB protein expression with lung cancer sub-types that has different amounts of REST protein.

2 Materials and Methods

Cell Culture. In this study, four types of human lung cell lines have been used; Normal cell lung lines were normal human bronchial epithelium (NHBE), normal lung fibroblasts (MRC5), SV40 transformed human bronchial epithelium (BEAS2B and SV40-HBE), SV40 transformed Normal lung fibroblasts (MRC5VA) and SV40 transformed embryonic lung fibroblast (WI38-VA13). Small cell lung cancer (SCLC) cell lines Lu-165, GLC19, NCI-H69,

NCI-H345, COR-L88, COR-L47 and U2020. A lung carcinoid line NCI-H727. Finally non-small lung cancers (NSCLC): NCI-H460, NCI-H322, NCI-H647, NCI-H2170, COR-L23 and A549. All cells were maintained in RPMI culture medium with 10 % BCS and were incubated in 5 % CO2 at 37°C.

Protein extraction: whole cell extracts were prepared by rinsing cells in PBS before they were lysed in 1x laemmli buffer (50 mM Tris pH 6.8, 2% SDS, 10% glycerol and made up to 50 ml) and heated at 1100C for 10 minutes with vortexing every 2 minutes. Then they were diluted 1:5 in laemmli buffer. Nuclear and cytoplasmic protein fractions were extracted by scraping cells in 5 ml of ice cold phosphate- buffered saline (PBS) and collection by centrifuging the pellet was resuspended in 500 µl NP40 (150mM NaCl, 1% NP-40, 50mM Tris pH7.5, 1.5mM EDTA ,2 ug/ml Aprotonin, 2µg/ml Leupeptin, 1mM PMSF, H20). Samples were incubated on ice for 10 min with vortexing. To pellet nuclei samples were centrifuged at 16,000 xg for 5 min and the supernatant collected as the cytoplasmic fraction. To obtain the nuclear extract the cell pellet was resuspended in 40 µl Dignam buffer (20 mM HEPES pH 7.9, 25% glycerol, 0.42 M Nacl, 1.5 mM EDTA and protease inhibitors 0.5 mM DTT) and incubated in a cold room for 40 mins with vortexing at 2000 rpm. Supernatant was collected as the nuclear fraction. All protein extracts were quantified by BCA assay.

Western Blotting: Western blots were performed to detect the expression of seven proteins expression (USP4, USP11, USP15, USP49, UCHL1, and REST) in extracts from normal lung cell lines, SCLC, carcinoid and NSCLC. 10 µg of whole cells protein extract, 5 µg of cytoplasmic protein fraction or 2.5 µg of nuclear protein fraction was separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis using either 8 % or 10% polyacrylamide gel depending on molecular weight of protein of interest. Gels were run for 1 hour at 200 V. Perfect protein and Rainbow molecular weight markers were loaded in first and last lane of each gel for size standardization. Proteins were transferred to nitrocellulose membranes (Schleicher & Schuell) for 60 min at 21-22 V in transfer buffer (14.4 gm Glysine, 3.03 gm Tris , 200 ml methanol and mad up to 1000 ml). Membranes were cut to three pieces at 75 kDa and 150 kDa. The first piece from 20-75 kDa was blotted with anti actin and tubulin. The second piece from 75-150 kDa was blotted with anti-DUBs (USP4, USP15 and USP11). The third piece from 150-225 kDa was blotted with anti- REST. Nitrocellulose membranes were blocked with 5% nonfat dried milk (NFDM) in TBST (TBS 0.1% Tween 20) for all proteins except REST, which was blocked with 3% NFDM in phosphate- buffered saline (PBS) to block nonspecific sites. Then blots were incubated with primary antibody: mouse monoclonal anti Beta-Actin (1:10000, Abcam), mouse monoclonal anti-Tubulin (1:10000) were from Sigma. Rabbit anti-USP11 (1:1000), rabbit anti-USP15 (1:1000) and rabbit anti-USP4 (1:2000) were from Bethyl. Polyclonal rabbit anti-REST (1:2000) was from Millipore, and polyclonal goat anti-USP49 (1/200). Polyclonal rabbit anti-UCHL1 (1:1000) was from Abcam. The USP4, USP11, USP15, USP49 and UCHL1 primary antibodies were diluted in TBST with 5% NFDM and incubated overnight at 40C. While the REST antibody was diluted in TBS with 3 % NFDM and incubated overnight at 40C. We used anti-mouse with actin and tubulin as secondary antibodies. Anti-rabbit for all DUBs and REST protein. Proteins were quantified using Odyssey® Infrared Imaging. Then all proteins normalized to actin.

3 Results

3.1 Expression of DUBs.

DUB expression was tested by immunoblotting in lung cancer and then, correlate with mRNA, which we measured previously in the first rotation by qRT-PCR to investigate any correlation between transcript-protein levels. To test whether there was any correlation between DUB protein and transcript levels, DUB proteins in whole lung cancer cell lines and normal lung cell lines was examined using immunoblot analysis to detect one protein within a mixture of others. In this study, whole cell protein extracts were used from twenty different lung cell lines: six normal cell lines (three epithelial and three fibroblast cell lines), seven SCLC, six NSCLC and one lung carcinoid cell line. We investigated expression for five DUBs USP15, USP11, USP4, USP49 and UCHL1 that may reverse REST ubiqutination. Replicate gels were run for several reasons. Firstly, USP15, USP11 and USP4 proteins migrate with similar molecular weights 100-120 kDa. Secondly, USP49 and UCHL1 required a different percentage of gel because they had small molecular weight. REST protein was detected from the same gel as USP11. REST protein was detected in both normal and NSCLC, while it was absent in all SCLC and carcinoid lung cell lines as shown in figure 1. USP11 and UCHL1 proteins were overexpressed in SCLC compare with normal and NSCLC lung cell lines. Also, there was a variety of USP4 and USP49 expression in different cell lung lines. USP15 protein was more in normal lung cell lines compare with lung cancer cell lines.

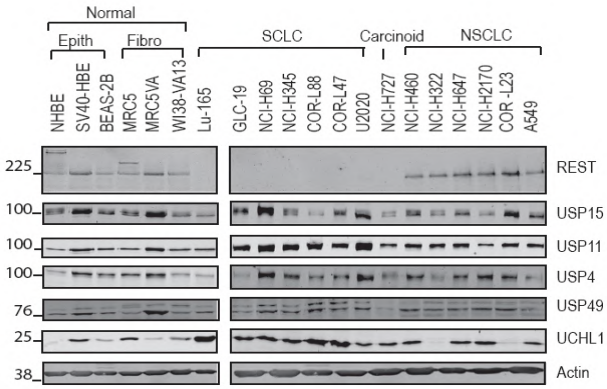

Figure (1). Immunoblot analysis of different DUB protein in whole cell protein lysate from 20 cell lung cell lines. Blots demonstrate REST, USP15, USP11, USP4, USP49 and UCHL1 protein in normal epithelial and fibroblast (Epith, Fibro); small cell lung (SCLC); carcinoid and non-small lung cell cancer (NSCLC). Actin protein was used as control to correct for protein loading. All proteins were quantified using Odyssey and normalized to actin then expressed to relative a sample of NCI- H647 which was analyzed on every gel.

Next, we quantified proteins using Odyssey and correlated to mRNA from previous project which measured by qRT-PCR. Overall, there was not a good correlation between DUB proteins and their transcript levels in the 17 lung cell lines (table1). The exceptions were UCHL1 protein, which has some evidence of correlation in normal and NSCLC lung cell lines, but it was lost in SCLC (figure 2). Interestingly, USP11iso 2 had some correlation in NSCLC. This may be the isoform which is transcript.

	Lung cell line sub-type			
DUB transcript	normal	SCLC	NSCLC	all
USP4 iso1	0.0771	0.4648 (-ve)	0.1762	0.059
USP11 iso1	0.035	0.048	0.0185	0.0003
USP11 iso2	0.0022	0.034	**0.3769 (+ve)**	0.0317
USP15 iso1	**0.9811 (+ ve)**	0.0026	0.1604	0.0105
USP15 iso2	0.5038 (-ve)	0.0031	0.0329	0.0213
UCHL1	**0.46 (+ve)**	0.0151	**0.2951 (+ve)**	0.0087

Table (1). Correlation of DUB protein-transcript levels. R^2 values are shown for lung cell lines estimate between USP4 iso1, USP11 iso1 and 2, USP15 iso 1 and 2, and UCHL1 transcript determined by qRT-PCR and whole cell lung protein extracts in 17 lung cell lines.

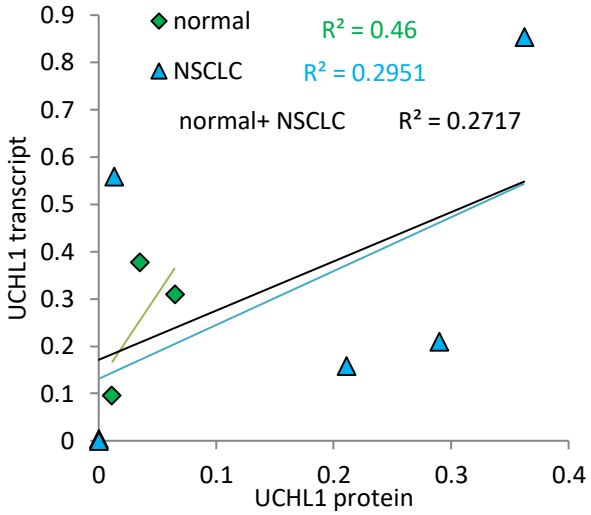

Figure (2). Example of transcript-protein levels correlation. UCHL1 Whole cell protein extracted from normal (n=3) and NSCLC (n=6) lung cell lines. UCHL1 transcript was quantified by qRT-PCR and UCHL1 protein was quantified by immunoblot. Both UCHL1 transcript and protein were normalized to Actin as the reference gene/ protein.

3.2 Determination of Correlation Induvial DUB Protein-REST Levels

Afterwards, we tested whether there was any correlation between total DUB protein and REST protein in a panel of lung cell lines. Looking for DUB levels may be related to REST. We found that there was a significant positive correlation for all DUBs except for UCHL1 (P= 0.31) in normal lung cell lines (table 2). However, this association was reduced in NSCLC for all DUBs except USP49 (P= 0.029) (figure 3).

Table (2). Correlation between DUB and REST protein levels. R^2 values are shown the correlation of DUBs (USP4, USP11, USP15, USP49 and UCHL1) and REST protein in a panel of 20 lung cell lines.

DUB	Lung cell line sub-type				
	Normal	SCLC	NSCLC	all	SCLC + NSCLC
USP4	**0.7871 (+ve)**	N/D	**0.0823 (+ve)**	0.063	0.097
USP11	**0.9345 (+ve)**	N/D	0.0007	0.118	**0.394 (+ve)**
USP15	**0.8018 (+ve)**	N/D	**0.3132 (+ve)**	0.0265	**0.532 (+ve)**
USP49	**0.7157 (+ve)**	N/D	**0.7343 (+ ve)**	0.0002	**0.656 (+ve)**
UCHL1	**0.2525 (+ve)**	N/D	0.066 (–ve)	0.2122 (-ve)	0.022(-ve)

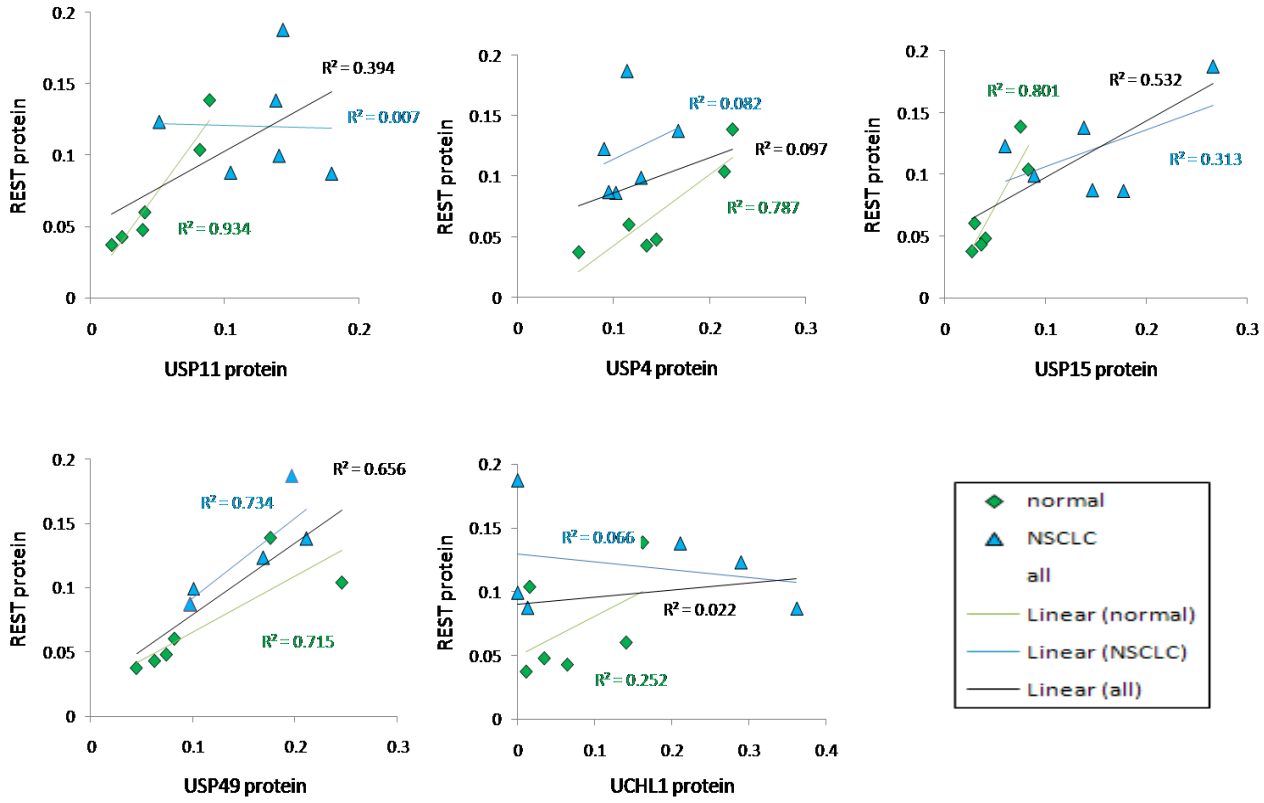

Figure (3). Correlation between DUB and REST protein levels. R^2 values are shown the correlation between whole cell DUB protein (USP11, USP4, USP15, USP49 and UCHL1) and REST protein. All DUB protein was normalized to actin then expressed to relative a sample of NCI- H647 which was analyzed on every gel.

3.3 Determination of DUB Distribution Between Nuclear and Cytoplasmic Protein Extracts from Lung Cell Lines

To determine whether these DUBs were predominately associated with cytoplasm or Nucleus in lung derived cell lines we prepared fractionated cell. It found some evidence all DUBs in the nuclear fraction except UCHL1 in all cell lines (figure 4). Immunobloting for REST was carried out twice, once when the fractions were fresh (a), and REST (b) was detected after freezing and thawing samples. There were some correlation between USP11 and REST in nuclear fraction therefore; we tested USP11 expression in normal lung cell lines in cytoplasmic and nuclear fractions (figure 4 B). Also, there was associated between USP11 and REST proteins in nuclear fraction.

3.4 Correlation of DUB and REST Protein in Fractionated Cells

Tested the correlation between DUBs (USP11, USP15 and USP49) and REST protein in cytoplasmic and nuclear fractions was performed. It had found that, a positive correlation of USP11 (P= 0.001) and USP15 (P= 0.005) with REST protein in SCLC and NSCLC nuclear extracts rather than in cytoplasm fraction. Also, there was positive correlation in SCLC with USP49 protein as shown in (table 3).

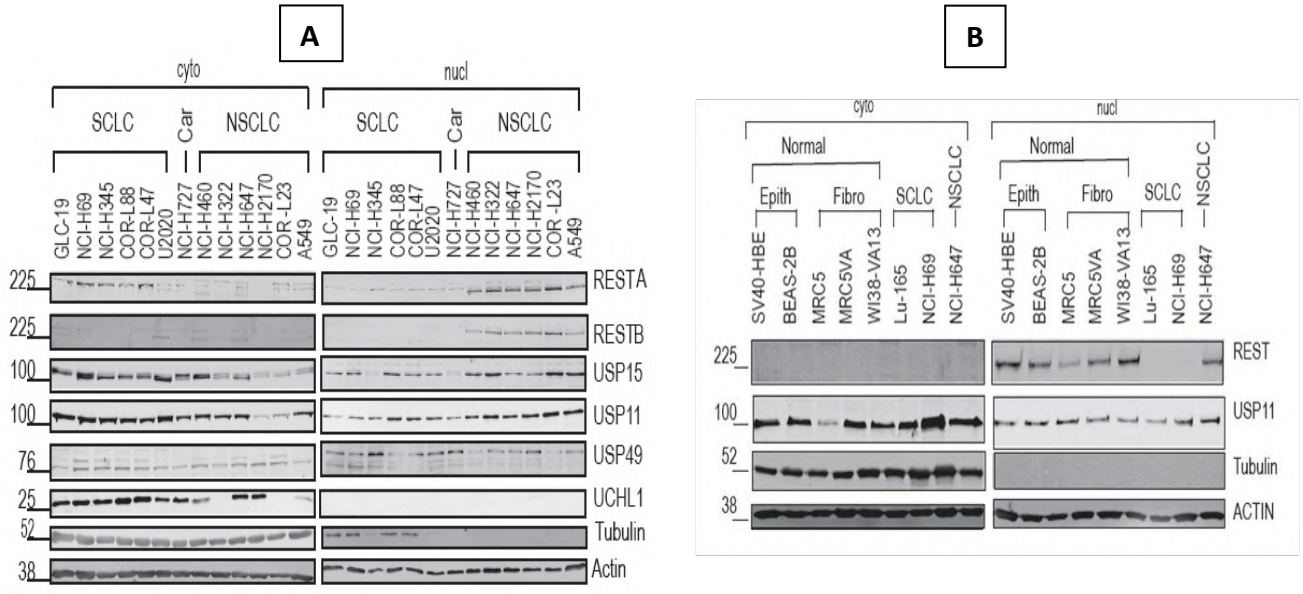

Figure (4). Enrichment of DUB proteins in cytoplasmic or nuclear fractions of cells. All DUB proteins (USP15, USP11, USP49 and UCHL1) and REST protein were tested in cytoplasmic (cyto) and nuclear (nucl) fractions of; A) SCLC, NSCLC and carcinoid (Car) lung cell lines. B) In normal lung epithelia (Epith) and fibroblast (Fibro) lung cell lines. Blotted with actin for normalization and tubulin to check fractionation**.**

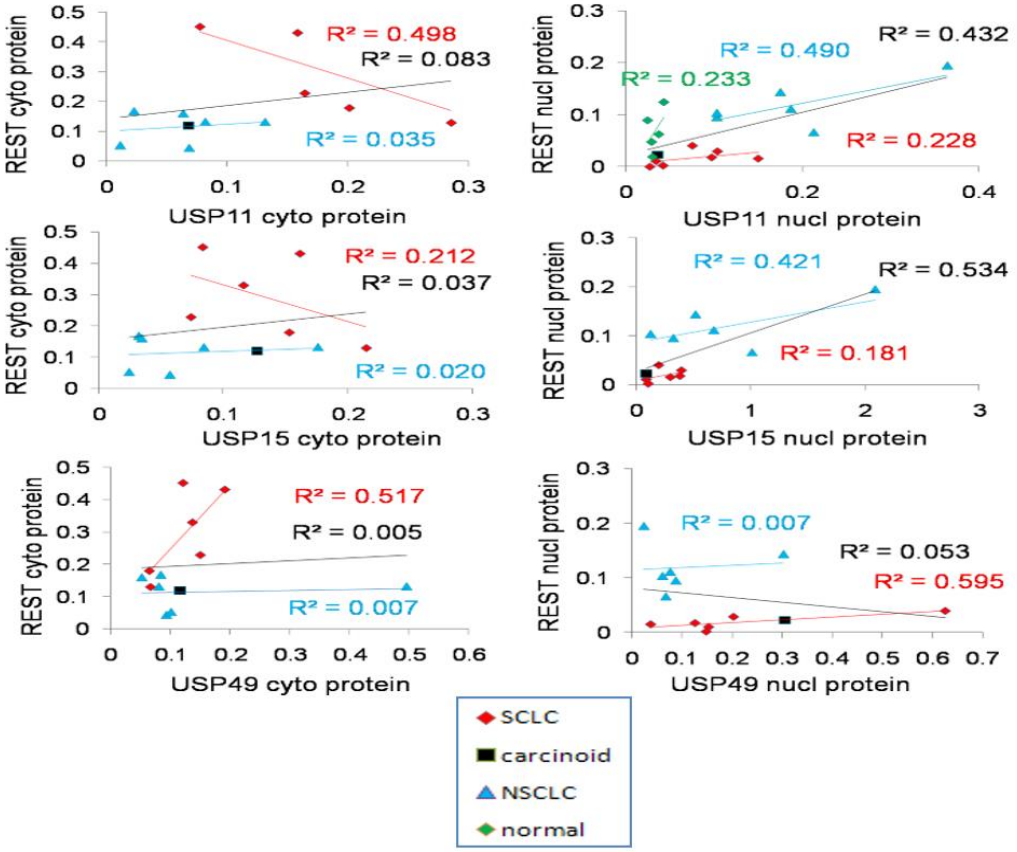

Figure (5). The correlation between DUB and REST proteins in cytoplasmic (cyto) and nuclear (nucl) fractions. All DUB proteins were normalized to Actin and then expressed relative to NCI- H647 on the same gel.

Table (3). The correlation between DUB and REST proteins in cytoplasmic (cyto) and nuclear (nucl) fractions. R^2 values of the correlation of REST- DUB proteins.

Sub-type (CYTO)	Normal	SCLC	NSCLC	all	SCLC+ NSCLC
USP4	not done	not done	not done	not done	not done
USP11	Not detected	0.4989 (-ve)	0.0354	0.0832	0.0832
USP15	Not done	0.2123 (-ve)	0.209	0.0379	0.049
USP49	Not done	**0.5175 (+ve)**	0.0076	0.0058	0.004
UCHL1	Not done	0.1417	0.0008	0.3277	0.359
Sub-type (NUCL)	Normal	SCLC	NSCLC	all	SCLC+ NSCLC
USP4	not done	not done	not done	not done	not done
USP11	0.233	**0.228 (+ve)**	0.4908 (+ve)	**0.4326 (+ve)**	**0.6732 (+ve)**
USP15	not done	**0.1811 (+ve)**	0.4212 (+ve)	**0.534 (+ve)**	0.0514
USP49	not done	**0.5958 (+ve)**	0.007	0.007	0.007
UCHL1	not done	not detected	not detected	not detected	not detected

4 Discussion

Studies of protein expression in human cancers have led to identification of many polypeptides as markers, or as useful tool for diagnostic. In this study, the expression of USP15, USP11, USP4 and UCHL1 proteins in a panel of 17 lung cell lines was measured. We compared data with the transcript of these DUBs, which we had measured previously. We found that there was not a good correlation between the difference DUB isoforms transcript and protein levels. There were some evidences of correlation between USP11iso 2 transcript and USP11 protein. This suggests that it could be most USP11, which was expressed is USP11 iso2, but we cannot predict the level. Our data suggests that estimation of transcript is not a good indication of DUB protein level. There was agreement of our data with research done recently by Shebl and *et al* (Shebl *et al.*, 2010) on cytokines in HPV which shows that, the correlation between gene and protein levels were variable among different cytokines. Whereas, our data were in disagreement with study by Celis and Coworkers (Celis *et al*., 2000) microarray study in bladder cancer, they found a good correlation between protein and transcript levels.

This may result of translation- modification or could result of protein -ubiquitination which could influence the stability of protein. A significant correlation was reported between whole cell DUB proteins) (USP15, USP11, USP4 and USP49) and REST protein in normal lung cell lines and in cancer cell lines (NSCLC and carcinoid) except with UCHL1 protein. This may due to UCHL1 and REST proteins are little in the same comportment because when we test UCHL1 in nuclear and cytoplasm fraction we found that, UCHL1 protein was manly in cytoplasm while REST protein was manly in nuclear. USP11 has an important role in regulation and stabilizing the HPV-16E7 by reducing ubiqutination (Lin, 2008)and it also controls an IkB (Yamaguchi, 2007). It has been identified of USP15 in regulation of E6 protein (Vos, 2009) and it is also implicated in stabilization of APC (Huang, 2009).

When we compare the correlation between whole cell, cytoplasm and nuclear protein, it was obvious that USP11 (P= 0.001) and USP15 (P= 0.005) showed a significant positive correlation with REST in SCLC and NSCLC nuclear fraction. The significant correlation of these DUBs with REST may be consistent with their putative role in stabilizing REST. The specificity of DUB in stabilization of proteins dysregulated in cancer could make them potential drug targets.

5 Conclusions

In summary the transcript level was in general not a good indicator of DUB protein level. There was a significant positive correlation of REST with DUB proteins from whole cell extracts for USP15, USP11, USP4 and USP49 in normal lung cell lines. Also in both normal and NSCLC cell lines total USP15, USP11 and USP49 showed positive correlation with REST. However, USP11 protein was highly abundant in REST -deficient SCLC. In contrast, both USP11 and USP15 showed a positive correlation with REST in SCLC and NSCLC nuclear extracts. A positive correlation of DUBs with REST would be consistent with their putative role in stabilizing REST.

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Conflict of Interest: The author declare that there are no conflicts of interest.

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Antibacterial Activity of *Cyclamen rohlfsianum* against two Species of *Xanthomonas*

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A B S T R A C T

Xanthomonas is one of the most important genera that leads to severe losses in many agricultural crops, the exertion to control is constrained to the utilisation of industrial pesticides, which have brought human and environmental health problems, and the emergence of resistant strains. This work was carried out to evaluate the activity of the aqueous extracts of leaves and tubers of *Cyclamen rohlfsianum* at concentrations (50, 100, 200 and 400) mg/ml against *(Xanthomonas campestris* pv. *vesicatoria* and *Xanthomonas axonopodis* pv. *eucalyptus)* by a sensitivity test by a disk diffusion method. The results showed that *Cyclamen rohlfsianum* extracts have good inhibitory activity against tested bacteria, the tubers extracts are more efficient than leaf extracts, a concentration of 400mg/ml was the most effective to control bacteria. *Xanthomonas axonopodis* pv. *eucalyptus* was the most sensitive to aqueous extracts. The study concluded that *Cyclamen rohlfsianum* showed promising antibacterial activity and effective control programs for the control of diseases caused by *Xanthomonas.*

1 Introduction

Plants are influenced by numerous illnesses caused by organisms, the predominance of illnesses can reach 70-80%. Depending on climate conditions and the phytosanitary condition of crops (Nazarov *et al*., 2020). Bacterial plant diseases are most severe in tropical and subtropical places, it's recorded in all countries agricultural crop high losses, especially in developing countries (Campos and Ortiz, 2020), from common symptoms of bacterial plant diseases are wilting, necrosis, spotting, rotting, blight, blisters (El-Meneisy *et al*., 2005). *Xanthomonas* are a large genus of Gram-negative bacteria, yellow-pigmented, rod-shaped, a single polar flagellum (Bradbury, 1984). The genus consists of 27 species that cause serious diseases in almost 400 plants including a wide variety of important crops such as rice, citrus, cabbage and pepper, including *Xanthomonas campestris* pv. *vesicatoria* and *Xanthomonas axonopodis* pv. *eucalyptus*, which causes diseases to tomato and eucalyptus (Ryan *et al*., 2011; Ferraz *et al*., 2018), Measures to combat it are limited to the use of antibiotics and industrial pesticides, especially those made up of copper compounds, due to their harmful side effects, the emergence of resistant strains, the trend has been to use natural alternatives with low environmental impact represented by medicinal plants (Gochez *et al*., 2018; Puigvert *et al*., 2019). *Cyclamen spp*. a small genus consisting of 21 species distributed in the Mediterranean region, Europe, Western Asia and North Africa. used as an ornamental plant in gardens (Yesson *et al*., 2009), a potential source of natural antioxidants and antimicrobial (Stanojevic *et al*., 2018). *Cyclamen rohlfsianum* is an endemic strain, only grows in Al-jabal Al-akhdar region, Libya, known locally as Al-Rakhf, a perennial tuberous herbaceous plant, belonging to the family Primulaceae (Salih and Abdulrraziq, 2021), It's classified as a poisonous plant because it contains Cyclamen glycoside (Chant, 1979), the plant has also been reported to show pharmacological properties to the therapy of diabetes, Its tubers are used in the process of fermenting milk to produce cheese (Elabbar *et al*., 2014). Several studies

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have been conducted in an attempt to combat various diseases caused by *Xanthomonas spp.* using plant extracts, where a study concluded in Libya an extract of leaves of *Arum cyreniacum* can be relied on to control of *X.campestris* pv.*vesicatoria* which cause tomato spots disease (Abdulrraziq *et al*., 2021). Furthermore, findings from a study that was conducted in India confirmed the effectiveness of alcoholic extracts of six types of medicinal plants in eliminating black rot of cauliflower caused by *Xanthomonas campestris* pv. *campestris* (Didwania *et al*., 2013). The results of a study conducted in Korea showed of a methanolic extract of *Pharbitis nil* seeds possesses a high inhibitory ability against *Xanthomonas aboricola* pv. *pruni*, that causing bacterial spot disease of peach (Nguyen *et al*., 2017). The results of another study indicated the possibility thyme oil and lemon grass oil effectively retarded pomegranate bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae* (Chowdappa *et al*., 2018).

Therefore, the study aimed to evaluate the efficacy of aqueous extracts of *Cyclamen rohlfsianum* to bio-control against two species *Xanthomonas*, which had never hitherto been established.

2 Materials and Methods

The study was carried out in Biology Department/ Faculty of education / Omar Al-Mukhtar University. Plant washing (leaves- Tubers) with distilled water and dried inside the laboratory under room temperature, grinded by an electric grinder and saved for use.

Figure (1): *Cyclamen rohlfsianum.*

2.1. Aqueous Extraction:

10 g of dry powder of the plant was added to 100 ml of sterile distilled water in a glass flask, put on a vibratory shaker for 24 hours at 35 ° C, then filter and shaken in a centrifuge at 3000 rpm for 10 minutes. The next step was filtered with Whitman No.1 filter paper and drying in a Rotary evaporator to get dry powder (Jigna *et al*., 2005). The concentration of 400 mg/ml was prepared by dissolving 4g of powder in 10 ml distilled water.

2.2. Bacterial Isolates:

Xanthomonas campestris pv. *vesicatoria* and *Xanthomonas axonopodis* pv. *eucalyptus*, from the collection of the Department of Plant Protection, Omar Al-Mukhtar University.

2.3. Antibacterial Susceptibility Testing:

The mediums were sterilized for 15 minutes in an autoclave at 121°C. bacteria were grown on Mueller-Hinton agar medium. For screening, sterilised filter paper disks (6 mm) and impregnated with the extract were placed on the surface of inoculated bacteria mediums. The dishes were incubated for 24 hours at 28°C with three replications per dish, With the use distilled water to control, then a measure of diameters of inhibitory zones minus the diameter of the disc (Driscoll *et al*., 2012).

Statistical Analysis:

The study's experiment was designed according to the Completely Randomized Design (CRD). The statistical analysis was performed using (Minitab 17) program and ANOVA test was carried out means were compared using Tukey's test at $P<0.05$.

3 Results

The results of antibacterial activity were recorded as a zone of inhibition in mm around the extract disk against the tested bacteria compared with the control. As shown in table(1), there were significant differences in the inhibitory activity of extracts of leaves and tubers of *Cyclamen rohlfsianum* against plant pathogenic bacteria, according to the type of microbe, concentration and type of part used. The concentration of 200 and 400 mg/ml of the tubers extract recorded good inhibitory activity against two species *Xanthomonas* with a diameter ranging (3.1-4.4) mm. The results also showed that the Tubers extract with a concentration of 50 and100 mg/ml had no inhibitory effect against the tested bacteria, except for a concentration of 100mg/ml against *X.axonopodis* pv. *eucalyptus* with a diameter (2.9) mm. The results showed that the leaves extract with a concentration of 50,100 and 200mg/ml had no inhibitory effect against the tested bacteria, except for a concentration of 200mg/ml against *X.axonopodis* pv. *eucalyptus*, which showed low sensitivity with a

diameter (1.4) mm, while both types of *Xanthomonas* were affected by the concentration of 400mg/ml with a diameter of inhibition that did not exceed (2.0) mm.

Table (1). Antibacterial activity of *Cyclamen rohlfsianum* aqueous extracts agints *Xanthomonas*. (Mean ± Standard Deviation).

Extract \ Bacteria		*X. campestris pv. vesicatoria* (mm)	*X. axonopodis pv. eucalyptus* (mm)
Leaves	**50 mg/l**	0.0 d	0.0 d
	100	0.0 d	0.0 d
	200	0.0 d	1.4±0.5 c
	400	1.2±0.1 c	2.0±0.3 c
Tubers	**50 mg/l**	0.0 d	0.0 d
	100	0.0 d	2.9±0.3 b
	200	3.1±0.3 b	3.6±0.4 ab
	400	3.5±0.2 a	4.4±0.3 a
Control		0.0 d	0.0 d

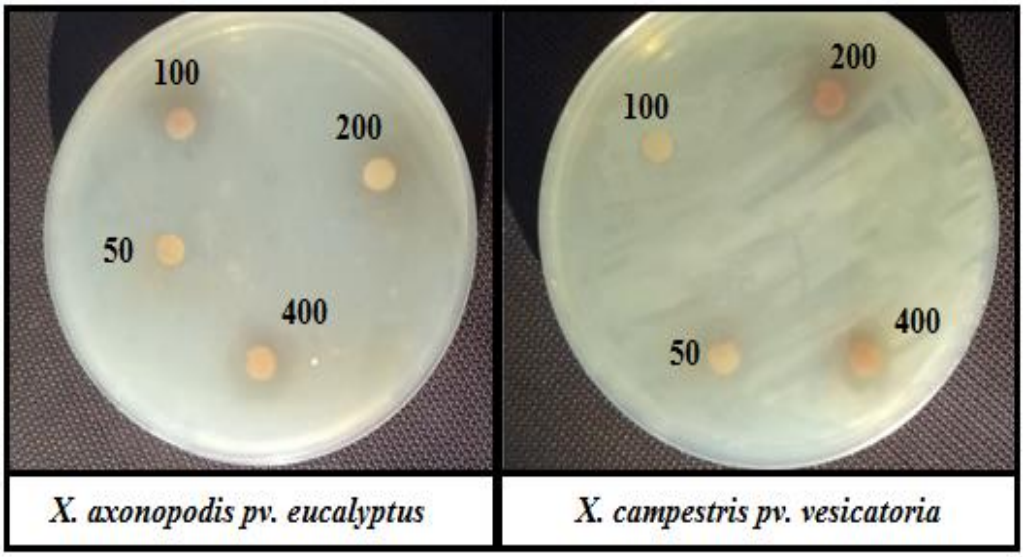

Figure (2): Effect of tubers extract of *Cyclamen rohlfsianum* against *Xanthomonas***.**

4 Discussion

Al-Jabal Al-Akhdar is considered as one of the most important Libyan regions in terms of its diversity of native medicinal plants, which it does not obtain a received of full studies Bio-activities (Agile and Mericli, 2017), So this study was conducted to which showed of *Cyclamen rohlfsianum* extracts possess good inhibitory activity against two species of Xanthomonas, This result was in agreement with (Yemata and Fetene, 2017; Abo-Elyousr *et al*., 2020 ; Leksomboon *et al*., 2001), where they confirmed the medicinal plants have promising antibacterial activity, and could be considered an effective tool in integrated control programs for a sustainable system of Plant diseases caused by *Xanthomonas* spp., The Inhibitory activity of *C. rohlfsianum* may be due to its content of alkaloids the presence of phenolics, triterpenoids, saponins, steroidal, compounds, Kaempferol, Genistein, Hesperetin, Oleanolic acid and 4, 7, 8-Trihydroxyflavone. (Elabbar *et al*., 2014). The results also showed the tuber extract was more efficient than the leaves extract, this result agreed with (Lee *et al*., 2016) when using the Tubers and leaves of a type of seaweed Zostera marina against some microbial species. The inhibitory activity increased with an increase in concentration, the concentration of 400mg/ml was the most effective against plant pathogenic bacteria. *X. campestris* pv. *vesicatoria* was

the most resistant, *X.axonopodis* pv. *eucalyptus* was the most sensitivity to aqueous extracts of *Cyclamen rohlfsianum*.

5 Conclusions

This study concludes that *Cyclamen rohlfsianum* (tubers and leaves) possesses inhibitory activity against the plant pathogenic bacteria, and the most efficacy is reached with increasing concentration. The best inhibition activity of aqueous extracts against tested bacteria was obtained with 400 mg/mL. Commonly, *X.campestris* pv. *vesicatoria* was the most resistant, while *X.axonopodis* pv. *eucalyptus* was the most sensitivity to aqueous extracts. Therefore, Data in this work indicated that the use of *Cyclamen rohlfsianum* could be a valid alternative for bio-control of plant pathogenic bacteria.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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Risk Factors for Ciprofloxacin and Gentamycin Resistance among Gram Positive and Gram Negative Bacteria Isolated from Community-Acquired Urinary Tract Infections in Benghazi City

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A B S T R A C T

Urinary tract infections (UTIs) are a severe public health problem caused by a range of pathogens. The aims of the study were to investigate the prevalence of urinary tract infection and to see the pattern of Ciprofloxacin and Gentamycin susceptibility against uropathogens in Benghazi city. The study took place between 2021 April and October 2021. In-vitro antibacterial activity and resistance patterns of these two well-known antibiotics were studied and compared using the disk diffusion method. Laboratory reports and clinical data of patients with a positive urine culture (≥105 CFU/mL) were included in the study. Ciprofloxacin and gentamycin were tested against fourteen common bacterial pathogens, isolated from clinical samples of patients from Benghazi city Libya. A total of isolates were detected in 264 (75.4%) samples. Out of these, 75.4% were female and 24.6% were male. The majority of the study participants were in the age range of between 36-53 years (33.7%). The Increased bacterial resistance to ciprofloxacin has been shown. Most strains of *E. coli* and *Staph aureus* were resistant to ciprofloxacin and sensitive to gentamycin thereby showing that gentamycin is more effective than ciprofloxacin. This study showed that *E. coli* followed by *staph aureus* and *K. pneumonia* were the predominant uropathogen of UTIs in this geographical area. Most of the uropathogens were susceptible to Ciprofloxacin. The results showed that there is an alarming subject of resistance to Gentamicin against UTI patients in this area. Clinicians should be aware of the existing data and treat patients according to susceptibility patterns.

1 Introduction

Urinary tract infections (UTI) are among the most frequent bacterial infections affecting people both in the community and in hospitals. (Laupland *et al*., 2007) it is estimated that about 150 million people are diagnosed with UTI worldwide per annum. (Gupta *et al*., 2001) The problem of antibiotic resistance is severe in Libya. (Khalifa *et al*., 1993) A recent World Health Organization (WHO) report on antimicrobial resistance

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(AMR) surveillance specified nine bacteria of international concern which are responsible for some of the most common infections in community and hospital settings (WHO, 2014). Escherichia coli, the pathogen most often implicated in UTIs, is one of the nine. In all six WHO regions (Africa, Americas, Eastern Mediterranean, European, South-East Asia and Western Pacific), high rates of antimicrobial resistance have been observed in this pathogen (WHO, 2014). Ciprofloxacin belongs to the group of drugs called fluoroquinolones. Ciprofloxacin the most commonly prescribed fluoroquinolone for UTIs because it is available in oral and intravenous preparations. (Schaeffer, 2007) It is well absorbed from the gastrointestinal tract after oral administration. It also has a documented safety profile, broad Gram-negative organism coverage and high urinary excretion rate. (Schaeffer, 2007) During the last decade, the resistance rate of *E. coli* to fluoroquinolones such as ciprofloxacin has increased. (Mcquiston *et al*., 2013) Gentamicin Antibiotic Class Aminoglycoside. Mechanism of Action is the Inhibition of protein biosynthesis by irreversible binding of the aminoglycoside to the bacterial ribosome 30S subunit. (Wurtz R *et al*,. 1997) It was established through many studies that gentamicin produces oxidative stress in different cell types of the body accompanied with direct gonad toxic effect. (Sweileh, 2009; Denamur *et al*., 2011; Sobel, 2014). Aims of the study were to investigate the prevalence of urinary tract infection and to see the pattern of Ciprofloxacin and Gentamycin susceptibility against uropathogens in Benghazi city.

2 Materials and Methods

2.1 Study Area

The study was conducted at the Microbiology Department of Al saleem Medical Laboratory, Benghazi between 2021 April and October 2021. A total of 264 samples were included in this study.

2.2 Sample Collections

Urine specimens were collected from patients attending the al saleem Medical Laboratory. Urine was collected from patients into a sterile clean wide-mouth container. Upon collection, immediately urine was conveyed to the Microbiology Department.

2.1 Culture

Urine specimens were cultured within one hour of specimen collection on blood agar, MacConkey agar and CLED plate. They then incubated aerobically at 37oC for 24-48 h, whereas chocolate agar cultures were incubated at a 5% CO2 candle jar. (Denamur *et al*., 2011; Sobel, 2014). A culture that grew >105 units (CFU/mL) was considered significant bacteriuria.

2.4 Identification of bacterial isolates

After obtaining the pure strains, Gram-negative rods were identified with the help of a series of biochemical tests such as coagulase, catalase, oxidase, indole production, urease production, Triple sugar iron, simmons citrate utilization, motility, mannitol Salt agar, hydrogen sulphide production, nitrate/nitrite production, methyl red and voges Proskeur. (Mcquiston *et al*., 2013; (Wurtz *et al*, 1997; Sweileh, 2009) Morphologically identical colonies of the suspected strains were taken from the agar plates and were suspended in nutrient broth and vortexed. Then, the suspensions were inoculated into the butt and slant of the biochemical testing media. The inoculated media were aerobically incubated at 37°C and after overnight incubation bacteria were identified following the standard flow chart. Gram-positive cocci were determined based on their Gram reaction in catalase and coagulase tests (Cheesebrough, 2006; Baron *et al*., 1994).

2.4.1 Gram's Stain

The smear was made from the isolate on a clean grease-free slide and allowed to be air-dried and fixed. The smear was flooded with crystal violet as a primary stain and was allowed to stain for 2 minutes and rinsed with water. A mordant (lugol's iodine) was flooded, allowed to stay for 1 minute, and rinsed with water. Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediately with clean water A smear was then inundated with secondary stain (neutral red) and was allowed to stain for 2 minutes and then rinsed in water and allowed to air dry. (Oladeinde *et al*., 2011).

2.4.2 Coagulase Test

Staphylococcus spp were further tested for the production of free coagulase enzyme using tube coagulase test. Coagulase test, a drop of plasma was placed on a clean dried slide. A drop of saline was placed next to the drop of plasma as a control. A portion of the isolated colonies was mixed in each drop with a loop, starting with the saline until a smooth suspension was obtained. Then, the suspension was mixed well and rocked gently for 5-10 seconds. (Eltahawy and Khalaf 1988).

2.4.3 Oxidase Test

A piece of filter paper was moisture with a substrate (1% tetramethyle -p-phenylene -diamine dihydrochloride). A wooden stick was used to remove a small portion of bacterial colony and streak across the wetted filter paper streaked area on wetted filter paper was observed for the color change to deep blue. (Oladeinde *et al*., 2011).

2.4.4 Dnase Test

Using a sterile loop, test and control organisms (ATCC 2923) were spot-inoculated and incubated at 35-37 overnight. The surface of the plate was covered with 1mol/ml hydrochloric acid solution and excess was tipped off. Clearing around each colony was observed within 5 minutes of adding the acid. (Oladeinde *et al*., 2011)

2.4.5 Analytical Profile Index (API) 20e Test

5ml ample of API Na cl, 0.85% medium, was opened. A single well- isolated colony from an isolation plate was removed using a pipette. It was carefully emulsified in 5ml ample of API Na cl 0.85% to obtain a homogeneous bacterial suspension. Using the same pipette, both tube and cupule of the test CIT, VP and GEL were full with the bacterial suspension. Anaerobiosis was created in ADH, LDC, ODC, H2S, and URE tests by overlaying mineral oil. The incubation box was closed and incubated at 36° C for 24 hours (Eltahawy and Khalaf 1988).

2.4.6 Urease Test

The surface of the urea slant agar was streaked with a portion of well-isolated colonies. The slanted cap was left on the loose and incubated at 35°C for 18-24 hours. (Oladeinde *et al*., 2011)**.**

2.4.7 Carbohydrate Utilization Test

0.1ml of a heavy saline suspension of the test organism was added to each of the four tubes containing glucose, lactose, maltose and sucrose carbohydrate disk and no to the fifth tube and was incubated at 37°C for 5 hours. It was examined at 30-minute intervals for up to 5 hours from red to yellow indicating carbohydrate utilization (Hummers *et al*., 2005).

2.4.8 Citrate Utilization Test

The surface of the Simmons citrate agar slant was streaked with a portion of a well-isolated colony. The slant cap was left on loosely and was incubated at 35°C for 18-24 hours. (Eltahawy and Khalaf 1988).

2.5 Antibiotic Susceptibility

Antibiotic susceptibility testing was done for the bacterial isolates identified from urine cultures with significant bacteriuria using the Kirby-Bauer disk diffusion method. (Bauer, 1966). The procedure for antimicrobial susceptibility testing is as follows: Briefly, 4–6 morphologically identical colonies of bacteria from pure cultures were collected with an inoculating loop and transferred into a tube containing 5 mL of nutrient broth, then mixed gently until a homogenous suspension was formed, and incubated at 37°C. Using a sterile nontoxic dry cotton swab, a sample of the standardized inoculums (turbidity was adjusted to obtain confluent growth) was taken and streaked on the entire surface of the dried Mueller–Hinton agar plate three times, turning the plate at 60° angle between each streaking to ensure even distribution. The inoculums were allowed to dry for 5–15 minutes with the lid in place. Using sterile forceps, the selected antibiotics disks were applied to the plates at a distance of 15 mm away from the edge and 24 mm apart from each other. After incubating the plates at 37°C for 24 hours, the diameters of the zone of bacterial growth inhibition around the disks were measured to the nearest millimetre. The susceptibility or resistance to the agent in each disk was determined, and the isolates were classified as sensitive (S), intermediate (I), or resistant (R) according to the standardized table. A ruler's zone of inhibition was measured in mm. (Donne *et al*., 2017; CLSI, 2014). The antibiotics tested were Gentamycin- CN (10µg). CIP Zone in diameter in mm (MIC) (R<=12, I 13-14, S>=15) and ciprofloxacin- CIP (5mcg). CN Zone in diameter in mm (MIC) (R<=15, I 16-20, S>=21).

file:///D:/F+NA%20U%202021/cip,cn,f,na/Gentamicin.

Statistical Analysis

The data was analyzed using SPSS programs version 20.

3 Results

A total of 264 (100.0%) urine samples were positive in 2021 (April-October) in the selected area.

3.1 Distribution table of Urinary Tract Infection (UTI) patients by genders

Isolates were detected in 264 (75.4%) samples. Out of these, 199 (75.4%) were female and 65 (24.6%) were male.

Table (1). Distribution table of Urinary Tract Infection (UTI) patients by genders

Gender	Frequency	Percent
Female	199	75.4
Male	65	24.6
Total	264	100.0

3.2 Distribution of the Cases by Age Group

Urinary tract infection and its association with age are presented in Table 2. Males aged 36-53 years old had a somewhat high prevalence (89/264:33.7%) of urinary tract infections. In the age group 72-89 years (n=23), the incidence of urinary tract infection is somewhat reduced to (8.7%).

Table (2). Distribution of the cases by age group.

Age	Frequency	Percent
0-17	33	12.5
18-35	85	32.2
36-53	89	33.7
54-71	34	12.9
72-89	23	8.7
Total	264	100.0

3.3 Distribution of Isolates in Clinical Specimens Collected from Patients

In the present study, Enterobacteraeae 65.1% was the most predominant 65.1% isolates. *Escherichia coli* spp (41.7%) and *Staph aureus* (15.9%) were the predominant organisms isolated from the study subjects. The other bacterial isolates include *Klebseilla pneumonia* (9.8%), *Klebsiella* spp (6.8%), *Strep agalactia* (5.7%), *Strep pyogen* (4.9%), *strep pneumonia* (4.2%), *Staph saprophyticus* and *Pseudomonas aeruginosa* (2.3%) equally, *Enterobacter* spp and *proteus* spp (1.5%) equally, *Acinetobacter* spp (1.1%), and *Citrobacter* spp (0.4%) as indicated in Table 3.

Table (3). Distributions of Isolates in Clinical Specimens Collected from Patients.

Bacteria	Frequency	Percent
***E. coli* spp**	110	41.7
Staph aureus	42	15.9
Klebsiella pneumonia	26	9.8
***Klebsiella* spp**	18	6.8
Strep agalactia	15	5.7
Strep pyogen	13	4.9
Strep pneumonia	11	4.2
Pseudomonas aeruginosa	6	2.3
Staph saprophyticus	6	2.3
***Enterococcus* spp**	5	1.9
***Enterobacter* spp**	4	1.5
***Proteus* spp**	4	1.5
***Acinetobacter* spp**	3	1.1
***Citrobacter* spp**	1	.4
Total	264	100.0

3.4 Prevalence of Urinary Tract Infection Among Tested Patients in Relation to Month

May (32.6%) was the most month in our study in which UTI cases were recorded followed by June (22.3%) and September (15.5%).

Table (4). Prevalence of urinary tract infection among tested patients in relation to the month**.**

Months	Frequency	Percent
April	39	14.8
May	86	32.6
June	59	22.3
July	8	3.0
August	30	11.4
September	41	15.5
October	1	.4
Total	264	100.0

3.5 Gender Distribution by Months

According to gender, the most UTIs was recorded in May (25.3%) followed by June (17.4%) in females. While in the males, the most UTIs were recorded in May (7.1%) followed by June and August (4.9%) equally.

Table (5). Gender distribution by months

Gender			Total
Months	**Female**	**Male**	
April	30	9	39
May	67	19	86
June	46	13	59
July	6	2	8
August	17	13	30
September	33	8	41
November	0	1	1
Total	199	65	264

3.6 Prevalence of Different Uropathogens Among Male and Female Patients. `

In this study, the urinary tract infections of female patients (199) were more prone to male patients (65). In females, the most predominant uropathogen were *E. coli* 91 (34.4%) followed by *Staph aureus* 26 (9.8%) and *Klebsiella pneumonia* 23 (8.7%). In the male, the most prevalent uropathogens were *E. coli* 19 (7.1%) followed by *Staph aureus* 16 (6%) and *Strep pneumonia* 8 (3%). The study noted that] male patients were more infected by the entire isolated organism except organism *Enterococcus* spp.

Table (6). Prevalence of different uropathogens among male and female patients**.**

Gender			
Uropathogen	**Female**	**Male**	**Total**
***E. coli* spp**	91	19	110
Staph aureus	26	16	42
Klebsiella pneumonia	23	3	26
***Klebsiella* spp**	16	2	18
Strep agalactia	10	5	15
Strep pyogen	9	4	13
Strep pneumonia	3	8	11
Pseudomonas aeruginosa	5	1	6
Staph saprophyticus	4	2	6
***Enterococcus* spp**	3	2	5
***Enterobacter* spp**	4	0	4
***Proteus* spp**	3	1	4
***Acinetobacter* spp**	1	2	3
***Citrobacter* spp**	1	0	1
Total	199	65	264

3.7 Distribution of Different Age Groups of UTI Patients by Months

36-53 was the most age group of UTI patients recorded in the present study, and it recorded the most frequent cases in May.

Table (7). Distribution of different age groups of UTI patients by months.

Age						
Month	**0-17**	**18-35**	**36-53**	**54-71**	**72-89**	**Total**
April	10	6	9	6	8	39
May	13	28	29	12	4	86
June	5	24	15	9	6	59
July	0	4	4	0	0	8
August	1	9	16	3	1	30
September	4	14	15	4	4	41
October	0	0	1	0	0	1
Total	33	85	89	34	23	264

3.8 Distribution of Isolates by Ages

The most isolated age group from which *E. coli* was among 18-38 (12.5%), followed by 36-53 (10.9%), followed by 0-7 and 54-71 (6.4%) equally and the least isolated were at 72-18 (4.5%).

Table (8). Distribution of isolates by ages.

Age	**0-17**	**18-35**	**36-53**	**54-71**	**72-89**	**Total**
***Acinetobacter* spp**	0	1	2	0	0	3
***Citrobacter* spp**	0	0	1	0	0	1
***E.coli* spp**	18	33	29	18	12	110
***Enterobacter* spp**	0	1	3	0	0	4
***Enterococcus* spp**	0	2	2	1	0	5
***Klebsiella* spp**	2	7	4	1	4	18
Klebsiella pneumonia	6	8	5	4	3	26
Pseudomonas aeruginosa	0	1	4	0	1	6
***Proteus* spp**	0	1	3	0	0	4
Staph aureus	6	15	16	4	1	42
Staph saprophytics	1	3	1	1	0	6
Strep agalactia	0	6	6	1	2	15
Strep pneumonia	0	3	7	1	0	11
Strep pyogen	0	4	6	3	0	13
Total	33	85	89	34	23	264

3.9 Distribution of Susceptibility Pattern of Gentamicin Against Uropathogens by Months

The bacteria showed the most resistance to Gentamycin in May, while the most sensitive isolates were recorded in May.

Table (9). Susceptibility pattern of Gentamicin against uropathogens.

Month/Susceptibility	**Gentamycin**				
	Miss	**Intermediate**	**Resistant**	**Sensitive**	**Total**
April	2	5	8	24	39
May	42	8	8	28	86
June	36	3	10	10	59
July	3	0	1	4	8
August	19	1	6	4	30
September	31	4	5	1	41
October	0	0	1	0	1
Total	133	21	39	71	264

3.10 Antibiotic Sensitivity, Resistance and Intermediate Sensitivity of Bacteria Isolated from Urine Culture to Gentamycin.

The susceptibility patterns of the bacterial isolates to Gentamycin antibiotic are presented in Table 5. From the results, 26.9% of isolates were most sensitive to

Gentamycin. Percentage resistance of isolates to Gentamycin antibiotics was 14.8%. The activity of Gentamycin against the isolates was somewhat acceptable.

Table (10). Antibiotic sensitivity, resistance and intermediate sensitivity of bacteria isolated from urine culture to Gentamycin.

Gentamycin		
Susceptibility patterns	**Frequency**	**Percent**
Miss	133	50.4
Intermediate	21	8.0
Resistant	39	14.8
Sensitive	71	26.9
Total	264	100.0

3.11 Distribution of Susceptibility Pattern of Ciprofloxacin Against Uropathogens by Months

Table (11). Susceptibility pattern of Ciprofloxacin against uropathogens

Ciprofloxacin					Total
Month	**Miss**	**I**	**R**	**S**	
April	5	4	16	14	39
May	5	8	33	40	86
June	12	7	23	17	59
July	4	3	1	0	8
August	6	2	9	13	30
September	2	7	14	18	41
October	0	0	0	1	1
Total	34	31	96	103	264

Note: R: Resistant; I: Intermediate; S: Sensitive.

3.12 Antibiotic sensitivity, resistance and intermediate sensitivity of bacteria isolated from urine culture to Ciprofloxacin.

The susceptibility patterns of the bacterial isolates to Ciprofloxacin antibiotic are presented in Table 6. From the results, 39% of isolates were most sensitive to Ciprofloxacin. Percentage resistance of isolates to Ciprofloxacin antibiotics was 36.4%, the activity against the isolates was also somewhat acceptable.

Table (12). Antibiotic sensitivity, resistance and intermediate sensitivity of bacteria isolated from urine culture to Ciprofloxacin.

Ciprofloxacin		
Susceptibility	**Frequency**	**Percent**
Miss	34	12.9
Intermediate	31	11.7
Resistant	96	36.4
Sensitive	103	39.0
Total	264	100.0

3.13 Sensitivity of Gram Positive and Gram Negative Organism Groups to Ciprofloxacin

The Ciprofloxacin antibiotic has sensitive against almost all of the isolates, *E. coli* (17%), *staph aureus* (4.9%), *Klebsiella pneumonia* (4.1%). Ciprofloxacin exhibited good antibacterial activity against *Escherichia coli* more than Gentamycin, but these activities to both of them were also not 100%.

Table (13). Sensitivity of gram positive and gram negative organism groups to Ciprofloxacin

Ciprofloxacin					
Bacteria	**Miss**	**Intermediate**	**Resistant**	**Sensitive**	**Total**
Acinetobacter spp	0	0	1	2	3
***Citrobacter* spp**	0	0	1	0	1
***E. coli* spp**	17	13	35	45	110
***Enterobacter* spp**	0	0	1	3	4
***Enterococcus* spp**	2	0	1	2	5
***Klebsiella* spp**	0	2	9	7	18
Klebsiella pneumonia	2	7	6	11	26
Pseudomonas aeruginosa	0	1	1	4	6
***Proteus* spp**	0	2	0	2	4
Staph aureus	5	4	20	13	42
Staph saprophytics	2	0	2	2	6
Strep agalactia	4	0	3	8	15
Strep pneumonia	0	1	8	2	11
Strep pyogen	2	1	8	2	13
Total	34	31	96	103	264

3.14 Sensitivity of Gram Positive and Gram Negative Organism Groups to Gentamycin

The Gentamycin antibiotic has sensitive against almost all of the isolates, *E. coli* (13.2%), *staph aureus* (5.3%), *Enterobacter* spp (6%), *Klebsiella pneumonia* (1.8%). Gentamycin has antibacterial activity against *Escherichia coli* but this activity is not 100%

Table (14). Sensitivity of gram positive and gram negative organism groups to Gentamycin.

Gentamycin					
Bacteria	**Miss**	**Intermediate**	**Resistant**	**Sensitive**	**Total**
***Acinetobacter* spp**	2	1	0	0	3
***Citrobacter* spp**	1	0	0	0	1
***E. coli* spp**	51	9	15	35	110
***Enterobacter* spp**	1	0	1	2	4
***Enterococcus* spp**	1	0	2	2	5
***Klebsiella* spp**	12	2	2	2	18
Klebsiella pneumonia	15	2	4	5	26
Pseudomonas aeruginosa	3	1	1	1	6
***Proteus* spp**	3	0	1	0	4
Staph aureus	20	2	6	14	42
Staph saprophytics	2	0	1	3	6
Strep agalactia	7	0	5	3	15
Strep pneumonia	8	1	1	1	11
Strep pyogen	7	3	0	3	13
Total	133	21	39	71	264

3.15 Distribution of Isolates by Months

The highest isolation of *E. coli* was in May, followed by June.

Table (15). Distribution of isolates by months**.**

	Month							
Bacteria	**April**	**may**	**June**	**July**	**August**	**September**	**October**	**Total**
Acinetobacter spp	1	0	0	0	0	2	0	3
***Citrobacter* spp**	0	1	0	0	0	0	0	1
***E. coli* spp**	20	41	30	0	5	14	0	110
***Enterobacter* spp**	0	2	0	0	0	2	0	4
***Enterococcus* spp**	0	0	4	0	0	1	0	5
***Klebsiella* spp**	1	6	7	0	2	2	0	18
Klebsiella pneumonia	4	8	5	1	3	5	0	26
Pseudomonas aeruginosa	2	3	0	0	0	1	0	6
***Proteus* spp**	0	1	0	0	1	2	0	4
Staph aureus	5	12	7	3	10	4	1	42
Staph saprophytics	0	4	0	0	2	0	0	6
Strep agalactia	2	3	1	1	5	3	0	15
Strep pneumonia	1	3	4	1	0	2	0	11
Strep pyogen	3	2	1	2	2	3	0	13
Total	39	86	59	8	30	41	1	264

3.16 Distribution of Susceptibility Patterns of Isolates to Gentamycin by Sex.

In this study, a total of 264 isolates from urine specimens were tested in vitro by the disk diffusion test to determine the susceptibility of these bacteria to Ciprofloxacin and Gentamycin.

Isolates that were resistant to Gentamycin were observed in females more than males.

Table (16). Distribution of susceptibility patterns of isolates to Gentamycin by sex.

Gentamycin					
Susceptibility pattern	**Miss**	**Intermediate**	**Resistant**	**Sensitive**	**Total**
Female	100	18	27	54	199
Male	33	3	12	17	65
Total	133	21	39	71	264

3.17 Distribution of Susceptibility Patterns of Isolates to Ciprofloxacin by Sex.

Isolates were resistant to Ciprofloxacin also was observed in females more than males.

Table (17). Distribution of susceptibility patterns of isolates to Gentamycin by sex.

Ciprofloxacin					
Susceptibility patterns	**Miss**	**Intermediate**	**Resistant**	**Sensitive**	**Total**
Female	30	22	66	81	199
Male	4	9	30	22	65
Total	34	31	96	103	264

4 Discussion

Urinary tract infections are primarily caused by gram-negative bacteria, but gram-positive pathogens may also be involved. More than 95% of uncomplicated UTIs are monobacterial. The most common pathogen for basic UTIs is *E. coli* (75%–95%), followed by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus, Enterococcus faecalis,* group B streptococci, and *Proteus mirabilis*. Sobel, 2014).

This study aimed to determine the causative bacterial agent of urinary tract infection among different groups in Alsaleem Medical Laboratory, Benghazi. Bacterial pathogens were isolated from 75.4% of the requested urine culture. The overall prevalence of UTI was 75.4% in this study. This was similar to the prevalence of UTI reported from Das RN *et al* isolation rate was 71.6%, (Das *et al*., 2006) and Latika *et al* 76.29%. Other studies done in Karnataka, western India, and South India reported 71.72%, 76.2 and 71.72%, respectively. (Latika *et al*., 2015; Razak and Gurushantappa 2012).

The selection of empiric antibiotics for UTIs should be based on the severity of the infection and local susceptibility patterns. When antibiotics are indicated, short courses are effective for uncomplicated UTIs, especially cystitis, and otherwise healthy women.

The common of patients with UTIs were females in the current study. This is expected and is likely the result of the anatomy of the female urinary tract compared to their male counterparts, particularly the shorter female urethra and closer proximity to the anus. (Hummers *et al*., 2005).

This finding is in line with previous in multiple countries, (Oladeinde *et al*., 2011; Bitew *et al*., 2017; Alanazi *et al*., 2018; Al Yousef *et al*., 2016) where *E. coli* was identified as the primary causative bacterium of UTIs, followed by *Staph aureus* and *Klebseilla pneumonia*, (Al Yousef *et al*., 2016; Al-Harthi and Al-Fifi 2008; Carlos *et al*., 2007; Abir *et al*., 2021) which correlates with findings from another study in Iran which revealed that uropathogens with a predominance of *Escherichia coli* (38%) and *Staphylococcus* spp (35%). (Mihankhah *et al*., 2017).

As we expected, *Escherichia coli* was by far the most frequent pathogen isolated in the medical centers evaluated and is probably the most frequent pathogen causing UTIs in Latin American hospitals. (Abir *et al*., 2021; Sader *et al*., 1999).

Antibiotic resistance is common in developing countries such as Libya, where drugs are available freely without prescription. An antibiotic stewardship program could provide educational programs and cascade the reporting of antibiotic susceptibility results as effective strategies to improve antibiotic prescribing behavior. The drug susceptibility profile of Gram-negative and Gram-positive bacteria tested in the present study was variable. Ciprofloxacin is the most commonly used antibacterial drug in treating community-acquired UTIs. (Hryniewicz *et al*., 2001) Gram negative organisms, particularly *E. coli* are commonly associated with UTI in children in developing countries. (Carlos *et al*., 2007; Jeena *et al*., 1995; Jeena *et al*., 1996; Kala and Jacobs 1992; Rabasa and Shattima 2005).

Our investigator was showed, 6.4% of *E. coli* was isolated from patients their ages between 0-17. More than half of the *Escherichia coli* and *K. pneumonia* isolates were resistant to ciprofloxacin followed by gentamycin in the current study. *E. coli* and *Klebsiella pneumonia* were resistance to the fluoroquinolones (Ciprofloxacin) were observed for 13.2 % - 3.4 % respectively, while they were resistant to the Aminoglycosid (gentamycin) was observed for 5.6% - 1.5%. *Escherichia coli* are also a significant cause of other kinds of nosocomial infections. (Sader *et al*., 1998; Pfaller *et al*., 1998) In addition to *Escherichia coli*, fluoroquinolone resistance was also high among other species, and cross-resistance to newer compounds was very common. The increasing fluoroquinolone resistance documented in this study may, due to the extensive use of fluoroquinolones, ultimately risk and the use of this important class of antibiotics in the region. However, further epidemiologic studies are necessary to improve our understanding of this problem.

The fluoroquinolones (Ciprofloxacin) was effective against many strains more than aminoglycosides (gentamicin), with 14.8%. Similar findings were seen in studies by (Abir *et al*., 2021; Butler *et al*., 2015), who concluded that the organisms exhibited utmost resistance (80.00%) against Ciprofloxacin.

This study matches results reported by (Khalifa *et al*., 1993) who revealed that Ciprofloxacin was the most effective antimicrobial agent. Therefore, we observed a higher frequency of ciprofloxacin-sensitive in *E. coli* (17%) when compared to *Klebsiella pneumonia* (4.1%). (Falagas *et al*., 2010).

Additionally, 13.2% of the *E. coli* was resistant to ciprofloxacin. Although with a different methodology, the present study demonstrated similar *E. coli* resistance rates compared to the Hummers-Pradier clinical study. (Carlos *et al*., 2007; Hummers *et al*., 2005).

The results of this study agreed with other studies that dictated that uropathogens are always predictable and *E. coli* are the leading causes, besides other common Gram negative organisms as *Klebsiella*, Enterobacter, Proteus and *Citrobacter* species (Sahm *et al*., 2001). All isolated bacteria in this study belonged to Enterobacteriaceae that can live in the digestive tract, rectum, and vagina or around the urethra. Infection occurs when these bacteria enter the normally sterile urinary system and multiply (Patterson and Andriole 1987). Similarly, Enterobacteriaceae is the predominant (78.7%) isolates, of which *E. coli* was the most (64.0%) common organisms followed by *Klebsiella* species (17.9%) (Mohammed *et al*., 2016; Thakur *et al*., 2013) and *P. aeruginosa* (2.3%), *Acinetobacter baumannii* and *Proteus* species are very often isolated in hospitals (Rampure, 2013)

The gram-negative bacteria were the most common isolates in the current study, obtained in the present study was different in rates with other reports from different areas. (Mohammed *et al*., 2016; Guermazi *et al*., 2018; Salim *et al*., 2017; Mostafa *et al*., 2016) Gram-positive bacteria were *Staphylococcus aureus* 42 (15.9%) followed by *Strep agalactia* 15 (5.7%) of the isolate's strains.

Gentamicin is an antibiotic widely used in Australian hospitals. It is known to lawyers due to its damage to the apparatus inside the kidney (nephrotoxicity). (Sweileh, 2009) In general, the broad-spectrum ciprofloxacin was the antibiotic with the highest activities, followed by Gentamycin in the present study.

This study detected the dominance of *Escherichia coli* spp (41.7%) and *Klebsiella pneumonia* (9.8%) (Table 2), which was almost identical compared with other research in Libya and other countries. In Northwest Libya, Abujnah *et al.* have found a predominance of *Escherichia coli* (56%) and *Klebsiella pneumonia* (19%). *Escherichia coli* spp (41.7%) followed by *Klebseilla pneumonia* (9.8%). In another study in Messalata, Libya, Mahammed *et al*, have reported the predominance of Escherichia coli (56%) and Klebsiella pneumonia (17%). (Mohammed *et al*., 2016) In Southern Tunisia, the authors have found *Escherichia coli* (68%) and *Klebsiella pneumonia* (13%) as predominance uropathogens among patients of UTIs. (Guermazi *et al*., 2018) A study in Iran has reported uropathogens with a predominance of *Escherichia coli* (38%) and *Staphylococcus* spp (35%). (Mihankhah *et al*., 2017) *Staph aureus* (15.9%) and *Strep agalactia* (5.7%) were the most dominant Gram positive uropathogen isolated in our study. Unlike in other studies which isolated coagulase-negative Staphylococcus and Enterococcus as the most dominant Gram-positive uropathogen. (Ayoyi *et al*., 2017; Okonko *et al*., 2009).

Our study also showed a high prevalence of UTI in females than males 199 (75.4%) and 65 (24.6%), respectively. This correlates with findings from other studies that revealed that UTI frequency is more significant in females than males. (Gilbert *et al*., 2018; Prakash and Saxena 2013).

This result also agrees with what was previously reported by Mahmoud and colleagues in 2016. Many other researchers have also reported similar findings. (Butler *et al*., 2015; Foxman, 2014) The reason behind this high prevalence of UTI in females is due to the proximity of the urethral meatus to the anus, shorter urethra, sexual intercourse, incontinence, and bad toilet. (Nili and Asasi 2002).

The drug susceptibility profile of Gram-negative and Gram-positive bacteria tested in the present study was variable. For instance, increased bacterial resistance to ciprofloxacin has been shown. This study is opposite to the results reported by (Guermazi *et al*., 2018) who revealed that Ciprofloxacin was the most effective antimicrobial agent. Therefore, we observed a higher frequency of ciprofloxacin-resistant in *E. coli* (57.14%) when compared to *K. pneumoniae* (16.67%).

5 Conclusions

In conclusion, the problem of antibiotic resistance is very serious in Libya and appears to be on the rise. These results showed that there is a high prevalence of occurrence of urinary tract infections among patients in a selected area of Benghazi city. This study finding showed that *E. coli* was the predominant uropathogen was isolated from most samples. Most of the uropathogens were susceptible to Ciprofloxacin and seem somewhat appropriate for the empirical treatments of community-acquired UTI. The results showed that there is an alarming subject of resistance of Gentamicin against UTI patients in this area. Clinicians should be aware of the existing data and treat patients according to susceptibility patterns. To reduce the risk of a UTI, it's best to wipe genitals from front to back

after using the bathroom, drink plenty of fluids, urinate before and after sex and get antibiotics.

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Some Physico-Chemical Analyses of Groundwater Sources in Al-Marj Region in the North-East of Libya

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A B S T R A C T

The main aims of this study are to evaluate some Physico-chemical quality of groundwater in Al-Marj city in Libya for drinking, domestic and irrigation uses. All results of the measured physical parameters (Temperature, turbidity, and odor) were within the recommended range by Libyan standards, and WHO guideline values but the chloride (Cl) and ammonia (NH3) concentrations in some sites are exceeded the recommended values by Libyan standard. This is indicating that the potential contamination by municipal sewage or agricultural activity.

Most of the groundwater samples were within the recommended value by Libyan standards and WHO guidelines, except one groundwater sample, which was collected from site 6W (sample No.10), in the west direction of Al-Marj city. This groundwater sample recorded the turbidity values 5.85 NTU, and this value was higher than the permissible value by Libyan.

The average results for the physical parameters the average of turbidity results was higher than 3.0 NTU at site 6W (sample No.10), and the rest of the physical parameters of the other sectors, were within the recommended range by the Libyan standards in all other sites.

The results of the chemical parameters were within the Libyan permissible limits, except the Cl^- average levels were higher than the Libyan standard, which were ranged from 256.8 to 372 mg/l in the Eastern and western sectors in the study area. Also, the ammonia was elevated to the Libyan standard in eastern sites which was ranged from 1.66 to 9.72 mg/l.

1 Introduction

For all living creatures including the human being, water is considered as one of their basic needs., Therefore, water is one of the most important and valuable substances on the earth (WHO.,2014; WHO.,2011). Recently, there has been a great increase in the demand for safe drinking water. Although water plays an important role in life due to its essential for human survival, many are denied access to sufficient potable drinking water supply and sufficient water to maintain basic hygiene (WHO., 2011). However, around 71% of the earth's surface is covered by water (B.Nirmala et al ., 2012). The freshest water is available as groundwater in aquifers. Several water resources such as oceans, ice caps, glaciers, groundwater, and lakes, and rivers contained 98.93%, 1.05%, 0.009%, and 0.0001%, respectively (R. M. Harrison, 1995).

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This underground freshwater has been considered as one of the purest forms available in nature to meet the overall demand of household, industrial, recreational, agricultural, and environmental activities (S.C.Izah et al .,2015 ; R.D.Pratima et al ., 2014).

Around the world, 1.5 million people mostly are children, die annually from diarrheal diseases regarding water-related diseases (WHO., 2006). However, approximately 1.1 billion people in

The world relies on unsafe drinking water sources from lakes, rivers, and open wells (An R Lawrence et al., 2001). About 22 African countries, including Malawi, are failed to provide safe and clean drinking water to 50% of their population (T.Kanyerere et al., 2012). This is caused by the drinking and using of water contaminated by faucal matter. In addition, by inadequate sanitation. Naturally occurring chloride ion in water is caused by the dissolving of minerals containing chloride, also, chloride ion comes from sewage and industrial effluents, and fertilizers. (Hale, R.L.,2006)

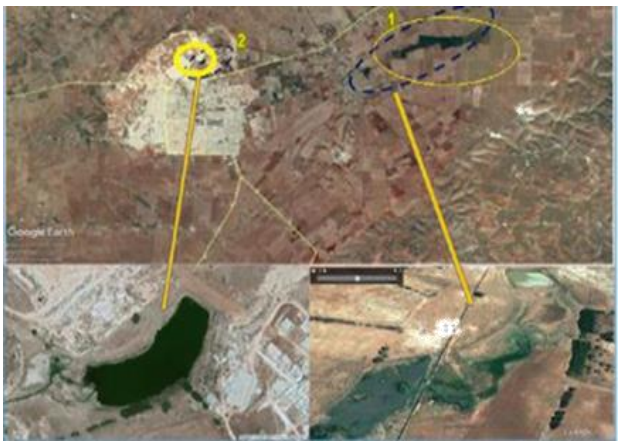
2 Materials and Methods

2.1 Study Area

The study area was in the Al-Marj region, which is located at Al-Akhder in the eastern north part of Libya, at 32.50 ^{0}N –20.82 ^{0}E; (The General People's Committee, the General Information Authority;2006). It can be seen from Fig (1) and (2) that the huge area of municipal sewage discharge in two side-lakes is as random damping sites in Al-Marj city. These lakes are the main contamination sources in the Eastern sector of this city, and the secondary contamination source in the western sector of this city.

In the study area, groundwater is the main source of water supply for all water uses, therefore, this excessive demand became too much load on the groundwater of this area, and the pure water that comes from the Al-Marj Region desalination plant is not enough for Al-Marj city, and its surrounding area. Therefore, the quantity and quality of this source of groundwater might be changed without a propel control or protection.

A



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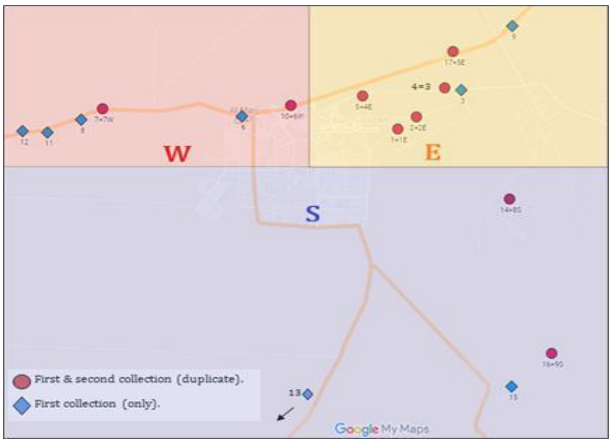


Figure (1) . (A): The huge areas of municipal sewage in the study area. (1) Is the main contamination source, and No (2) is the secondary contamination source. (B): Groundwater sampling locations of the study area. The studied area was divided into three sectors: Eastern(E), Western(W), and Southern(S).

2.2 Groundwater Sampling

Samples of groundwater from the study area were selected to represent the condition of groundwater quality, and ground surface contaminants, especially, municipal sewage effluent. All sampling wells are boreholes with electrical pumps, and the samples were collected twice (Fig.2) from nine sites. The first collection was collected during the dry season (fall 2017), and the second collection (nine samples) was collected from the same wells to assess the wet season in summer 2018. All samples were collected in clean and autoclaved glass bottles and carried over from samples locations to the lab before 24 hours of sampling time.

2.3 Analytical Methods

For Physico-chemicals and, all groundwater samples were collected in cleaned and autoclaved glass bottles, and stored in a cold box, and carried over to the laboratory of the Environmental and Biological Chemistry Research Center (EBCRC). However, ((pH, Electrical Conductivity (EC), Dissolved Oxygen(DO), Total Dissolved Solids(TDS), Ammonia (NH3), Total Hardness(T.H), Alkalinity (Alk), Chloride (Cl^{-}), Odour and Colour Temperature, and Turbidity) parameters were measured and recorded at the sample point. The quality of groundwater wells is determined by measuring some Physico-chemical parameters of

collected samples, by using standard analytical methods for water quality as follow.

2.3.1 Physical Analyses and Chemical Analyses

For physical parameter analyses of groundwater samples, the physical parameters (odor, color, temperature, and turbidity) were measured by using the methods as shown in Table (1). For chemical analysis Table (1) includes the chemical parameters and the methods, which are used to determine the chemical quality of groundwater in this research, and the mathematical calculation for some chemical parameters.

2.3.2 Principals of Used Analytical Methods in this Study:

1. Volumetric Titration, the titration end-point is identified by the development of color resulting from the reaction with an indicator (A Malik et al., 2012; Health Association., 1999).

2. Colorimetric Methods: these are based on measuring the intensity of the color of a colored target chemical or reaction product. The optical absorbance is measured by using light of a suitable wavelength (WHO 2001 Health Association, 1999). Electrometric Methods: Also, the voltages are produced when the electrodes are immersed in a solution or water sample (Health Association., 1999). Nephelometric Method: This method is based on a comparison of the intensity of scattered light by the sample under defined conditions, with the intensity of scattered light by a standard reference suspension under the same conditions. The higher the intensity of scattered light is thehigher in turbidity.

Table (1). A used method for chemical tests of groundwater samples.

Parameter	Method	Instruments apparatus	UNIT
pH	Electrometric	Multi-parameter	-
Total Dissolved Solids (TDS)	Electrometric	Multi-parameter	mg/l
Electrical Conductivity (EC)	Electrometric	Multi-parameter	µS/cm
Dissolved Oxygen (DO)	Electrometric	DO-meter	mg/l
Ammonia (NH3)	Colorimetric	Spectrophotometer DR 2800	mg/l
Total Hardness (T.H)	Titration	Titration by EDTA	mg/l
Alkalinity (Alk)	Titration	Titration by sulphric acid	mg/l
Chloride (Cl-)	Titration	Mohr titration by AgNO3	mg/l
Temperature	Electrometric	Multi-parameter	Celsius (°C)
Turbidity	Dispersion (Nephelometric)	Turbid meter	Nephelo metric Turbidity Unit (NTU)
Odor and Colour	Personal Observation	Look and smell	Good or acceptable/ Bad or unacceptable

3 Results and Discussion

The analytical results for groundwater samples that were collected from the study area in Al-Marj city in two collections are described in this study. For the first collection, nine groundwater samples were collected for analytical purposes, and the results are shown in the following section. The list of nine duplicated (First collection (dry) and Second collection (wet) groundwater samples, which were collected from the study area are shown in Table (2).

Table (2). Numbers of duplicated sampling sites in both collections.

The first collection (dry)	1	2	4	5	17	10	7	14	16
Location of the samples collections	1E*	2E	3E	4E	5E	6W*	7W	8S	9S
The second collection (wet)	1	2	4	5	17	10	7	14	16

E*, East, W*, West and S*, South (Sample locations).

The analytical results for physical, chemical parameters indicators of both (First collection (dry) and Second collection (wet)) collections are described in the following sections.

3.1 First Collection Result

The analytical results for physical, chemical parameters indicators for the collected nine groundwater samples (n=9) are shown in Tables (3).

From the analytical results of physical parameters as shown in Table (3), it can be seen that all results of the measured physical parameters (Temperature, turbidity, and odor), were within the recommended range by Libyan standards, and WHO guideline values (WHO., 2011; Health Association., 1999). Also, the color of all groundwater samples was colorless.

3.1.1 Chemical parameters' Results for the First Collection. (Chloride and Ammonia):

For the chemical parameters the analytical results of the same samples, are shown in Table (3). However, it can be seen that the most results of the measured chemical parameters, were within the permissible limits recommended by Libyan standards (WHO ., 2011 ; Health Association ., 1999). Nevertheless, as shown in Table (3), the chloride and ammonia concentrations in some sites are exceeded the recommended values by Libyan standard. This is indicating that the potential contamination by municipal sewage or agricultural activity.

3.2 Second Collection Results:

The results of the duplicated (First collection (dry) and Second collection (wet) groundwater samples, for physical and chemical parameters of the second collection, are shown respectively in Table (3).

3.2.1 Physical parameters' Results of the Second Collection:

Form physical parameter results as shown in Table (3), it can be seen that most groundwater samples were within the recommended value by Libyan standards and WHO guidelines, except one groundwater sample, which was collected from site 6W (sample No.10), in the west direction of Al-Marj city. This groundwater sample recorded the turbidity value is 5.85 NTU, and this value was higher than the permissible value by Libyan standard, and however, this is related to the presence of microorganisms, which may threaten human health and life. As well, the watercolor of all groundwater samples was colorless.

3.2.2 Chemical parameters' results of the Second Collection:

The results of chemical parameters for the duplicated (First collection (dry) and Second collection (wet)) nine groundwater samples are shown in Table (2). However, the most values of measured chemical parameters value were in the recommended value by Libyan standards, except the chloride concentration levels in groundwater samples numbers (5, 10, and 17) (4E,6W, and 5E) were recorded values of 284, 372 and 265.9 mg/l, respectively, which were higher than the recommended value by Libyan Standards. These three groundwater samples are located in the west and east sections of Al-Marj city near the municipal sewage water damping sites, and these recorded higher concentrations of chloride indicate the influence of municipal sewage water on the groundwater in these sections. Nonetheless, as shown in Table (3) it can be seen that the other chemical parameters' results were within the permitted limit by Libyan standards. This is maybe due to the consequence of dilution of groundwater by rainwater in the wet season, and therefore, this is not meaning these groundwater sources are safe for drinking purposes.In this study, the results of duplicated (First collection (dry) and Second collection (wet)) samples were correlated with each other in order, to give clear data about the contaminated locations of the groundwater sources in Al-Marj city.

Correlation between Physical Parameters' Results of Both Collections

It can be seen from Table (3) that the average temperature and turbidity results were within the permissible range by Libyan standards. But, according to WHO guidelines the most turbidity values go above the permissible limit at <1 NTU.

Correlation bChemical Parameters' Results of both Collections.

The results' averages (mg/l ± SD) for all chemical parameters of the collected groundwater samples for both collections are shown in Table (3) and therefore the correlation of the results between each chemical parameter are as follow:

pH Results Correlation (pH ± SD) between both Collections.

From the correlation results of pH values in groundwater samples of both collections Table (2) that the pH average values in most sites gave a. However, the average pH results were within the acceptable limits recommended by Libyan standard and, it is ranged between 7.0 ± 0.21 to 7.6 ± 0.35.

3.6 TDS Results Correlation (mg/l ± SD) between both Collections.

The total dissolved solids (TDS) generally comprise chloride, consequently, it arises from municipal sewage water contamination and agricultural activities. However, as shown in Figure (2. a) and from Table (3) the TDS results average were ranged between 400 ± 7.07 to 920 ± 41.72 mg/l. However, the groundwater samples from the East and the west sites of Al-Marj city have the higher values of TDS, and however, the average TDS results were giving a strong correlation between both collections. This indicates that the groundwater sources in the east and west of Al-Marj city are contaminated by sewage water, which comes from the damping sites of municipal wastewater in these directions.

The Electric Conductivity of Water Results in Correlation (µs/cm ± SD) between both Collections

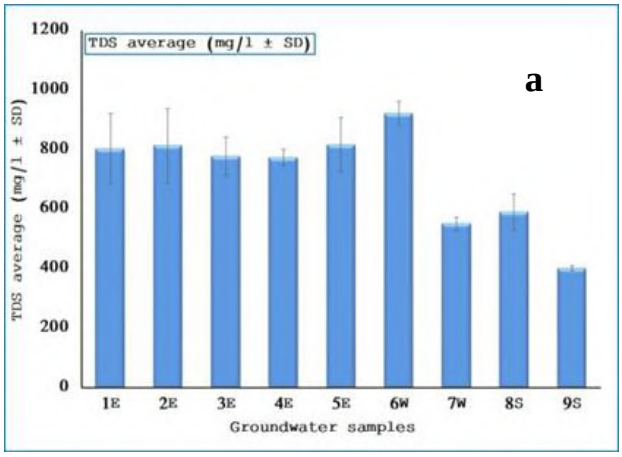
The electric current is conducted through the movement of ions in water, whereas the ions in water are originating from dissolved inorganic matters, hence, are contribute to conductance, and therefore, the EC gives an indication of the concentration of the ions, or the dissolved salts (TDS) in the water, mainly chloride ion. In this study, the average of EC results is shown in Figure (2. a) and from Table (3). The EC averages were ranged between 848 ± 52.8 to 1870 ± 126.1 µs/cm, whereas the values of sites which are located around the municipal wastewater damping sites are higher than other sites. Moreover, the EC concentrations' averages in groundwater gave a very strong correlation between both collections. Therefore, these EC and TDS results are indicating that the contaminated sites in the east and west of Al-Marj city are due to the municipal wastewater damping sites.

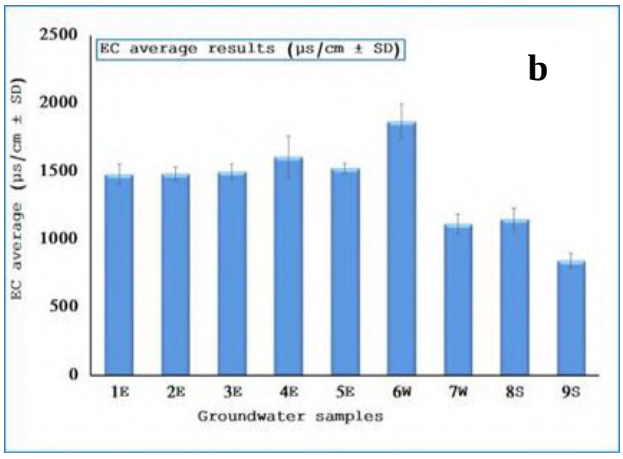
Chloride Ion Results Correlation (mg/l ± SD) between both Collections

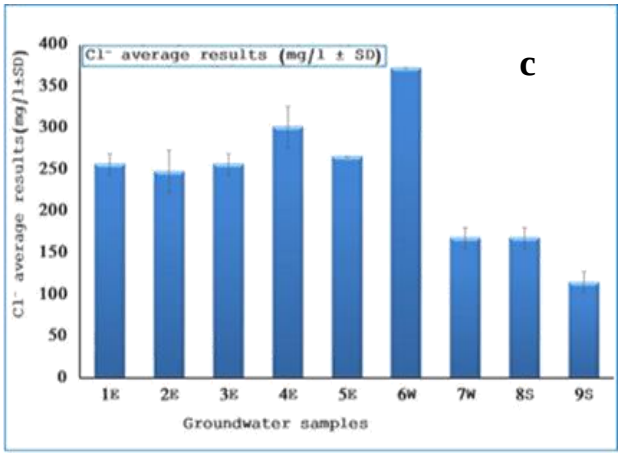
chloride is a significant indicator of water contamination by sewage, consequently, to microbiological [25] contaminations. However, it can be concluded from Figure (2. c), and Table (3) the averages of Cl- ion concentrations in groundwater samples in five duplicated samples from east and west sites near the municipal sewage damping zones, were exceeded the Libyan standard limit of chloride (250 mg/l) and between 115 ± 12.5 to 372 ± 0.4 mg/l. These levels of Cl- concentrations gave a strong correlation in both seasons. Furthermore, the higher averages of Cl- ion concentrations were recorded in groundwater sample sites near the contaminated areas in the east and west directions. This result is indicating to reaching of municipal sewage to the groundwater in the vicinity areas of the contamination sources.

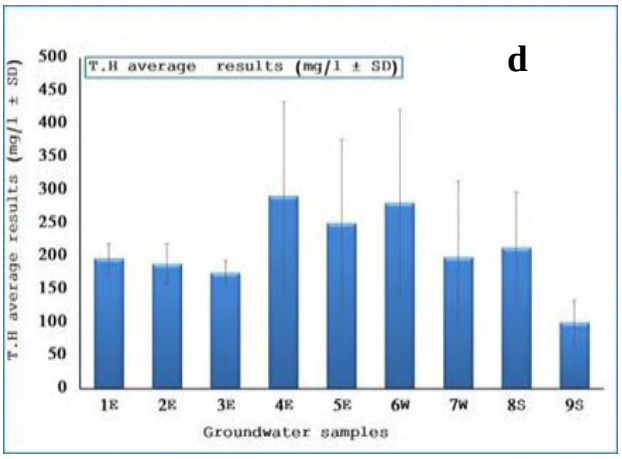
Ammonia Results Correlation (NH3-N mg/l ± SD) between both Collections

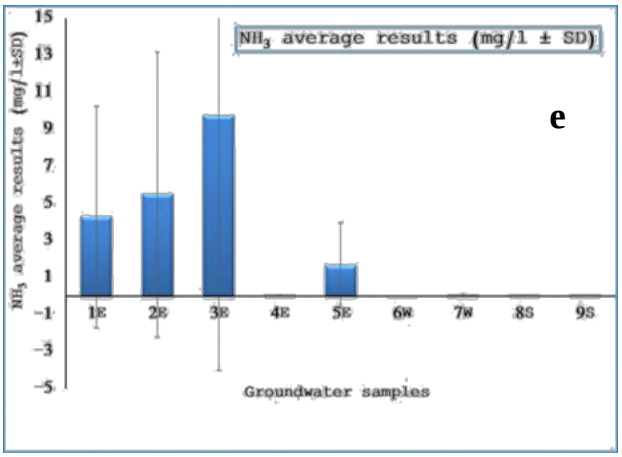
The high concentrations of ammonia are rarely in groundwater in nature, and if it is found at concentrations above 0.1 mg/l as N that indicates an unnatural source of ammonia such as sewage or industrial contamination [24]. As shown in Figure (4. e) and Table (3) it can be seen that the NH3 levels are exceeded the recommended limit value by Libyan standard in most groundwater sampling sites in the study area, especially which are located in the east direction of Al-Marj city (at Al-Marj old city) around the municipal sewage damping site. Also, ammonia averages were ranged between 0.0 ± 0.002 to 9.72 ± 13.8 mg/l, in addition, there is no correlation between both collections. Therefore, the presence of ammonia is a very strong indication of the presence of ammonia in groundwater in the study area is introduced by sewage leaching, consequently, pathogenic microorganisms are expected to be existent.











All Figures (4). (a), (b), (c), (d) and (e) Correlation of results (mg/l ± SD) between both collections. Figures (4). (a): TDS (b): EC, (c): Cl, T.H: (d) and (e): NH3, results (µs/cm ± SD) between both collections.

Table (3). Comparing Chemical parameters' results of the First collection (F) and second collection (S) with Libyan standards and WHO guidelines.

Samples	pH	pH	TDS	TDS	EC	EC	DO	DO	Cl	Cl	ALK	ALK	TH	TH	NH3	NH3
	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S
1	7.2	7.3	884	715	1535	1429	1.8	6.4	266	248	115	110	212	180	8.5	0.06
2	7.2	7.3	900	725	1520	1450	2.9	3.2	266	230	154	110	210	166	10.9	0.05
4	6.8	7.1	822	731	1542	1463	1.2	5.2	266	248	120	111	188	161	19.5	0
5	7.1	7.3	791	752	1719	1502	6.4	5.6	319	284	86	118	392	190	0.0	0.05
17	7	7.4	879	750	1553	1500	6.2	1.2	266	266	103	100	340	160	3.3	0.04
10	7.2	7.1	949	890	1959	1781	6.2	5.8	372	372	100	128	380	181	0.0	0
7	7.3	7.4	566	533	1169	1068	6.7	7.1	177	160	94	100	280	118	0.0	0.11
14	7.5	7.6	632	547	1212	1094	6.2	4.2	177	160	75	82	272	154	0.0	0
16	7.3	7.8	395	405	885	811	6.7	5.1	106	124	96	90	76	124	0.0	0.02
Libyan's	8.5-6.5		1000		-		-		250		-		500		1.5	

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Biological Studies of Harmala peganum Exracts as Antibacterial Agent

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A B S T R A C T

Harmala peganum is one of the most well-known medicinal plants in traditional Chinese medicine. The goal of this study was to look at the antibacterial effects of a methanol extract of several components of *H. peganum*, such as the stem and leaf, against certain common human pathogenic bacteria. The antibacterial capabilities of methanol extracts of the specified sections were tested using the disc diffusion method, as well as their synergism activity when combined with synthetic antibiotics. The leaf and stem extracts were the only portions of *H. peganum* that showed antibacterial action against all of the microorganisms tested, even at the lowest concentrations. Leaf extract out performed stem extract in terms of antibacterial activity against the majority of gram-positive bacteria tested. The extracts were tested for their biological activity against microorganisms such as *Streptococcus pneumoniae, Pseudomonas aeruginosa, Salmonella typh,* and *Escherichia coli*. Except for the distilled water extract of *H. peganum*, the results demonstrated that the extracts are active against all bacterium strains. However, the antibacterial activity of *Harmala peganum* leaf and stem extracts was higher than that of antibiotics used against the pathogens tested.

1 Introduction

Indicates that man used plants and herbs to treat some diseases that afflicted him or his pets, and he used them either in their natural form, or extracted as essential oils for a period of time approximately 622 Year. (Bin Marash, 2012). Medicinal and aromatic plants are used in folk medicine, and these fields go beyond perfuming, cosmetics, seasoning, and food, where activity is due to the presence of antioxidants and antimicrobials in their tissues, they are considered the natural source of natural antioxidants. (Omar, 2012.) The science of medicinal herbs in its modern concept is advancing a lot in different parts of the world, increasing interest in the study of medicinal plants and their use in treating various diseases, as plants contain a very large number of medically effective components that reflect the great therapeutic potential of these plants, it is known that some plant drugs have a greater therapeutic capacity than those of manufactured medicines in treating some diseases, and plant drugs contain nutrients and vitamins as well as active ingredients (Bin Marash, 2012). The recommendations of the medical and pharmacological conferences held in recent years have called for the necessity to limit the intake of these manufactured drugs, whose use has been proven to cause harmful side effects, and recommended the return to medicinal plants with interest in them as a safe source for the manufacture of medicines, and to make them in the service of health in a scientific way by applying And based on established scientific principles, where phyttochimie plays a vital role in extracting active substances or elements from the plant, the actif principe. (Nazni et al., 2006). This is by using different chemical, analytical and physical methods, and then comes the biological and pharmacists' role to conduct biological experiments (Ibn Arabiya, 2013). For this purpose, we decided through this work to study a plant that has been common since ancient times in many Arab countries, namely the H. *peganum* plant (Newall et al.,1996). It was widely used in ancient folk medicine the *Harmala* plant is considered a herbaceous flowering plant that spreads in Central Asia, Africa, and

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the Middle East, and it has also spread America and Australia. (Elsayem et al., 2012). It is one of the herbs used in folk medicine since ancient times for pain relief and sterilization, and its use has spread in cases of back pain, asthma, colic, and wheezing, and as a stimulant among many, and scientific research has found that the different parts of this plant carry different therapeutic effects. (Englisch et al., 2000; Lopez-Molina et al; 2003). The *H. peganum* plant is used industrially to produce a red dye used in carpet dyeing. Scientific research has found that the camel plant and its extracts have great importance in drug extraction and manufacture. This is due to the therapeutic effects it carries, and include its therapeutic benefits Anti-bacterial, anti-virus and anti-fungal effect. (Alghazee et al., 2012).

2 Materials and Methods

Leaves and stems of every species of selected plant were separated and washed with distilled water several times, then dried in open air. Its height is 60 cm, with lobed leaves, a distinctive aroma, and its large white flowers. It gives white top fruits, with small black seeds. The plant grows wild in most areas of Libya. Fresh of *Harmala peganum* washed two times distilled water and subjected to shade drying at room temperature the dried plant material was powdered using a mechanical grinder (Akinpelu *et al*., 2 008; Alshammary and Ibrahim, 2014). The powdered materials of *Harmala peganum* were extracted with methanol 10 grams of each plant powders were added to 100ml of methanol (80% w/v). Crude extracts were evaporated at 45^0C with the rotary evaporator the extracts were collected and stored at 4^0C until further use (Akinpelu et al., 2008; Alshammary and Ibrahim, 2014). The antimicrobial activity of the plants extracts was determined using the agar disc diffusion method (Sathishkumar *et al*., 2008), where Mueller-Hinton (MH) agar plates were seeded with bacterial strain on each plate wells were made by sterile standard cork borer. Each well was filled with 30µl of the different concentrations (0.8, 0.4, 0.2, 0.1, 0.01, 0.001, 0.0001 and 0.00001 g/ml) of incubated for 24 - 48 h at 37°C for bacteria. The of inhibition zones were measured, the results are presented as mean of triplicate. The minimal inhibition concentration (MIC) values were evaluated according to published procedures (Koneman et al., 1997; Iscan et al., 2002 and Guven *et al*., 2005). The minimal inhibitory concentration (MIC) was determined only with micro-organisms that displayed studied plants extracts and the plates were then inhibitory zones. MIC was determined by dilution of the plants extracts and pipetting 30µl of each dilution into wells dilutions of the extracts within a concentrations range of (0.8 - 0.00001 g/ml). MIC was defined as the lowest concentration that inhibited the visible microbial growth (NCCLS, 2005).

Aim of the study

This study aims to find out the inhibitory effect of medicinal plant extract of the medicinal plant *H. Peganum* used in folk medicine on some types of antibiotic-resistant bacteria.

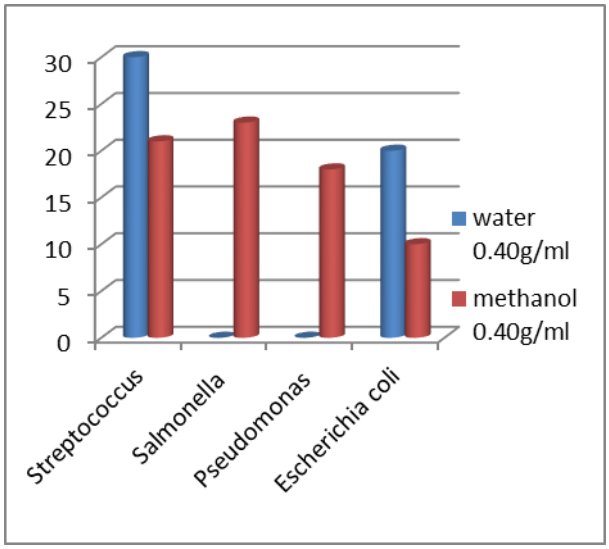

Figure (1). Antimicrobial activities of different concentrations of studied plant *Harmala* leaves extract against bacteria.

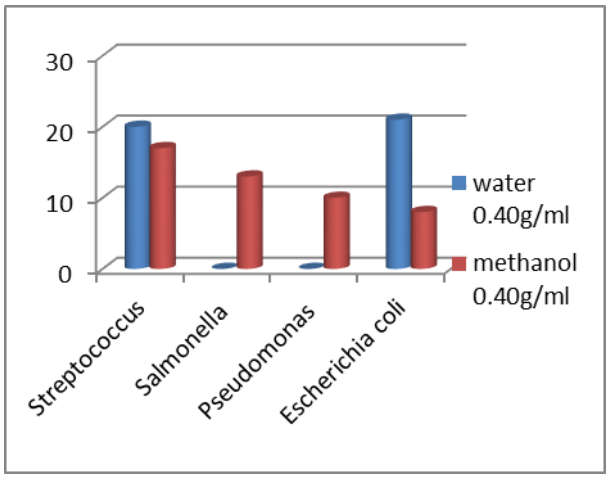

Figure (2). Antimicrobial activities of different concentrations of studied plant *Harmala* stems extract against bacteria.

2.1 Antibiotic Sensitivity Tests

In vitro antimicrobial susceptilit to four antibiotics in table. The inoculums was prepared by adding isolated colonies of the microorganism from an overnight nutrient agar plate into 2ml try tone soya broth (TSB). A sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. The swab was streaked over the entire surface of the sterile Mueller Hinton Agar plate. This procedure was repeated by streaking two more times, rotating the plate approximately each time to ensure an even distribution of inoculums. plates were

allowed to dry for 5 minutes and then the antimicrobial disks were dispensed onto the surface of inoculated agar plates using an oxide antibiotic. Plates were then incubated at 37°C for 18-22 hours. The diameters of the zones of inhibition are measured to the nearest mm using a venier calipers (junior), zones diameters were interpreted as being susceptible sensitive (S) or resistant (R) according to (NCCLS, 2001).

Table (1). Antibiotic sensitivity testing**.**

Kanamycin	K	30mg/ml
Gentamicin	CN	10mg/ml
Tetracycline	TE	30mg/ml
Cefotaxime	CTX	30mg/ml

3 Results

Antibacterial activity showed different concentrations of studied plants extract against bacteria. The results showed that the inhibition zone and MIC in all extract It was resistant to bacteria. Except for distilled water of *H. peganum* no zones of inhibition did not show any effect on the S. typh and *P. aeruginosa*. Where it was found that the activity of the plant extract of the leaves and stems of the *H. peganum* plant was higher than the effect of antibiotics.

Discussion

If we notice that the *Harmala paganum* plant was a good resistance to different types of bacteria in all concentrations except for two types of bacteria that were resistant to the effect of this plant similar results observed by (Memon et al., 2003).

Table (2). Demonstrates the effect of *Harmala* plant extract (distilled water, methanol) against the tested bacteria**.**

Used bacteria				**concentrations**	**Solvent**	**Plant**
The diameter of the inhibition zone						
Escherichia coli	*P. aeruginosa*	*S. typh*	*Streptococcus pneumoniae*	0.40g/ml	Distilled water	*Harmala*
20	N.A	N.A	30			
10	18	23	21	0.40g/ml	Methanol	

Where inhibition zone diameters in (mm) **N.A: no activity**

Figure (3). Shows the effect of *Harmala* plant extract against *Streptococcus pneumoniae*

Figure (4). Shows the effect of *Harmala* extract agains *S. typh*

Figure (5). Shows the effect of *Harmala* plant extract against *P. aeruginos*

Figure (6). Shows the effect of *Harmala* plant extract against *Escherichia c*oli

Antibiotic Sensitivity

Table (3) show the rates of sensitivity of gram negative and gram positive bacteria results showed that the sensitivity pattern of *S. pneumoniae* was sensitive to K, CN, TE and resistant CTX figures (7). However, *S.typh* was resistant to K, CN, TE and sensitive CTX figures(8). Whereas *P.aeruginosa* was sensitive to CN, CTX and resistant to K, TE figures (9). Whereas *Escherichia coli*sensitive to CN, and resistant K, TE, CTX figures (10). Antimicrobial resistance developed by microbes against antibiotics open serious debates in this issue and recognized as a serious problem byglobal medicinal and research community (Finch,2004).

Table (3). Antibiotic sensitivity against bacteria

Antibiotic	Symbol	Concentration	Organism			
			S. pneumoniae	*S.typh*	*P.aeruginosa*	*E. coli*
Kanamycin	K	30mg/ml	S	R	R	R
Gentamicin	CN	10mg/ml	S	R	S	S
Tetracycline	TE	30mg/ml	S	R	R	R
Cefotaxime	CTX	30mg/ml	R	S	S	R

S-Sensitive

R-Resist

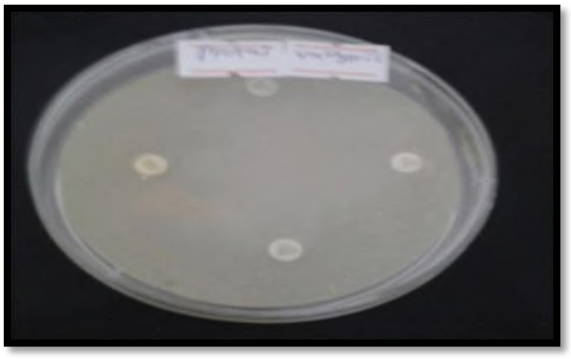

Figure (7). Antibiotic sensitivity testing of *S. pneumonia*

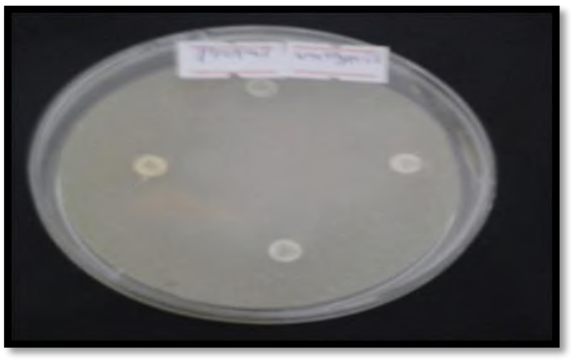

Figure (8). Antibiotic sensitivity testing of *S.typh*

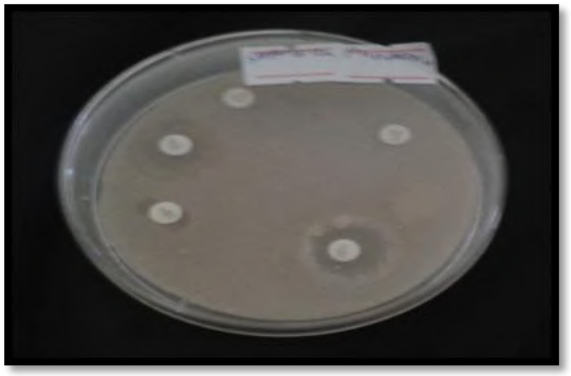

Figure (9). Antibiotic sensitivity testing of *E. col*

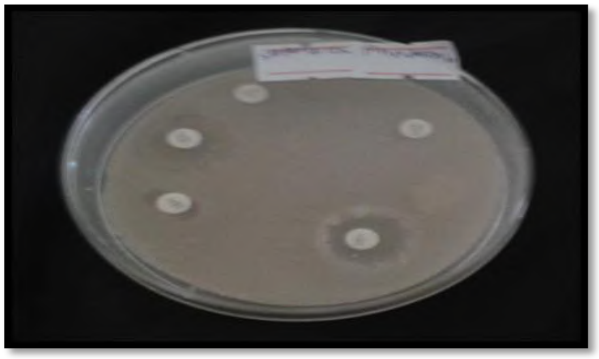

Figure (10). Antibiotic sensitivity testing of *P.aeruginosa*

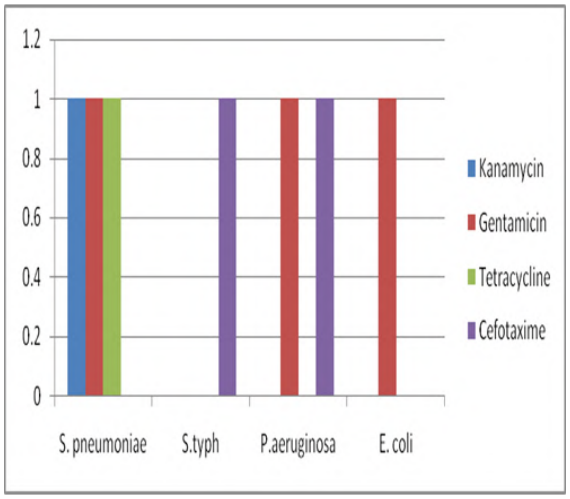

Figure (11). Antibiotics of against types different bacteria.

4 Conclusions

The present investigation proves that antimicrobial activity of leaves and stems *H. peganum* extracts was higher than that of antibiotic used against the tested microorganisms. The obtained results might be considered adequate to demonstrate that *H. peganum* extracts can be considered a good antibacterial agent, it can be used to an antibacterial overcoat against the strain that a major problem of resistance in hospitals.

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The Role of Inoculation with Glomus macrocarpum and Saprophytic Fungi on Growth of Wheat Plant Grown in Addition with Olive Mill Residues

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A B S T R A C T

This study was carried out to test the influence of olive mill dry residue (DOR), Aqueous extraction (ADOR) and (SDOR) fraction treated with saprobe fungi on growth of Wheat (*Triticum aestivum* L.) plants colonized by *G. macrocarpum*.These fungal genera *Aspergillus niger* and *Penicillium crustaceum* were reported to possess the ability of detoxifying by degrading its phenolic compounds found in olive mill dry residue (ADOR and SDOR)) fraction . The percentage of mycorrhizal colonization by *G. macrocarpum* strongly decreased in presence of DOR, but the level of AMF colonization likewise increased in presence of ADOR or SDOR. Our study demonstrates that, in controlled conditions, The use of certain saprobe and AM fungi allows the possibility of using DOR as an organic fertilizer.

1 Introduction

Soils in the mediterranean region contain small quantities of organic matter that is important for plant growth (Brunetti *et al.*, 2005). The importance of the olive mill in Arabic countries is known and gaining importance in other countries outside the Mediterranean area.

The cultivation and production of olive oil (Olea europea L.) is of great importance in the Mediterranean region (Sampedro *et al* 2008). Dermeche *et al.* (2013) assessed and compared products of olive oil, in which phenolic substances, carbohydrates, organic acids and mineral elements were distributed differently, depending on the cultivation methods, were compared in terms of the use of olive dry residue (DOR) for cultivation (Parides *et al.*, 1999; Bonanomy *et al.*, 2006).And it was found from previous studies conducted using DOR that it contains toxic substances for microbes due to its phenolic content (Perez *et al.*,1992; Martin *et al.*, 2002). By using saprobe fungi, the toxicity of DOR on the plant can be reduced. The saprophytes break down cellulose materials into simple sugars as a source of energy for microbes that include the mycorrhizal (AM) (Radford *et al.*, 1996). In addition to that DOR toxicity can be reduced by inoculation with saprophytes around 20 weeks (Sampedro *et al.*, 2004). DOR can improve plant resistance to attacking pathogens to stimulate root growth and plant growth due to its high content of phenolic compounds Sasanelli *et al* 2011. The AM root fungus helps host plants grow in a polluted environment (Shetty *et al.*, 1994) by supplying phosphorous to the plant (Querejeta *et al.*, 1998).

Previous studies confirm the role of AM fungi in the resistance of host plants to growth in a polluted environment (Shetty *et al.*, 1994) by increasing the plant's ability to absorb phosphodiester (Querejeta *et al.*, 1998). And the effects of DOR had an effect on soil

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microbes and texture (Karpouzas *et al.,* 2010). These effects were negative in sandy clay soils and were not observed in clay soils due to the absorption of polyphenols in these soils (Rousidou *et al.,* 2010). It is possible to reduce the phytotoxicity of toxic residues dependent on intracellular AM (Volante *et al.,* 2005). It appears that the rhizosphere AM increases the effect of toxicants in olive residues (Martín *et al.,* 2002). The symbiotic effects of saprophytes and AM in plant roots have the potential to increase the host's tolerance to heavy metals in soil (Fracchia *et al.,* 2004; Martínez *et al.,* 2004).

This study is to determine the role of AM *Glomus macrocarpum* and Saprobe fungi in resisting the effects of phenols present in DOR, through their effect on the dry weight of wheat plants.

2 Materials and Methods

DOR was collected from the olive oil plant (Al-Marj area), sieved and sterilized by autoclave at 120 ℃ and stored in a refrigerator before use. The main characteristics of DOR was as follows: total organic carbon, 52.7%; total nitrogen, 1.15%; total phosphorous, 0.17%; lignin, 18.3%; cellulose 21%; hemicellulose 14.3%; total phenols, 3.15%; fat, 0.4%; Ash is 11.5%.

The most abundant elements, the concentration of which is reported in g.kg-1 DOR were: potassium, 27.4; calcium, 17.3; magnesium, 2.7; iron, 1.4; sodium, 0.19; copper, 0.09; zinc, 0.07 and manganese, 0.08.

An aqueous extract was obtained from the dry and by shaking this residue DOR with distilled water in a ratio of 1:2 (w/v) for 8 hours at room temperature and filtered with layers of Fabric obtained with (ADOR) and solid residues (SDOR), these were incubated with ADOR and SDOR or with saprophytes.

The saprobe fungi used are: *Aspergillus niger* and *Penicillium crustaceum* obtained from Omar Mukhtar University (Department of Plant Protection Laboratory). They were propagated and stored on PDA agar and 2% malt extract agar (MEA). Done

Supplementation with ADOR and shaking at 125 rpm at room temperature with Chapek in the presence of 50% ADOR extract for two weeks. The fungi were collected by filtration and washed with distilled water. Fungi were grown in an Erlenmeyer flask (250 mL) containing 70 mL of ADOR extract for 15 days and shaken at 125 rpm. Each vial was inoculated with 0.60 g^{-1}, then perform SDOR incubation in 500 ml Erlenmeyer flasks containing 125 g of SDOR, the flasks were covered with sterile cotton and incubated under at 28 ℃ for 15 weeks. Sterile, incubated ADOR and SDOR were prepared under the same conditions.

Experiments were carried out in pots containing 500 g of soil that had been autoclaved for one hour (110℃). The soil used is a clay texture collected from the Al-Marj region (Northeast of Libya). In addition, sift the soil 2 mm thick, mixed well to remove the original AM. Properties of soil analysis were described (Page. *et al* 1982) 29% sand, 30% silt, 41% clay, pH 7.8, (ECe) 2.1 d Siemens/m at room temperature; 3.2 mg/kg P ,2.1% organic matter, and C.E.C 22.62 cm mol/kg (Mg, Ca, Na and K were 0.5, 2.2, 21.6 and 3.02 cm mol/kg respectively).

Wheat (*Triticum aestivum* L.) plants were used. The seeds were sterilized and picked before sowing. Plants were grown in a greenhouse. The pots were regularly watered with distilled water to maintain 70% of the field capacity by regular weighing of the pots, and they were fed with a nutrient solution (Hewitt, 1966).

A species of fungi (AM) (*Glomus macrocarpum*)

Obtained and prepared by the Faculty of Agriculture, University of Cairo, Egypt.5 g of mycorrhiza inoculum was added to each pot. In our experiment DOR, ADOR and SDOR were applied to the 500 g soil at concentrations of 0.15 and 30 g/kg^{-1}. Plants were harvested after 8 weeks and measured dry weight, roots were sampled and determination of root colonization with mycorrhizal fungi as described by (Phillips and Hayman 1970), and AM colonization by determining percentage of using a method by (Giovannetti and Mosse, 1980).

The percentage of AM colonization was calculated from the following equation: Percentage of AM colonization = (Root length colonized / Root length observed) ×100.

The experiment was designed in a randomized, whole-plant design, in which two experiments were used: (1) plants in sterile soil and (2) plants grown in sterile soil inoculated with 5 g of *G. macrocarpum* inoculum. These experiments were performed with three repetitions of each treatment.

Determination of phenol content: 0.5 g of DOR, ADOR and SDO was incubated for 24 h in 10 ml of distilled water/acetone to extract phenols under orbital shaking from DOR, ADOR and SDO according to (Ribereau-Gayon 1968).

Statistical analysis: using the ANOVA procedures according (Snedecor and Cochran 1972), treatments were compared by Duncan's method.

3 Results and Discussion

Root colonization by fungi was noted in roots of wheat plants inoculated with *G. macrocarpum* in presence of 15 and 30 $g.kg^{-1}$ of DOR, ADOR or SDOR (Table 1). The percentage of AMF colonization likewise decreased. Specifically, the AM colonization of plants

at 30 g. kg^{-1} of DOR (19.7%) (Table 1). The symbiotic relationship with the mycorrhizal fungus depends on the concentration of phenolic compounds in the soil (Leadir et al., 1997). Moreover, the decrease in the phenol concentration of ADOR And SDOR by saprobe fungi is one of the factors beneficial effect of these fungi on the formation of AM colonization reached by the roots of wheat plants (Table 4).But AM colonization was higher in presence of30g.kg^{-1} of ADOR or SDOR than in presence of 30 g.kg^{-1} of DOR (Table 1),and this was indicated by (Scervino *et al.*, 2005; Sampedro *et al*; 2008) that the effect of phenols on AM fungi depends on the type of plant and fungus.

Table (1). Shoot dry weight and (AMF) colonized root of wheat plants (*Triticum aestivum* L.) inoculated or not by *G. macrocarpium* with (DOR), (ADOR) and (SDOR).

Treats.	Addition level of olive residue (g.kg^{-1})	Shoot dry weight (mg)		Root coloniza-tion rate (%)
		with AM	Without AM	
Control	0	550[b]	304[a]	52.6[b]
with DOR	15	95.8[e]	106.7[d]	34.2[d]
	30	20.5[f]	43.9[g]	19.7[f]
A DOR	15	679.5[a]	252.6[b]	65.4[a]
	30	510.8[c]	174[c]	41.6[c]
S DOR	15	502.4[c]	89.5[e]	67.8[a]
	30	156.3[d]	62.7[f]	28.4[e]

Similar letters in the same column are not significantly different at 0.05 according to Duncan's multiple range test.

The application of olive mill residues reduce the shoot dry weight related to that of plants grown in absence of DOR (Table 1). The phytotoxic effect of DOR was higher than that of ADOR or SDOR (Table 1). This is due to the presence of toxic substances in DOR that are soluble in water, although other fatty acids and aldehydes also have a negative effect on plant growth (Komilis et al., 2005)

Table (2). Shoot dry weight and (AMF) colonized root of wheat plants (*Triticum aestivum* L.) inoculated or not with *G. macrocarpium* in presence of (ADOR) inoculated with *Aspergillus niger* (ADORAN) and *Penicillium crustaceum* (A DORPC)

Treats.	Addition level of olive residue (g.kg^{-1})	Shoot dry weight (mg)		Root coloniz-ation rate (%)
		with AM	Withou t AM	
Control	0	211.8[b]	146.3[b]	29.7[a]
ADOR	15	276.5[a]	124.4[c]	26.8[b]
	30	188.7[c]	78.6[e]	13.4[c]
A DORAN	15	269.3[a]	187.6[a]	34.7[a]
	30	127.8[e]	89.7[d]	26.3[b]
A DORPC	15	217.8[b]	179.5[a]	36.2[a]
	30	139.7[d]	180.6[a]	24.7[b]

Similar letters in the same column are not significantly different at 0.05 according to Duncan's multiple range test.

The dry weight of the buds was lower in the presence of SDOR than in ADOR, especially clearly in inoculated with *G. macrocarpum* as Compare with non-AM inoculated soil (Table 1). The results also indicated in previous studies that the detrimental effect of applying 25 g/kg of DOR in soil on the root system and dry weight of tomatoes and alfalfa (Martin *et al.*, 2002). The application of 15 g.kg–1 of ADOR to soil inoculated with *G. macrocarpum* increased the shoot dry weight to that of plants grown in control without any residues (Table 1).

Table (3). Shoot dry weight and (AMF) colonized root of wheat plants (*Triticum aestivum* L.) inoculated or not with *G. macrocarpium* in presence of (SDORAN) inoculated with *Aspergillus niger* and *P. crustaceum* (SDORPC).

Treats.	Addition level of olive residue (g.kg^{-1})	Shoot dry weight (mg)		Root coloniz-ation rate (%)
		with AM	Without AM	
Control	0	501.6[a]	309.7[b]	44.6[b]
SDOR	15	214.8[d]	112.3[e]	38.4[c]
	30	118.5[f]	58.7[g]	29.7[d]
SDORAN	15	312.2[b]	189.4[c]	52.4[a]
	30	188.6[e]	77.8[f]	49.7[a]
SDORPC	15	295.4[c]	345.3[a]	20.8[e]
	30	189.6[e]	163.2[d]	18.6[e]

Similar letters in the same column are not significantly different at 0.05 according to Duncan's multiple range test.

AM fungi seems to facilitate the action or transfer of toxic substances to wheat plants, increasing the sensitivity of plant to the toxicity of ADOR and SDOR, Opposite trend was noticed for the shoot dry weight with DOR. The shoot dry weight of plants grown in presence of 15 g.kg^{-1} of ADOR incubated with *Aspergillus niger* was higher than that of plants grown in absence of ADOR (Table 2). However, all doses of ADOR incubated whether *Aspergillus niger* or with *Penicillium crustaceum* increased the shoot dry weight relative to that of plants grown in absence of saprophytic fungi. In the present study both tested saprophytic fungi eliminated similar quantity of phenols from both ADOR and SDOR.

Table 4. Phenol content (g.kg^{-1}) of (ADOR) and (SDOR) incubated with *A. niger and P. crustaceum*

Treatments	ADOR	SDOR
Control	22.6[a]	17.5[a]
Aspergillus niger	9.3[b]	7.1[b]
P. crustaceum	8.7[b]	6.8[b]

Similar letters in the same column are not significantly different at 0.05 according to Duncan's multiple range test.

This study has revealed that saprobe fungi decreased the toxicity of ADOR and SDOR on wheat plants of incubation. Phenols appear to be the main substances responsible for the harmful effect of DOR on the dry

weight and the level of AM mycorrhization of plants, hence the valuable effect of saprobe fungi on both parameters seems to be related to the reduction that these fungi cause in the phenol concentration of DOR (Fiestas Ros de Ursinos ,1986). In the present study, the fact that The phenol content of ADOR was reduced by *Aspergillus niger* and *Penicillium crustaceum* 9.3 and 8.7g.kg–1, respectively and 7.1 and 6.8 g.kg–1, respectively with SDOR (Table 4). Incubation of ADOR and SDOR with saprobe fungi decreased its phenol content, these result are agreement with different plants were observed by (Aranda *et al.*, 2009) who found that the saprophytic fungi *Trametes versicolor* and *Pycnoporus cinnabarinus*.

Table (5). N and P content (g/kg^{-1}) of DOR after incubation with saprobe fungi for 8weeks.

Treatments	N	P
Control	14.8[a]	2.2[a]
Aspergillus niger	16.3[a]	2.4[a]
P. crustaceum	15.2[a]	2.7[a]

Similar letters in the same column are not significantly different at 0.05 according to Duncan's multiple range test.

After 8 weeks of incubation N and P content of DOR did not differ significantly from those of the control (Table 5). However, saprobe fungi did not decrease the N and P content of DOR, these results did not explain the decrease of the toxicity of DOR incubated with saprobe fungi for AM fungal colonized plants.

The application of ADOR and SDOR incubated with the saprophytic fungi during eight weeks increased the shoot dry weight of Wheat Plant inoculated with *G. macrocarpum* in comparison to AM colonized plants cultivated in absence of DOR, and, in conclusion, this olive mill residues allows the possibility to use as an organic fertilizer.

Conflict of Interest: The authors declare that there are no conflicts of interest

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The Unified Approach of Ionizing Radiation on Biological Matter: Action of Heavy Charged Particles on Mammalian Cells

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A B S T R A C T

Damaging effects to mammalian cells by heavy charged particles have been realized in terms of the mean free path for linear primary ionization (the spacing of ionizing events along the charged particle tracks) using *in vitro* radiobiological experimentation data. Damage is found to be optimum when the mean free path for linear primary ionization along the tracks in the cell nucleus matches the mean chord length of approximately 1.8 nm through a DNA segment. A simple semi-theoretical model is proposed to define absolute biological effectiveness based on effect inactivation cross section σ (μm^2) which is interrelated to the mean free path for linear primary ionization λ. For heavy charged particles, the model shows a saturation region for the effect cross section, $\sigma_s = 60\ \mu m^2$ for $\lambda \leq 1.8$ nm. The model explains the mechanisms leading to cell death via DNA strand scissions. In the saturation region, double strand breaks of the DNA are predominant, unrepaired or mismatched repair processes lead to maximum damage. At higher mean free path; $\lambda > 1.8$ nm, single strand breaks of the DNA is the main basic mechanism and thus repairable processes are possible.

1 Introduction

Even though that DNA is accepted as a critical target responsible for cell killing by ionizing radiation, the exact nature of cell death remains unknown (Ward, 1994; Pouget et al., 2004). Biophysical modelling could provide answers to how DNA strand breaks are related to cell killing (Chadwick and Leenhouts, 1981). Damage to mammalian cells is usually quantified with what is known as the Relative Biological Effectiveness (RBE), in terms of track average Linear Energy Transfer (LET) (ICRU-16, 1970; Barendsen, 1993; 1994). Problems associated with dose energy dependent quantities as seen by the former relation, RBE vs. LET, were subjects to debates (Kellerer, 1975; Watt et al. 1994; Simmons and Watt, 1994). The correlation between biological effects and a number of physical quality parameters led to propositions of several biophysical models (Katz, Ackerson et al., 1971; Kiefer, 1982; Kampf, 1982). The main objective of these models is to investigate the damage mechanisms, at molecular level, leading to cell death (Kramer and Kraft, 1991; Harder et al., 1992; Kellerer and Rossi, 1972). The conceptual foundations of many of the existing models were criticized by many researchers (Kraft et al., 1992).

In the last few decades, researchers have provided evidences that the double breaks of DNA opposite strands; dsb's, is responsible for cell death (Ward, 1990; Iliakis, 1991). Watt and his group suggested that the spacing of ionizing events along charged particle tracks can explain the details of the different mechanisms at nanometric scales. It is thus far better to define ionizing events at macro-molecular level with the mean free path for linear primary ionization (Watt et al, 1985).

Radiobiological experiments involving *in vitro* exposure of mammalian cells to different types of heavy charged

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particles (HCP's) would provide useful information to quantify damaging effects. The damaging effect which is also known as "reproductive cell death" characterizes an end-point effect by survival curves against dose, where survival fraction presented in logarithmic scale against radiation dose in linear scale. The shape of the curve depends on radiation type and to a certain extent on cells type. For sparsely ionizing radiations (SIR's), such as x-rays, and fast electrons, the curves have shoulder shapes, while HCP's such as slow protons, alpha particles have linear response type. It is continuously useful to use linear-quadratic fitting formula $F(S) = \ln(S/S_o) = -\alpha D-\beta D^2$, where α (Gy^{-1}), β(Gy^{-2}) are constant parameters for any specific curve. The first term represents the slope of survival curve at zero dose, which is also known as the radio sensitivity parameter. For linear survival curve, $\beta = 0$, and the equations becomes simply as $F(S) = \ln(S/S_o) = -\alpha D$.

The present study will focus on physical parameterization of the biological damage caused by HCP's. Hence presenting a model that unifies the action of HCP's on mammalian cells.

2 Materials and Methods

The cellular damage induced by ionizing radiation can be defined as the probability to produce cellular damage in units of area, which also known as inactivation cross section; σ_s. Inactivation cross sections, σ_s (in μm^2) of a variety of mammalian cells were calculated using the relation (Watt, 1996):

$$\sigma_s = \frac{L_T}{6.25\ \rho\ D_o}$$

where L_T is the track average linear energy transfer; LET (in keV/μm) for the equilibrium spectrum of charged particles involved, *do* is the initial dose (in Gy) and ρ is the density of biological matter (in g/cm^3).

Cross sections were determined for the initial slope of survival curves to avoid any problems associated with cell recovery. The initial slope for cell survival curve is simply the slope of curve at zero dose. For survival curves, whether the relationship is shouldered or linear type the slope is equivalent to α. Hence the inactivation cross section is evaluated at $D_o = 1/\alpha$. For wet cells, the density of medium is assumed of water.

The cell survival parameters were extracted by the author from published data in previous work (Watt and Alkharam, 1994). The corresponding cross sections were plotted as a function of the mean free path for primary ionization λ (in nm). The track average structure parameters; LET and λ, are estimated using Watt's group foundations (Watt, 1994; 1995a; 1995b).

The search for model imply trying a function F= F(λ), provided that the semi-empirical formula fits the curve observed by the σ–λ in terms of justifiable physical parameters.

3 Results and Discussion

Cross sections for the various mammalian cells including human cells are shown as a function of λ for HCP's. Visual inspection clarifies the grouping of σ and λ data within the spread of the physical and biological errors.

On examining the σ–λ concurrent relationship, there exist a clear inflection point around $\lambda_o = 1.8$ nm. In the saturation region, where $\lambda < 1.8$ nm, the maximum damaging effect is attributed to the mean chord of the strands in the DNA segment which can only identify that the double strand break (dsb) of the DNA are the critical lesions for inactivation for all HCP's. The spikes shown over this region are due to delta rays effect (δ-ray). These fast electrons produced in this region by HCP's would have their own tracks and thus multiply damaging effects. The damage mechanism for neutrons, shows an identical behaviour as HCP's for λ >1.8 nm but could never reach saturation damage as clearly seen in Figure-1. That is because of the limited range of protons at optimum λ. For λ > 1.8, the σ-λ relation shows a linear correlation on log-log scale graph. This part of the curve is attributed to the repairable single strand breaks of the DNA (ssb's) whether induced by the directly ionizing radiation or the water radicals along the track of DNA strands.

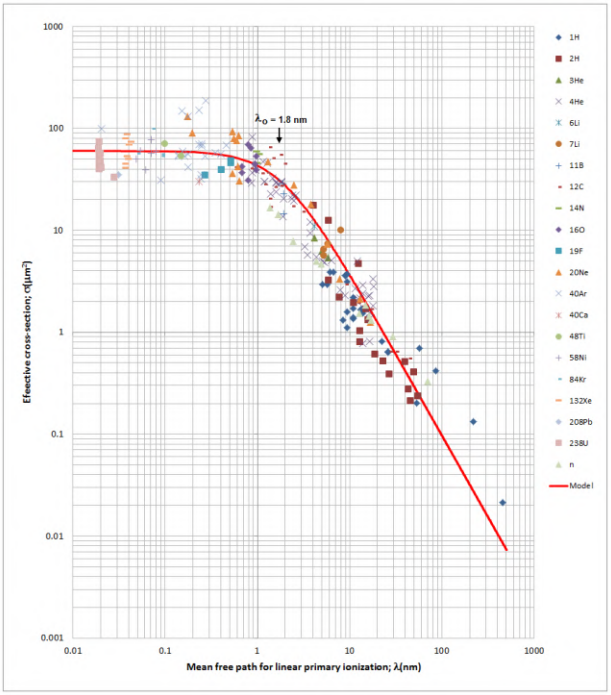

Figure (1). The effective cross section σ (μm^2) for mammalian cells vs. mean free path for linear primary ionization λ(nm) for HCP's.

Earlier investigation by the author shows similar behaviour of SIR's (Alkharam, 2022). The study concluded that SIR's like x-rays, γ–rays and fast electrons have smaller cross sections as compared to what would be expected by HCP's, as clearly shown in Figure 2. The study showed that only C_k ultra-soft x-rays with photons of energy around 0.278 keV can induce highest biological damage near the inflection point where λ_o = 1.8 nm. Higher energy photons, i.e., x-rays and γ-rays have much lower cross sections. The dominant interactions produced by SIR's are ssb's of the DNA.

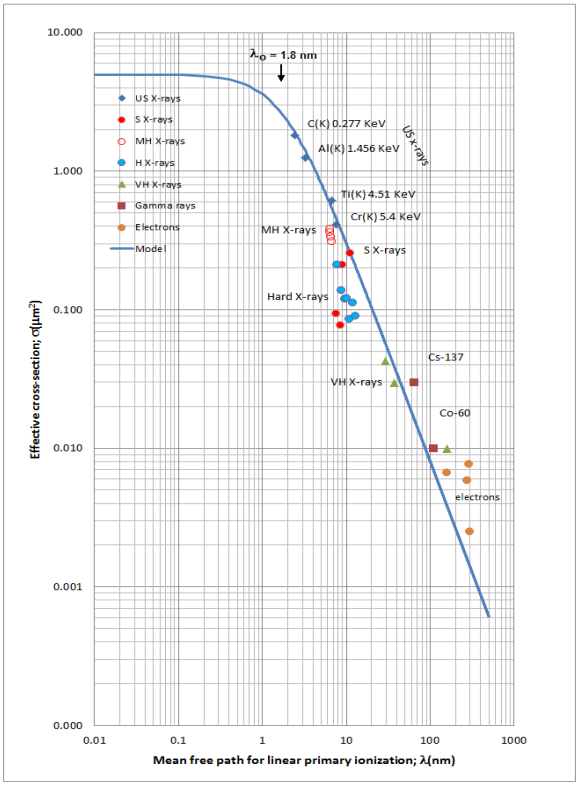

Figure (2). The effective cross section σ(μm^2) for mammalian cells vs. mean free path for primary ionization λ(nm) for SIR's (Alkharam, 2022). Only the sparsely ionizing data are extracted in the figure. The model carried in this research has been modified to match with the one used in this paper.

The grouping of data of both directly ionizing radiation and indirectly ionizing radiation and their action on mammalian cells as observed in both Figures 1-2; indicate damage to cells is independent of both the type of radiations and the type of cells. That is nothing to say more than a unified action of either types of ionizing radiation (HCP's and SIR's) on mammalian cells. The search of a simple mathematical model to fit data of both types of ionizing radiations leads to the following semi-empirical relation:

$$\sigma(\lambda) = \frac{\sigma_o}{\left(1 + \left(\frac{\lambda}{\lambda_o}\right)^n\right)}$$

where σ_o is the saturation cross section, λ_o is the value of the mean free path at inflection point (the spacing between the DNA strands), and n is a numerical value to be find for best fitting model.

The result of fitting these relations is presented by the solid lines in Figure 1 (red curve) and Figure 2 (blue curve). Both models are presented infn the same log-log scale for effect cross sections vs. mean free path for linear primary ioniztion in Figure 3 to asses the size of damage by both types of radiations and to compare their effectvness on mammalian cells.

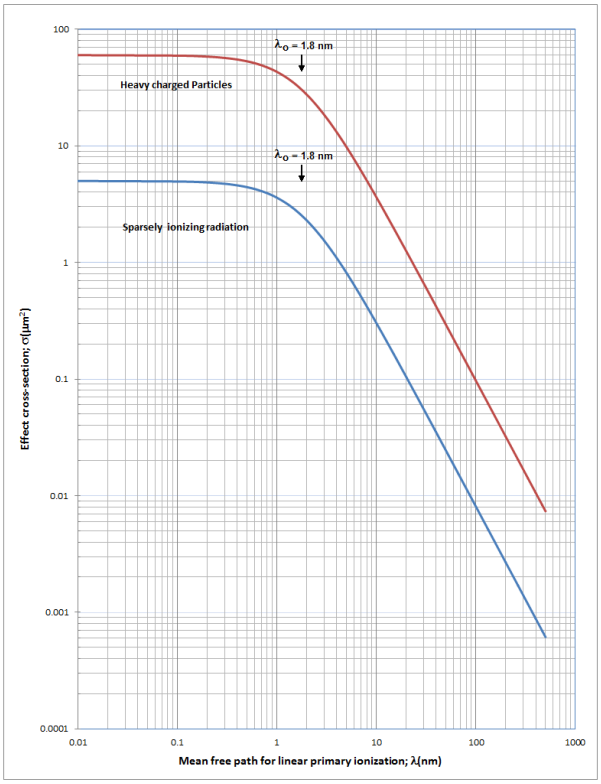

Figure (3). The unified model of radiation action on mammalian cells for both HCP's and SIR's as indicated by the solid lines.

Both curves show saturation damage for $\lambda \leq 1.8$ nm of different scales. The linear portions of the two curves have the same slope with gradient of -1.59 ± 0.06.

The merit of these values; for n = 1.6 the saturation cross sections of HCP's at $\sigma_o = 60 \pm 4$ μm^2, and of SIR's at $\sigma_o = 5 \pm 0.3$ μm^2, and an infliction point at λ_o = 1.8 ± 0.4 nm, indicate that the size of this damage have got to be related to nanometric dimensions. In other words, ionizing radiation initially induce dab's and ssb's in the DNA strands.

related to nanometric dimensions. In other words, ionizing radiation initially induce dab's and ssb's in the DNA strands.

HCP's is responsible for the induction dsb's in the DNA directly. The fact that only the DNA dsb leads to cell death, shows that HCP's have greater capability than SIR's to destroy normal or cancerous cells. The damaging capability of HCP's to mammalian cells is about 12 times of SIR's.

4 Conclusions

In this study, a simple model that unifies the action of radiation on mammalian cells was presented. The main features of this model is; its unique specification of the cellular damage in terms of biophysical parameters that relates molecular events such dsb's of the DNA to macroscopic biological effects such as cell death. In simple mathematical form; $\sigma(\lambda)=\sigma_o/(1+(\lambda/\lambda_o)^n)$, the model indicate that the maximum damage is represented by σ_o which equivalent to the geometrical cross section, $\sigma_g = 60\ \mu m^2$ of the cell nucleus. This maximum damage can only take place if the ionizing radiation have mean free path greater than 1.8 nm. The inflection point as indicated by the parameter λ_0, gives an insight of where the damage becomes prominent; for $\lambda < 1.8$ nm the damage is saturated and caused by dsb's of the DNA whereas for $\lambda > 1.8$ nm the damage could be repaired whether initiated by dsb's or ssb's of the DNA. Further investigations are needed to indicate the rules of water radicals in cellular damage. A more sophisticated model will be vital to further demonstrate the unification action of ionizing radiation on mammalian cells in terms of detailed physical parameters.

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Testing an Allelopathic Effect *Arum Cyreniacum* on Germination and Growth of *Pisum Sativum* L

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A B S T R A C T

Two experiments were conducted (laboratory - pots) at the lab of the biology Department/ Faculty of education/ Omar Al-Mukhtar University/ Al Bayda/ Libya, to aim testing allelopathic effects of aqueous extracts at concentrations of (6, 8 and 10%), adding the Crude powder to soil at a concentration of (3%) of (Tubers-Leaves) *Arum cyreniacum* on the germination of seeds and development seedlings of *Pisum sativum* with three replications according to a completely randomized design. The results of the laboratory experiment showed were significant differences in reduction of germination percentage, reduction of radical and plumule lengths between aqueous extracts, compared with control, also, all concentrations caused a clear delay in an average germination time. The results of a pots experiment showed a concentration (3%) reduced the percentage emergence of seedlings, and decrease root and shoot lengths, fresh and dry weights of seedlings. A concentration (3%) caused a decrease of content chlorophyll (a, b), and sodium, an accumulation of carotenoids, potassium and iron, compared to the control. The leaves had the highest inhibition rates compared to the tubers at inhibiting the growth of studied traits. This study concluded that A. cyreniacum has inhibitory effects against germination of *P.sativum.*

1 Introduction

Weeds are one of the most significant pests that obstruct human efforts to achieve adequate agricultural produce of high quality (Salih and Abdulrraziq, 2020). *Pisum sativum* L. is a Fabaceae family annual herb crop that is regarded as one of the most significant legumes. It is a vital source of energy as well as a high-protein food (Al-bozidy, *et al.*, 2019). Furthermore, it contains some critical minerals such as calcium, phosphorus, and iron, as well as 20-25 percent starch, 4-10 percent sugar, and 0.6-1.5 percent fat (Makasheva, 1983; Haque *et al.*, 2014). Susceptible to biotic and abiotic stressors, particularly allelopathic stress (Mohamed and El-Ashry, 2012; Benezit *et al.*, 2017). Allelopathy is described as a plant's direct or indirect, catastrophic effect on another plant caused by the release of toxins. (allelochemicals) which are secondary metabolites, which are present in all plant tissues including leaves, stems, flowers, roots, and seeds (Kumar *et al.*, 2018; Salih and Abdulrraziq, 2021).

Classified into various groups on the basis of their chemical properties, phenolics, alkaloids, terpenes, fatty acids, and indoles are the most ordinarily occurring allelochemicals in plants (kato-Noguchi, 2008). Allelopathic inhibition may be due to the toxic effects of a single compound or the interaction of a group of some chemicals (Li *et al.*, 2010) plants wild plays an important role in the formation of their natural habitats as it contains the allele-chemical compounds, enabling plants to compete with other species, where it inhibits crop growth and production (Ebid, 2016).

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Arum cyreniacum is a tuberous annual herb in the (Araceae) family that is used for both food and medicine. It is unique to the Cyrenaica region near agricultural regions, particularly in Libya's Al-Jabal Al-Akhdar region (Abdulrraziq and Salih, 2020). Allelochemical interactions with *Pisum sativum* have been documented in a variety of plants, including medicinal plants. In a study conducted in Saudi Arabia, aqueous extracts of two medicinal plants (*Artrmisia monosperma* and *Thymus vulgaris*) were found to lower growth capability, shoot and root length, total free amino acids, and proline in *P. sativum* (Al-Hawas and Azooz, 2018). Furthermore, an aqueous extract of *Ageratum conyzoides* has been demonstrated in a study (Singh, 2021) to have detrimental impacts on *P.sativum* germination, shoot length, root length, and biomass production. Furthermore, the aqueous extract of *Alhagi maurorum* lowered all growth indices of *P.sativum*, including photosynthetic pigments, total carbohydrate, and total protein (Khalil *et al*., 2017).

The purpose of this study was to see if aqueous extracts of *Arum cyreniacum* leaves and tubers at different concentrations (6, 8 and 10%) had an allelopathic effect on the germination of seeds of *Pisum sativum*, and if adding the crude powder of *Arum cyreniacum* to the soil at a concentration of (3 %) (tubers-leaves) had an allelopathic effect on the germination of seeds and the development of seedling.

2 Materials and Methods

2.1 Sample Collection:

Samples of *A.cyreniacum* (leaves- tubers) were collected from of Al-Bayda city, it is defined to classified according to (Abdulrraziq and Salih, 2020), washed with distilled water, then dried under natural conditions, grind with an electric grinder, finally preserved for use.

2.2 Laboratory Experiment:

2.2.1 Seed Selection:

Seeds of *Pisum sativum* were obtained from local markets, cleaned of impurities, and viability was tested by soaking in distilled water to get rid of empty seeds floating on the surface, were soaked in 1% sodium hypochloride solution for 3 minutes, washed with distilled water (Dafaallah *et al*., 2019).

2.2.2 Preparation of the Aqueous Extract:

The aqueous extract (leaves- tubers) was prepared separately by adding 100 g of air-dried powder to 1000 ml of distilled water for 24 h, after that the extract was filtered through filter paper and placed on a Shaker for 24 hours. Then it was centrifuged at the speed of 2000 rounds per minute for 15 minutes. The extract was passed through Whatman filter paper No.1. The obtained extract concentration was considered as the stock solution (10%) (Al-Hawas and Azooz, 2018). Then it was appropriately diluted with distilled water to give final concentrations of 6, 8, and 10%.

2.2.3 Seed germination:

Normally, 10 seeds per Petri dish, were lined with two Whatman No.1 filter papers, incubated at room temperature, three replications for each treatment, dishes were subjected to daily observation for 10 days and follow-up of germination in terms of addition of extracts to the treated dishes. add distilled water to Control as needed for each dish (Othman *et al*., 2018), germination was calculated by recording a number of germinated seeds in all treatments starting from the second day, which the first germination occurred, germination criterion is the appearance of radical outside seed cover (Ganatsas *et al*., 2008) at end of the experiment took final results of following qualities:

Germination percentage (PG %) = number of germinated seeds / total number of seeds × 100 (Yousif *et al*., 2020).

Mean germination time (MGT) = the total number of germinated seeds per day / total number of germinated seeds at end of the experiment (Das *et al*., 2017).

radical and plumule lengths: The root and plumule lengths were taken using a graduated ruler, the averages were calculated by taking 5 seedlings from each plate.

2.3. Pots Experiment:

The soil samples were finally sterilized at (90ºC for 48 h) to remove any microorganisms and weed seeds. Ten seeds of *P.sativum* were sown in plastic pots (16 cm in diameter) in pure culture practices with about 1000 g of sandy loam soil thoroughly mixed (w/w) with 3 % of electrically crushed crude powder of leaves and tubers of *A.cyreniacum.* One treatment was run as a control with zero percent of crude powder with three replicates. The plants were watered every two days on average with normal tap water. The experiment was performed under normal laboratory conditions (20±2°C temperature, 75±2% relative humidity, and 14/10 h light/dark photoperiod). After 21 days, the homogenous seedling was carefully collected from each treatment, washed with tap water to remove the adhering soil particles, and then, by distilled water, gently blotted with filter paper. The seedlings were separated into shoots and roots for the determination of seedling fresh weight as well as seedling length. Other samples were dried at 65ºC till constant weight to determine the seedling dry weight (Alaila *et al*.,2021).

2.3.1 Photosynthetic Pigments

The photosynthetic pigments (chlorophyll a and chlorophyll b) were determined spectrophotom etrically according to (Metzner *et al*., 1965).

2.3.2 Estimation of Minerals in Plant

Seedlings were carefully and thoroughly cleaned, blotted dry between absorbing paper and their dry weights were measured after oven drying at 70°C for 72h.Oven dry samples of seedlings were finely ground and assayed for-mineral ion content by the wet digestion method. Minerals (K, Fe and Na) were determined using an atomic absorption spectrophotometer and expressed on the basis of dry weight (Humphries, 1956).

Statistical Analysis:

The study of two experiments were designed according to the complete random design (CRD). Statistical analysis was performed using Minitab 17 program and ANOVA variance analysis tables. The averages were compared using Tukey's test at P <0.05.

3 Results:

The results of this study showed that aqueous extracts of A.cyreniacum have highly inhibitor activity to reduce germination percentage, radical and plumule Length, with increasing the average germination time of *P.sativum* seeds, after 10 days from the start of the experiment, compared to control. The data recorded in the table (1) that the concentration of (6%) of leaves and (6 and 8%) of tubers extract had no inhibitory effect on a germination percentage, but caused a clear delay in the average germination time, from 3.0 days of control to about 5.0 days. While this percentage decreased of leaves extract at a concentration (8, 10%) from 100% for control to 73, 26%, respectively. The concentration 10 of tubers extract also recorded a decrease in germination percentage with 60%. The results also showed that all concentrations caused a clear delay in the average germination time. The corresponding allelopathy effects on radical and plumule length were recorded. Data demonstrated that the radical and plumule length decreased significantly upon applying different concentrations of the extracts, especially, at 10% which did not show any plumule germination.

Table (1). Effect of *Arum cyreniacum* extracts on germination of *Pisum sativum* seeds.

Extract	Conc. (%)	Germination (%)	Mean Germination time	Radical length	Plumule length
Control		100 a	3.0 d	5.6 a	7.4 a
Leaves	6%	100 a	5.7 bc	2.5 bc	2.9 c
	8%	73 b	7.3 a	1.4 de	1.1 d
	10%	26 d	7.0 ab	0.7 f	0.0 e
Tubers	6%	100 a	5.0 c	3.0 b	3.4 b
	8%	100 a	5.5 c	1.9 cd	2.8 c
	10%	60 c	7.0 ab	1.2 ef	0.0 e

As shown in Table (2) and Figure (1) the results of adding crude powder (tubers - leaves) of *A.cyreniacum* to soil at a concentration (3%) on seedling emergence, Shoot and root length, fresh and dry weight of *P. sativum*, after 21 days from agriculture at pots, noted reduced of seedlings emergence from 100% for a control to (40, 66%) for the treatment of leaves and tubers, respectively. The results also indicate a decrease in shoot and root length, fresh and dry weight of *P.sativum*.

Table (2). Effect of *Arum cyreniacum* extracts on the germination and growth rates of *Pisum sativum* on pots.

Extract	Conc.	Number seedling (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
Control		90.0 a	17 a	26 a	43.5 a	21.3 a
Leaves	3%	40.0 c	4.5 c	2.7 c	19.4 c	8.5 c
Tubers	3%	66.0 b	7.6 b	5.2 b	24,1 b	13.0 b

Figure (1). Effect a concentration (3%) of *Arum cyreniacum* extracts on the germination and growth rates of *Pisum sativum*.

As shown in table (3), the results of an effect of adding the dry crude powder of *A.cyreniacum* (tubers - leaves) to soil at a concentration (3%) on the content of chlorophyll (a, b and carotenoids), and Minerals (K, Fe, and Na) of *P.sativum*. where the results showed a decrease of content chlorophyll (a, b) and an accumulation of carotenoids of *P.sativum* leaves, also led to an accumulation of potassium, iron, while a decreased sodium compared to the control.

Table (3). Effect of *Arum cyreniacum* extracts on a content of pigments, and some of the minerals *Pisum sativum*.

Extract	Conc.	Chlorophyll a	Chlorophyll b	carotenoids	K	Fe	Na
Control		1.13 a	1.44 a	1.84 c	88 c	1.3 c	0.8 a
Leaves	3%	0.69 c	0.81 c	3.3a	122 a	3.7 a	0.2 b
Tubers	3%	0.95 b	1.05 b	2.6 b	97 b	3.2 b	0.3 b

4 Discussion

Weeds and wild present around the fields exert their allelopathic influence on agricultural crops (Hayyat *et al.,* 2020). So this study was conducted to test allelopathic effects of aqueous extracts and the crude powder added to the soil from *A.cyreniacum*. which showed that the aqueous extracts of (leaves and tubers) *A.cyreniacum* have an allelopathic effect against germination rates of *P.sativum*, this result agreed with the findings of (Salih and Abdulrraziq, 2020) that the allelopathic compounds present in the aqueous extracts of many wild plants had high toxicity against growth *P.sativum*, Which has a negative role in impeding of germination of seeds and seedlings, by affecting a variety of biochemical and physiological attributes, and also caused cellular membrane injury in the germinating seeds (Ullah *et al.,* 2015). The powder added to the soil also had negative effects on the growth of *P.sativum* seedlings, These results were supported by (Khali *et al.,* 2017) were indicated to inhibit germination and seedling growth of (*Pisum sativum* L.) when using *Alhagi maurorum* extract. the concentration (3%) also led to Deterioration in the content of pigments and mineral elements, which this result agreed with (Mendez and miranol, 2015; Al-Hawas and Azooz, 2018).

5 Conclusions

We conclude from this study that *A.cyreniacum* has clear inhibitory effects against germination of *P.sativum*. Leaves are the most toxic than tubers, so this study recommends excluding *A.cyreniacum* and limiting its spread near agricultural lands, because of clear inhibitory effects that reduce crop productivity.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Review Study of Chiral *N*-Heterocyclic Carbene (NHC) Ligands in Stereoselective Metal-Catalyzed Reduction Reactions

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A B S T R A C T

Stereoselective metal-catalyzed reactions using *N*-heterocyclic carbene (NHC) ligands have shown significant recent advances, due to the ability of NHC ligands as strong σ-donor species to coordinate with a wide variety of transition metals. Therefore, the design of new ligands and the subsequent strategies for their synthesis enables new applications of their metal complexes in catalysis to be investigated. This study focuses on the applications of different classes of Ir-, Pd-, Au- and Rh-NHC ligand complexes as promising catalysts in the asymmetric hydrogenation, hydrosilylation and transfer hydrogenation reactions.

1 Introduction

During the last two decades, *N*-heterocyclic carbenes (NHCs) as highly active and stable species have attracted considerable attention by chemists, due to their importance as versatile spectator ligands in the area of asymmetric metal-catalyzed reactions (César et al., 2004). Since the first reported stable NHC **1** by Arduengo (Figure 1) (Arduengo et al., 1991), tremendous advances on the design of NHCs and the application of their complexes in asymmetric catalysis have been achieved (Clavier et al., 2005). NHC ligands such as imidazolylidenes **2**, benzimidazolylidenes **3**, imidazolinylidenes **4** and triazolinylidines **5** are strong σ-donors and poor π-acceptors that form strong carbon-metal bonds. Therefore, catalysts derived from these ligands have better thermal and air stability than others containing phosphine ligands (Perry & Burgess, 2003). Many complexes derived from NHC ligands have been synthesized and applied in stereoselective catalysis, yielding high enantioselectivities. These include NHC-rhodium and -ruthenium complexes for asymmetric reduction of olefins and ketones.

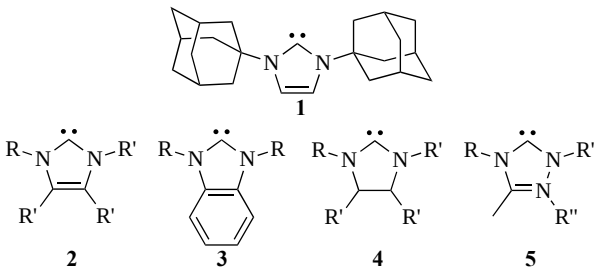

Figure 1. The first reported NHC ligand **1** and other NHC ligands **2**-**5**.

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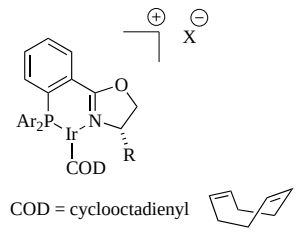
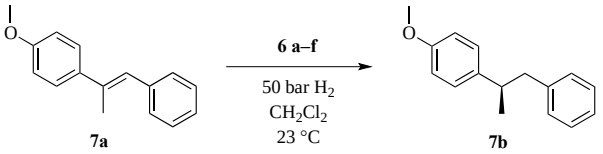
2 Stereoselective Hydrogenation

Stereoselective hydrogenation of alkenes is one of the earliest and important catalytic reactions, enabling the potential for the generation of stereogenic carbons. Ir-catalyzed hydrogenation of alkenes using phosphine-oxazoline ligands (Ir-PHOX complexes **6a-f**) was developed by Pfaltz and found to be one of the highly selective catalysts for wide variety of non-functionalized alkenes such as **7a**, affording the corresponding product **7b** in up to 98% ee and 99% yield (Scheme 1) (Lightfoot et al., 1998).



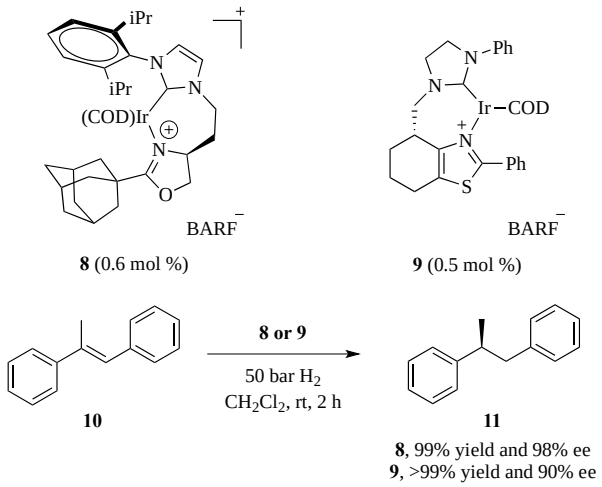
	Ar	R	X
6a	Ph	*i*Pr	PF_6
6b	Ph	*t*Bu	PF_6
6c	*o*-Tol	*i*Pr	PF_6
6d	*o*-Tol	*t*Bu	BARF
6e	Ph	*i*Pr	BARF
6f	*o*-Tol	*t*Bu	BARF



99% yield and up to 98% ee

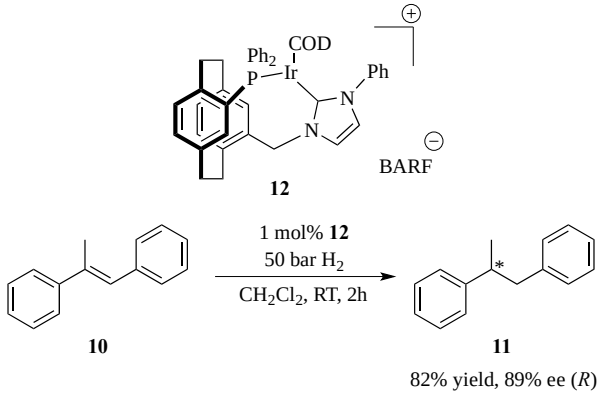
Scheme 1. Stereoselective hydrogenation of alkenes **7a** with Ir-PHOX complexes **6a-f**

Gade and Bellemin-Laponnaz (2007) reported the synthesis of a new type of NHC-Ir complex **8** containing NHC-oxazolines, and tested its catalytic activity in the stereoselective hydrogenation of non-functionalized alkenes, such as (*E*)-1,2-diphenyl propene (Gade & Bellemin-Laponnaz, 2007). This complex proved to be highly active and more selective than others from the same family, due to the electronic nature and the steric hindrance effects. The electronic effect forces the alkene towards the NHC to enable the steric effect to control the enantioface discrimination. The presence of the bulky adamantly group made the catalyst more efficient and highly selective, yielding **11** in excellent yield and enantioselectivity (99% yield, and 98% ee) (Scheme 2) (Gade & Bellemin-Laponnaz, 2007; Normand & Cavell, 2008). The Ir-NHC-thiozole complex **9** catalyzes the asymmetric hydrogenation of olefins with up to 90% ee and greater than 99% yield (Källström & Andersson, (2006).



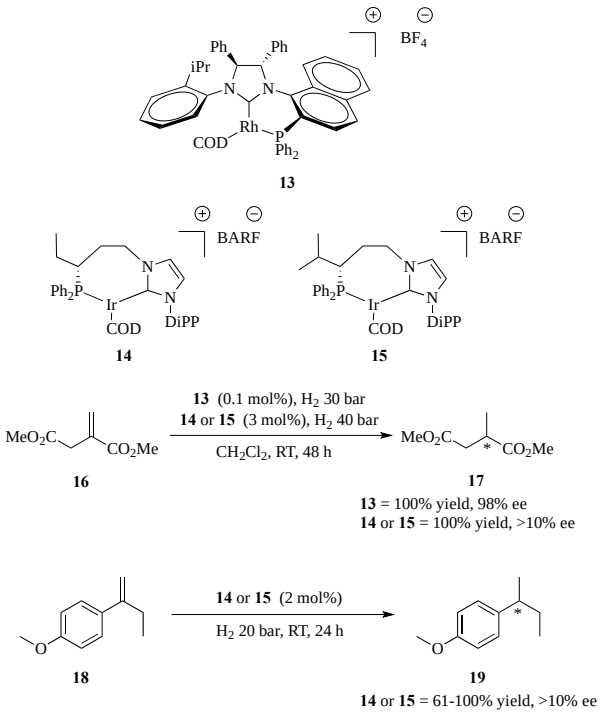
Scheme 2. Stereoselective hydrogenation of olefin **10** with chiral bidentate oxazoline- and thiazole-NHC-Ir complexes **8** and **9**.

Focken et al. (2004) reported the synthesis of iridium imidazolylidene complex **12** from a chiral phosphinoimidazolium salt (Focken et al. 2004). The asymmetric hydrogenation of simple and functionalized alkenes such as (*E*)-1,2-diphenyl-1-propene **10** gave the alkane **11** in high yield and enantioselectivity (up to 82% yield and up to 89% ee) using CH_2Cl_2 as a solvent at 25 °C (Scheme 3).



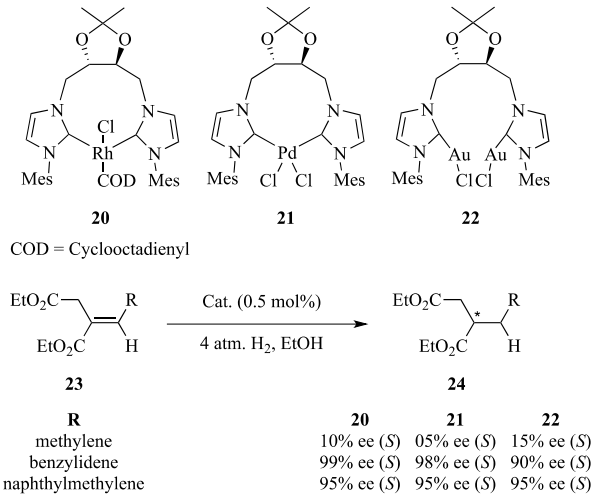
Scheme 3. The stereoselective hydrogenation of **10** catalyzed by complex **12**.

The *P*-functionalized Rh complex **13** hydrogenates dimethyl itaconate **16** with 98% ee. A wide variety of functionalized alkenes have been examined with complex **13**, giving the corresponding alcohols in up to 99% ee (Normand & Cavell, 2008). However, the Ir catalysts **14** and **15** displays poor catalytic activities towards the deactivated substrates **16** and **18**, giving the corresponding products **17** and **19** in lower enantioselectivities (> 10% ee) (Scheme 4) (Passays et al., 2011).



Scheme 4. The catalytic hydrogenation of the functionalized alkenes **16** and **18** with complexes **13**, **14** and **15**.

Arnanz et al. (2010) synthesized stable diimidazolidinylidene ligands linked by a *trans*-2,2-dimethyl-1,3-dioxolane and prepared chelated rhodium, palladium and gold complexes (*bis*-NHC-complexes) **20**, **21** and **22** (Arnanz et al. 2010). The efficiency of these catalysts was tested in the hydrogenation of (*E*)-diethyl-2-*R*-succinates **23** and showed high enantioselectivities, up to 99% (Scheme 5).



Scheme 5. The asymmetric hydrogenation of (*E*)-diethyl-2-(*R*)-succinates **23** with bis-NHC-complexes **20-22**.

3 Stereoselective Hydrosilylation

Stereoselective metal-catalyzed hydrosilylation of ketones using diphenylsilane is an important and efficient alternative process to the catalytic hydrogenation for the preparation of the corresponding chiral alcohols. This is advantageous as it uses mild conditions and avoids the over use of high pressure hydrogen (Kuang et al., 2009; Malkov et al., 2008).

Song et al. (2011) prepared three NHC-Rh complexes **25a-c** derived from dibenzimidazolium salts and tested their catalytic activities in the asymmetric hydrosilylation of acetophenone **27** under the reaction conditions given in table 1 (Song et al. 2011; Liu et al. 2009). The results show the importance of Rh-complexes as good catalysts for the catalytic hydrosilylation of acetophenones. Complex **25b** was found to be more selective than complex **25a** at 20 °C with 60% ee, due to the steric hindrance of the bulky substituents (entry 2), while 0% ee is observed with complex **25c** at the same temperature (entry 3), presumably because the bulky substituents are far from the metal center and are therefore outside the coordination sphere. By making the temperature lower with increasing the reaction time, the reduction product **28a** can be afforded in better enantioselectivities (54-70% ee) (entries 4-7) (Song et al. 2011).

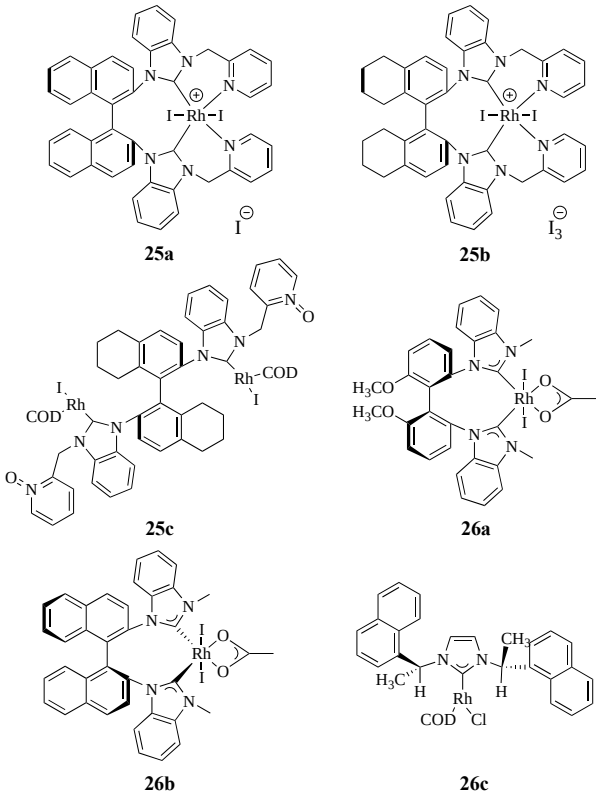
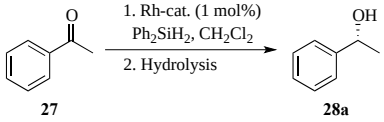


Figure 2. NHC-Rh complexes **25a-c** derived from dibenzimidazolium salts and bis NHC-Rh complexes **26a-c**

Shi and co-workers (2009) reported the use of *bis*-NHC-Rh complexes **26a-c** for the hydrosilylation of ketones (Normand & Cavell, 2008; Liu et al. 2009; Duan et al. 2003). It was found that using CH_2Cl_2 as the solvent, complex **26a** was ineffective (20% yield and 0% ee) (entry 8), while the reduction was proceeded with 55% ee and 70% yield using toluene. Furthermore, up to 98% and 32% enantioselectivities were afforded using complex **26b** and **26c** respectively (entries 10 and 11) (Liu et al. 2009).

Table 1. Stereoselective hydrosilylation of acetophenone **27** with Rh-catalysts **25a-c** and **26a-c**.

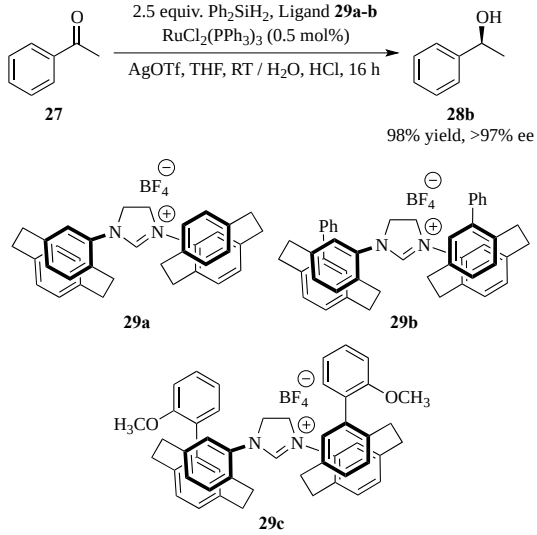


Entry	Complex	T °C	Time h	ee %	Yield %
1	**25a**	20	24	46	96
2	**25b**	20	24	60	90
3	**25c**	20	24	0	94
4	**25a**	0	36	54	82
5	**25b**	0	36	70	76
6	**25a**	– 20	72	56	54
7	**25b**	– 20	72	70	42
8	**26a**	25	48	0	20
9	**26a**[a]	25	48	55	70
10	**26b**	25	48	98	n.d.
11	**26c**	25	48	32	n.d.

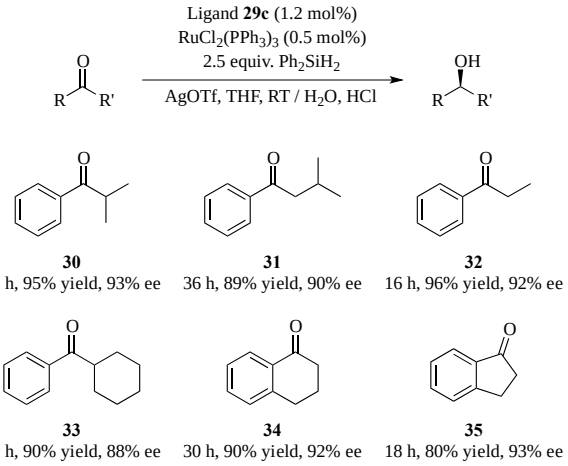
[a]Solvent = toluene

Bis-paracyclophane NHC-ligands **29a-b** have been synthesized from the known precursor (*Sp*-pseudo-orthobromoamino-[2.2]-paracyclophane). These ligands have been examined for Ru-catalysed asymmetric hydrosilylation of acetophenone **27** (under the conditions shown in scheme 6) to enantioenriched *S*-phenylethanol **28b** with 98% yield and 97% ee. Ligand **29c** was also used to optimize the reaction conditions. Different solvents were tested and THF proved to be the best for excellent enantioselectivities, where as using chloroform the selectivity reduced to 77%. Other solvents such as benzene, toluene and dioxane lowered the yield to 20%, 36% and 58% respectively, and poor selectivity was obtained using benzene and toluene. Further additives were also investigated such as using copper(II) triflate instead of silver(I) triflate, and this led to an increase in the reactivity with lowering the selectivity to 92% ee (Song et al. 2005).

The ligand **29c**-Ru combination has been also tested in asymmetric hydrosilylation of more hindered ketone substrates and gave high selectivities. For instance, isopropyl, sec-butyl and ethyl phenyl ketones **30-32** were afforded in excellent selectivities (up to 93% ee), as well as cyclohexylphenyl ketone **33**, benzocyclohexanone **34** and indanone **35** with enantiomeric excess of 88%, 92% and 93% respectively (Scheme 7).



Scheme 6. Hydrosilylation of acetophenone **27** with *bis*-paracyclophane NHC-ligands **29a-b**.

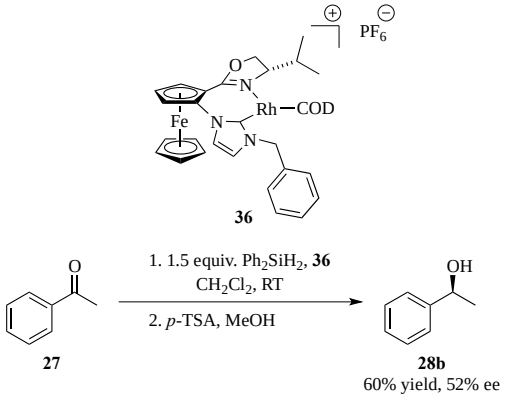


Scheme 7. Hydrosilylation of hindered ketone substrates with cyclophane ligand **29c**.

Kuang et al. (2009) reported the synthesis of NHC-oxazoline ligand **36** which has a rigid backbone consisting of an imidazole ring linked directly to ferrocene (Kuang et al., 2009). This new ligand was tested in the Ru-catalyzed asymmetric hydrosilylation of prochiral ketones, giving moderate enantioselectivity (52% ee) and reactivity (60% yield) (Scheme 8).

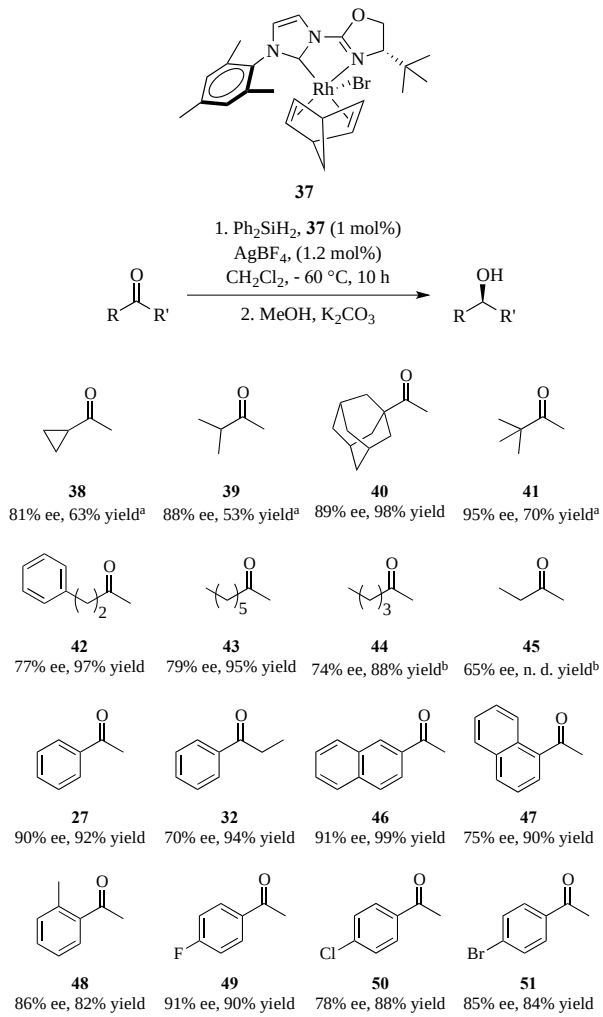
César et al. reported the synthesis of hydrosilylation catalysts derived from the condensation of both

oxazoline and imidazole rings (César et al., 2005). Among these catalysts, only **37** with the bulky ligand substituents such as mesitylene and *tert*-butyl gave the corresponding products in high yields and enantioselectivities. The catalyst **37** was exceptionally selected in the hydrosilylation of difficult unsymmetrical dialkyl and alkyl aryl ketone substrates. Dialkyl ketones have previously been reported to be difficult substrates to obtain high ee values in hydrosilylation reactions. The results shown in scheme 9 identified complex **37** as a highly selective catalyst for the nonaromatic ketones **38-45**. For example, cyclopropyl methyl ketone **38** gave the corresponding chiral alcohol with an ee value of 81% ee. The enantioselectivity can be superior using more highly steric alkyl groups such as *tert*-butyl methyl ketone **41**, reaching 95% ee. Furthermore, linear chain alkyl methyl ketone substrates **43-45** were obtained in moderate enantioselectivities (from 65% to 79%) (Scheme 9) (Gade & Bellemin-Laponnaz, 2007; César et al., 2005).



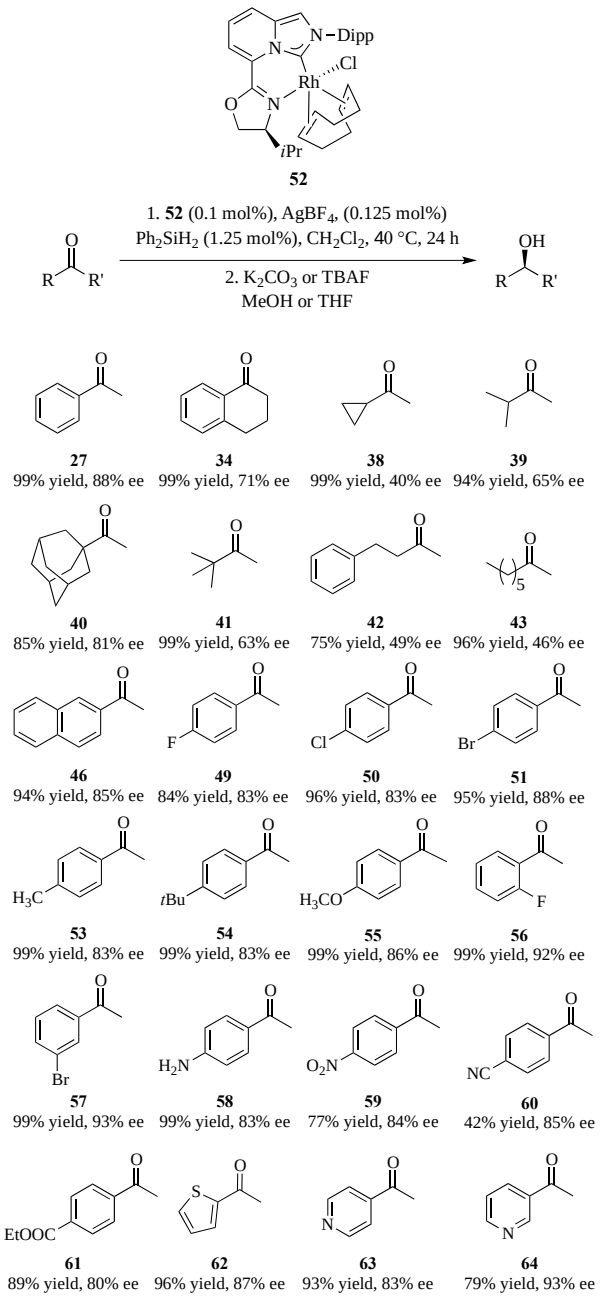
Scheme 8. Hydrosilylation of acetophenone **27** catalyzed by **36**.

In case of hydrosilylation of aryl ketones, complex **37** was similarly found to give high yields and enantioselectivities (César et al., 2005). For example, acetophenone **27** and 2-naphthyl methyl ketone **46** were produced in 90% and 91% ee (with 92% and 99% isolated yield) respectively. In addition, the effects of the reaction conditions such as solvent choice and temperature on the catalytic selectivity were also investigated with complex **37**. Solvent such as CH_2Cl_2 was found to be the best among different polar solvents that were tested, giving the best results. The effect of the temperature on the catalyst selectivity was also examined between –78 to 25 °C, and it was found that the highest enantioselectivity was obtained at –60 °C (up to 95% ee with different dialkyl and aryl alkyl ketones), while it drops with higher and lower temperatures (Gade & Bellemin-Laponnaz, 2007; César et al., 2005).



Scheme 9. Hydrosilylation of unsymmetrical dialkyl and alkyl aryl ketone substrates with catalyst **37** .[a]Moderate yield due to the volatility of the product. [b]Reaction carried out at –40 °C.

In a comparison with complex **37 (Scheme 9)**, Swamy et al. (2020), reported the synthesis of rhodium(I) complex **52**, with chiral bidentate NHC-oxazoline ligands (Swamy et al. 2020). This complex was found to be active for the asymmetric hydrosilylation of unsymmetrical dialkyl and alkyl-aryl ketone substrates. The corresponding alcohols were obtained in yields greater than 90% and up to 95% enantioselectivities (Scheme 10). It is worth noted that this catalyst was tested in different ketones bearing a wide range of functional groups as well as ketones bearing heterocyclic substituents such as **62-64**.

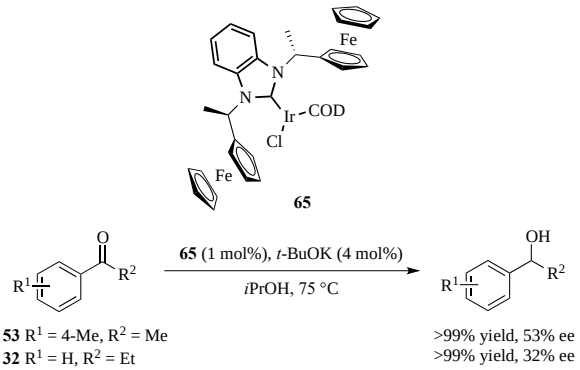


Scheme 10. Hydrosilylation of unsymmetrical dialkyl and alkyl aryl ketone substrates with catalyst **52**.

4 Stereoselective Transfer Hydrogenation

The catalytic asymmetric transfer hydrogenation of prochiral ketones is fundamentally an important synthetic reaction due to its simplistic operation with low cost reducing agents and the efficiency of this reaction as a synthetic route to optically active secondary alcohols (Jiang et al. 2009; Chiyojima et al. 2011). Although the asymmetric hydrogenation and hydrosilylation have shown a marked progress in the last two decades, only few studies were conducted on the asymmetric transfer hydrogenation using chiral NHCs.

Seo et al. (2003) reported the synthesis of the chiral ferrocenyl-NHC-Ir complex **65** for the asymmetric transfer hydrogenation of 4-methylacetophenone **53** and propiophenone **32**, giving the corresponding chiral alcohols with moderate enantioselectivities of 53% and 32% ee respectively. However, the selectivity was low or even racemic with other ketone substrates (Scheme 11) (Seo et al., 2003; Chiyojima et al. 2011).



Scheme 11. Stereoselective transfer hydrogenation of **32** and **53** with chiral ferrocenyl-NHC-Ir complex **65**.

Hodgson and Douthwaite (2005) synthesized a new class of chiral NHC-phosphine ligands **66a-e**, and investigated their applications in the asymmetric transfer hydrogenation of acetophenone (Figure 3). It was found that their complexes displayed poor selectivities with iridium(I) (11 - 37% ee) (Hodgson & Douthwaite, 2005).

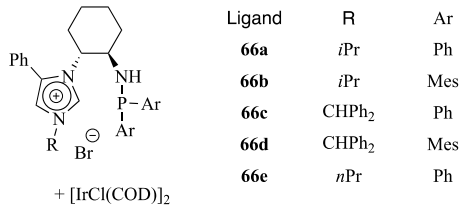
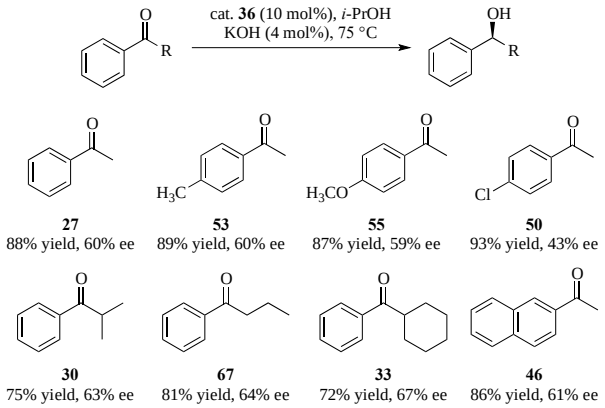


Figure 2. Chiral NHC-phosphine ligands **66a-e** and their application in asymmetric transfer hydrogenation.

The ferrocene based-NHC-Rh(I) complex **36** (previously shown in Scheme 8) was applied to the asymmetric transfer hydrogenation of aryl alkyl ketone substrates (Jiang et al. 2009). The catalytic activity of **36** was investigated under different reaction conditions and the highest selectivity was achieved by using KOH as a base at 75 °C. With these optimized conditions, various ketone substrates were examined (Scheme 12). The corresponding alcohols were afforded in slightly different yields and enantioselectivities, probably due to the effect of the electronic and steric properties of

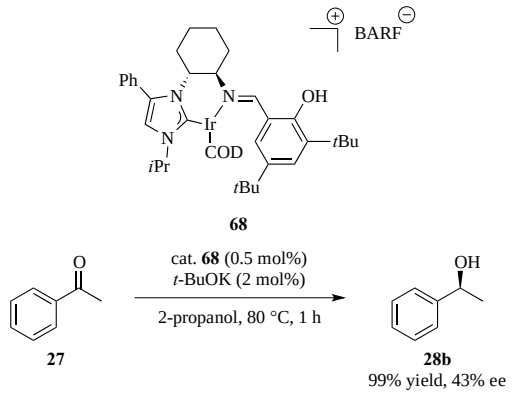
different aryl and alkyl ketones. The electron-donating substituents on the phenyl group of acetophenones such as methyl and methoxy (**53** and **55**) did not affect the yield or the selectivity of the corresponding product. On the other hand, the presence of an electron-withdrawing group such as chlorine (**50**) led to lower enantioselectivities and higher yields. Furthermore, the bulky R groups (*i*-propyl and *n*-propyl) on the ketone (**30** and **67**) can positively affect the selectivity with a negative effect on the reactivity. Finally, cyclohexyl phenyl ketone **33** gave the highest selectivity of 67% ee, while no considerable difference was shown on the reactivity and selectivity when the phenyl group replaced by naphthyl group **46** (Scheme 12).



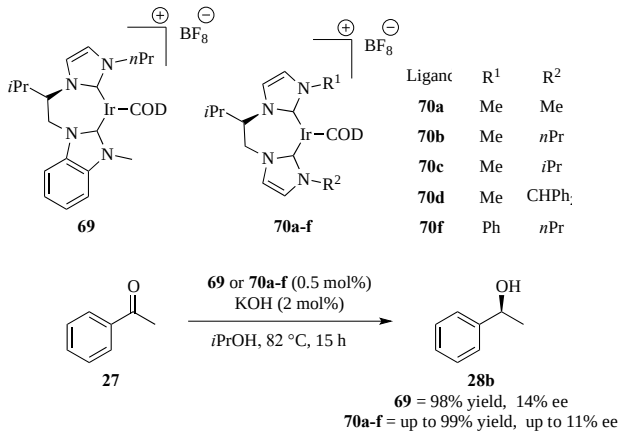
Scheme 12. The effects of the electronic and steric properties on the enantioselectivity of different aryl and alkyl ketones with NHC-Rh(I) **36**.

Dyson et al. (2009) reported the synthesis of iridium NHC–phenolimine complex **68** and tested its catalytic activities in the asymmetric transfer hydrogenation of acetophenone **27** (Scheme 13). The corresponding alcohol **28b** was obtained with moderate selectivity (43% ee) and high yield (99%). Complex **68** was also tested with other ketone substrates, in which lower enantioselectivities were obtained (Dyson et al., 2009).

Diez and Nagel (2010) reported the synthesis of novel chiral Ir(I)-*bis*(NHC) complexes **69** and **70a-f** and their applications in the catalytic transfer hydrogenation of acetophenone and its derivatives using isopropyl alcohol as a hydrogen donor. It was found that the corresponding alcohol products were obtained in high yields (up to 99%) with low enantioselectivity for acetophenone (11% ee), and higher enantioselectivities for more sterically hindered ketone substrates such as propiophenone, 2-methylpropiophenone, 2,2-dimethylpropiophenone, phenyl propyl ketone and mesityl methyl ketone (up to 68% ee) with all complexes shown in scheme 14 (Diez & Nagel, 2010).

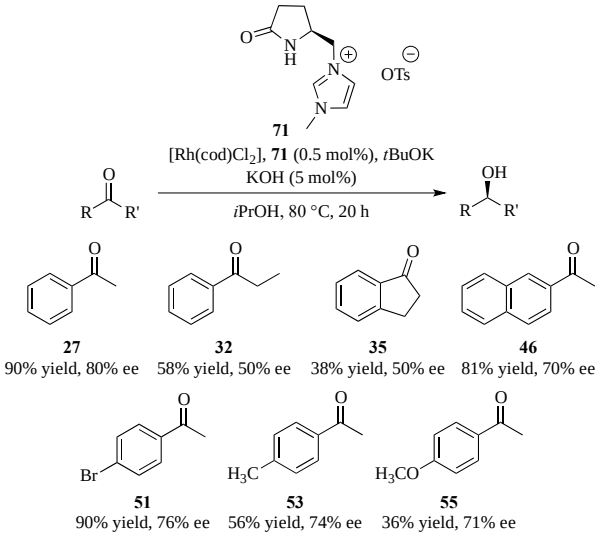


Scheme 13. Transfer hydrogenation of acetophenone **27** catalyzed by iridium NHC–phenolimine **68**.



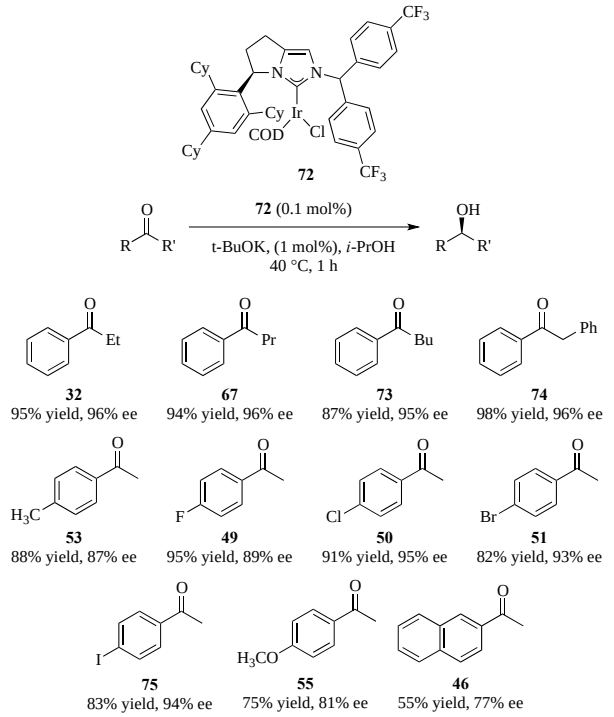
Scheme 14. The application of Ir(I)-*bis*(NHC) catalysts **69** and **70a-f** in asymmetric transfer hydrogenation of ketones.

The chiral NHC ligand **71**, prepared from (*S*)-pyroglutamic acid, was reported by Aupoix et al. (2011). This ligand was successfully applied in the rhodium-catalyzed asymmetric transfer hydrogenation of prochiral ketones using isopropanol as a hydrogen donor. High conversion and enantioselectivity were obtained for acetophenone (90% yield and 80% ee). Indeed, the results achieved using this ligand represented the highest enantioselectivities until the year 2011 for these types of reduction reactions. The imidazolium salt **71** was also tested in various ketone substrates giving the corresponding alcohols with good selectivities (up to 76% ee) (Scheme 15). Moreover, as previously mentioned, the electronic and steric properties of the ketone substituents can affect the yield and selectivity, such as electron-withdrawing groups, which led to lower enantiomeric excess and higher yields (Aupoix et al., 2011).



Scheme 15. Enantioselective transfer hydrogenation with NHC-Rh complex **71**.

Yoshida et al. (2015) have developed another successful example of NHC-Ir catalyst precursor **72** which was tested in the asymmetric transfer hydrogenation of acetophenone derivatives shown in scheme 16, giving the corresponding alcohols in excellent yields (up to 98%) and enantioselectivities (up to 96%) (Yoshida et al., 2015). The main reason of having such excellent results was using a bulky ligand substituent having an electron-withdrawing group.



Scheme 16. Enantioselective transfer hydrogenation of acetophenone derivatives with NHC-Ir complex **72**.

5 Conclusions

During the last two decades, different metal-NHC catalysts have been developed and applied in various types of asymmetric reactions. For example, Ir-NHC complexes, as well as other complexes have been used in the asymmetric hydrogenation of different functionalized and non-functionalized alkenes, affording the corresponding products in enantiomeric excess values ranged from 13-98% ee with up to 99% isolated yield. Rh-NHC catalysts proved to be highly active and selective in the stereoselective hydrosilylation of different ketone substrates, giving products with high enantioselectivities (up to 97% ee). Although the asymmetric hydrogenation and hydrosilylation reactions have shown considerable progress recently, only few studies have been conducted on the asymmetric transfer hydrogenation reactions using Ir-NHC catalysts, giving the corresponding products in moderate enantioselectivities (from 32-53% ee).

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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Comparison of two Genomic DNA Extraction Protocols from Single Dray Seed of Barley Crop

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A B S T R A C T

Modern plant breeding studies are largely based on plant genetic engineering programs. Extraction of DNA with high quality plays as a key factor in most of plant genetic Studies, therefore two different DNA extraction protocols based on CTAB buffer and SDS buffer were tested for the purpose of selecting the best DNA extraction protocol from dry seeds of nine variety of barely plant . Barley seeds were taken from Libyan seeds gene bank .After Barely samples were prepared DNA was extracted directly using CTAB and SDS solutions .The quality of extracted DNA was assessed by spectrophotometric measurements and gel electrophoresis system. The results of this study showed that purity of extracted DNA by CTAB method was clearly batter compared with SDS method. CTAB method seemed to be more effective for extracting DNA from barely dry seeds. High quality DNA obtained through use of CTAB method, while CTAB had overall better A260/A280 ratio (1.736-1.932). SDS method seemed to be not suitable for DNA extraction from dry seeds of barely crop.

1 Introduction

Long-term food security management has recently become as a key issue in the polices of several countries aimed at ensuring and providing enough food with suitable quality available to everybody. In the meantime, agriculture production is the main source of human food along with animal production. Barley *(Hordeum vulgare* L*)*. (Poaceae) is one of the most important and fundamental growing crops can be used for providing food and somehow enhancement of human feeding. Currently barley is a significant crop plant globally and it is mainly exploited as feed or as a raw material for malt production (Ullaholopainen, 2015). Moreover, barley considered as one of the fourth most important cereals in the world in terms of quantity produced and planted areas (Caterina *et al*., 2016). Practically plant biotechnology considered as one of the most important methods has been largely used for plant enhancement. Recently plant DNA extraction become one of the most important factors for improving scientific researches in plant genetic engineering to increase plant productivity. Good quality DNA is a prerequisite for all experiments of DNA manipulation. However, extraction of high-quality DNA with high yield is a limiting factor in plants genetic analysis (Abdel-Latif and Osman, 2017). In plant molecular researches and experiments, a paramount needed is the extraction of DNA of good purity and efficiency suitable for other analyses (Sharma and Purohit, 2012). High quality extracted DNA from plants considered as one of the most important technologies to be hold in plant improvement field, while Purity, integrity and quality and is often needed (Daniel *et al*, 2017).

Several DNA extraction methods have been used and applied, but the most commonly used basic plant DNA extraction protocols are those of Dellaporta *et al*., 1983 and Saghai - Maroof *et al*., 1984 along with many others that are modifications of the components of these protocols (Sharma and Purohit, 2012). Whatever all plant DNA extraction protocols comprise of the basic

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steps of disruption of the cell wall, cell membrane and nuclear membrane to release the DNA into solution. Typical plant DNA isolation methods must go through some steps like breaking the cell wall which usually done by using SDS (sodium dodecyl sulfate) or CTAB (cetylrimethyl ammonium bromide), protection of DNA from the endogenous nucleases with EDTA, removal of protein from buffer/tissue and separate the protein from DNA (Daniel *et al*., 2017). In general plant DNA is mainly isolated by procedures derived from the hot CTAB and SDS methods of Saghai – Maroof *et al*., 1984 and Dellporta *et al*., 1983 respectively along with many others that are modified to be suitable for DNA extraction with high quality (Sharma and Purohit, 2012) It is generally quite difficult to extract and purify high quality DNA from cereals because of the occurrence of polysaccharides ,proteins, and DNA polymerase inhibitors in the extracts (kamel *et al*., 2011). Presence of these compounds reduces the quality and quantity of DNA which often make the sample non-qualified sample (Sarwat *et al*., 2006).

While polysaccharides are the most common contaminants found in plant extracts and can make DNA pellets slimy and difficult to handle (Kamel *et al*., 2011). On the other hand, difference among the DNA extraction methods should be tasted and observed related to the studied plant species. (Daniel *et al*., 2017).

DNA extraction from dry seeds instead of leaf tissues has some advantages which are seed can be analyzed during the non- field season, selected and prepared for the next breeding cycle and it is possible to send seed samples internationally for comparative studies this being difficult for leaf samples (Von, 2003). Furthermore most of used and tested plant DNA extraction methods were mainly based on use of CTAB and SDS, but there some factors must be taken in consideration before selecting the extraction method such as the plant type, stage of plant growth , plant tissue and cost of DNA extraction method. Therefore, the aim of this study was to compare two different plant DNA isolation protocols and obtained high quality extracted DNA using Barley dray seeds. DNA of Barley dray seeds will be extracted using CTAB and SDS protocols

2 Materials and Methods

2.1 Experimental Site

The experiment was conducted at Biotechnology Research Center laboratories (BTRC) which located in Tripoli Libya for the purpose of assessment of two different DNA extraction protocols to obtain high quality DNA with satiable yield from seeds of barley crop.

2.2 Sample Collection.

Seeds of nine barley varieties (table 3) were taken from national gene bank, which is located in Tajoura / Libya. Directly seeds were transferred to biotechnology research center laboratories for other analyses.

Table (1). List of used barley variety for DNA extraction

Number	Variety name
1	Beecher
2	Wadiyesterday
3	Boom
4	Erwan
5	Oxidation-176
6	Maimoon Valley
7	Wai Al-H
8	Basil 3
9	My Guide

2.3 DNA Isolation Buffers

Extraction of DNA with high quality and satiable quantity started with preparation of some buffers .For successfully arrival to final step of DNA extraction every step of extraction was conducted carefully due to that, every mistake well largely effect the final assessment of DNA extraction protocol and surely the final result of the experiment .DNA extraction buffers in this experiment includes : detergent : CTAB cetyltrimethyl ammonium bromide and SDS sodium dodecyl sulfate which helps to disrupts the membranes , ß mercaptoethanol which used for denaturing the proteins by braking the bonds and removing the polyphenols, EDTA added for magnesium ions needed for DNA activity, Tris at pH 8 and salts like sodium chloride for neutralizing the negative charges. First of all DNA extraction stage began with Preparation of the original solutions. Practically there were three main solutions were prepared first.

1- NaCl 5M

5M NaCl was prepared by dissolved 292.2g of NaCl in 1000ml of double distil water. 29.2 g of NaCl were dissolved in 100 ml double distil water.

2-Tris1M [PH8.0]

Normally preparation of 1M Tris obtained through dissolve 157.6g of Tris in 1000ml double distil water with use of 49ml of HCl and keeping pH at 8.

3-EDTA 0.5M

The preparation of EDTA conducted through dissolve 186.1g of EDTA in 100ml of double distil water with use of 20g NaOH. pH was kept at 8

2.3.1 2% CTAB Extraction Buffer.

CTAB for DNA extraction buffer was prepared as shown in table 2.

Table (2). CTAB Extraction buffer.

Amount for 50ml fine concentration	Amount for 100ml fine concentration	Reagent
1g 2%	2g 2%	CTAB
14ml (1.4M)	28ml (1.4m)	5M Na CL
2ml (20mM)	4ml (20mM)	0.5M EDTA [PH8.0]
5ml (100mM)	10ml (100mM)	1M Tris – CL
ß– mercaptoethanol 2% and proteinase k 100mg /ml		

2.3.2 SDS DNA Extraction Buffer.

SDS extraction buffer was prepared as shown in table 3.

Table (3). SDS extraction buffer.

Reagent	Amount for 25ml (fine concentration)
1M Tris – cl (PH7.0)	10ml (100Mm)
5M Na CL	28ml (1.4M)
0.5M EDTA (PH8.0)	4ml (20Mm)
10% SDS	1.25ml (0.5% or 0.125g) complete the size to 100ml
ß-mercaptoethanol 2% and proteinase k 100mg /ml just before use	Note /2% means (100ml /2g) and therefore (25ml/0.5g)

TE buffer. TE. buffer.was.prepared.as.shown.intable4.

Table (4). TE buffer.

Reagent	Amount for 100ml
1M Tris (pH8.0)	1ml(10mM)
0.5M EDTA (pH8.0)	0.2ml (1mM)

Complete the size to (100ml) with double distilled water

Table (5). 50X TAE electrophoresis buffer.

Reagent	Amount for 1000ml
Tris-base	242g
Glacial acetic acid	57.1ml
0.5 M EDTA (pH 8)	100ml

Table (6). 6X loading buffer.

Reagent	Amount for 100ml
Glycerol	30ml
Bromophenol blue	0.25g

Complete the volume to 100ml by double distilled water and kept in freezer degrees -20c◦.

2.4 DNA Extraction and Purification.

Plant DNA was extracted depending on following steps

1-Barley seeds were washed by sterilized distil water. Dried cleaned seeds were gently grind to fine powder with a pastel and mortar tools.

2- Fine powder of grinded seeds was transferred to Eppendorf tube for the next steps of analyses.

3-The samples were divided to two groups, CTAB group and SDS group, while CTAB extraction buffer was added to CTAB group and SDS extraction buffer was added to SDS group.

4- 2% CTAB and 0.5% SDS extraction solutions were added separately to Eppendorf tube in quantity of (600µl) with a satiable intermittently mixture using vortex. Samples were put in a water bath at temperature of 55c◦ for a period of 60 minutes.

Note for all samples; 600µl of extraction solutions were added with addition of 60µl proteinase k and 12µl of (ß-mercaptoethanol).

5- Additions of 10ml chloroform: isoamyl alcohol (24:1) and mixed well (inverting and spin). Samples were put in centrifuge for 5 minutes at a speed of 13000 rpm under temperature (24 c◦)

6- 400µl from the clear solution (up aqueous phase) were transferred using pipet to new Eppendorf tube

7-Re –add 400µl of chloroform isoamyl alcohol (24:1) and spin as previously then transferee to a new tube.

8- Add 800µl Ethanol to each sample (Eppendorf tube)

9-Spin for five minutes then carefully transferee to the ethanol without damaging the DNA (pellet)

10-Repeat the washing process three times by using ethanol 70% with volume of 600µl and spin or discard

11-Dry the samples using soft paper and cold air.

12-Addition of TE to pellet with volume of (20-50µl) depend on the size of pellet.

13-Addition of RNase (50µg/ml) then place the samples in water bath 37c◦ for 30 -60 minutes.

2.5. Gel Electrophoresis Test.

Gel electrophoresis test was applied on barely samples in order to find out and check if DNA had extracted by the tested DNA extraction methods or not. Gel electrophoresis buffer was prepared as shown in table (5) and (6) Gel electrophoresis was prepared by using TAE electrophoresis buffer (Tris acetate-EDTA buffer) with (Agarose 0.7%) then solution was transferred to microwave to dissolve the media. Finally Addison of (Ethidium bromide) whit concentration of (0.5 µg/ml). Place the samples in coolant, and then add each sample

with 4µl loading dye. Inject samples, which were added by loading buffer in the hole, which located in the prepared gel by quantity of 5-10. Marker will be injected in the first hole for comparison, followed by the rest of the samples. The electrolytic device is set to a period of 20 minutes from 70-65V. Images of extracted DNA were taken immediately to report the result.

2.6 Spectrophotometer Analyses.

Finally, samples were moved to spectrophotometer system for measuring the purity of extracted DNA to each tested extraction protocol (CTAB and SDS). The results were recorded for all used barley varieties in this study. While the spectrophotometer analyses began with calibration of the system through addition of TE solution with volume of 50µl and with (blank) at degree (260nm). 2- 5µl of sample were put in tube and added with 48µl of TE solution. Mix well until the solution has mixed well .A230 it means the carbohydrates, A260 means DNA and A280 it means protein z. Dilution process were conducted through enter 2µl of prepared sample then press sample in the system and record the results .The concentration or purity ratio is (A260/A280) , and absorbance ratio at 260–280 nm (A260/A230 ratio) , while the results were measured with a Thermo Scientific Nano Drop™ 1000 Spectrophotometer (Thermo Scientific, Germany) using 1µL of each sample. The spectra were recorded for a range of 220–750 nm.

3 Results

3.1 Gel Electrophoreses Analysis.

The presence of DNA was checked by gel electrophoresis. After samples had prepared directly put in gel electrophoresis system for the purpose of insuring that DNA has been extracted by used DNA isolation methods. The gel images of placed samples were observed and taken to report the results figure (1), while the gel image of barely samples proved that the DNA had extracted from both used methods CTAB and SDS method which mane that the extraction steps of barely DNA were successfully applied. The gel electrophoresis step proved that the barley extracted samples are ready for other analysis and tests

3.2 Spectrophotometer Measurements.

3.2.1 CTAB (DNA Extraction buffer) Assessment.

It is well known that the type of DNA extraction methods or protocols have a great influence on both quality and purity of obtained plant genomic DNA. The isolation of high quality genomic DNA for molecular analyses still the main challenge, also obtaining good quality DNA is a prerequisite for PCR and DNA manipulation studies. Many studies reported that use of CTAB in plant DNA extraction produce DNA with high quality, purity and suitable quantity. The results of this research figure (2). Showed that the best results were obtained in samples supplemented with CTAB extraction buffer, which mean that use of CTAB for DNA extraction from barley seeds was successful. According to the obtained results, CTAB extraction method is very suitable for DNA extraction from dry seeds of barely crop. CTAB had overall better A260/A280 ratio (1.736-1.932) .The results showed also among CTAB samples there were no significant differences have been found, which explain that all of the samples produced DNA with quality and purity in the same levels.

The results of our study were in agreement with Sharma and Purohit, (2012). The others in their study found that among several DNA extraction methods which have been tested, using of CTAB methods were successfully applied for the extraction of DNA from plants having secondary metabolites. The study also proved that there is a highly possibility to produce DNA with high quality and suitable quantity through use of CTAB DNA extraction methods. Our obtained results were also in agreement with Hasibe. (2005), the researchers reported that CTAB method is rapid and yields DNA sufficiently pure for PCR amplifications. Furthermore Behrooz et al .(2012) found use of 2% CTAB in modified Murray and Thompson method gave the best results for DNA extraction compared with other tested methods.

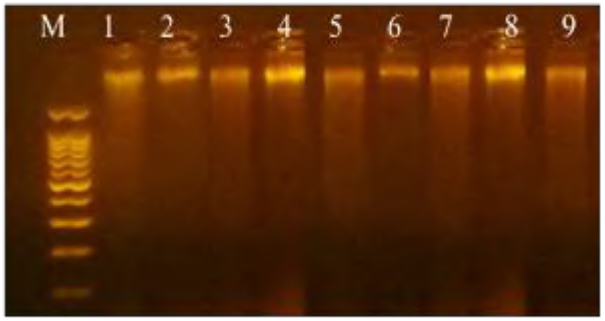

Figure (1). Agarose gel electrophoreses of DNA extracted from Barely varieties using M Bench Top PCR markers Lane 1, Beecher; Lane 2, Wadiyesterday; Lane 3, Boom; Lane 4, Erwan; Lane 5, Oxidation-176; Lane 6, Maimoon Valley; Lane 7, Wai Al-H; Lane 8, Basil 3; Lane 9, My Guide. Using cleaver scientific LTD. UV Transilluminator.

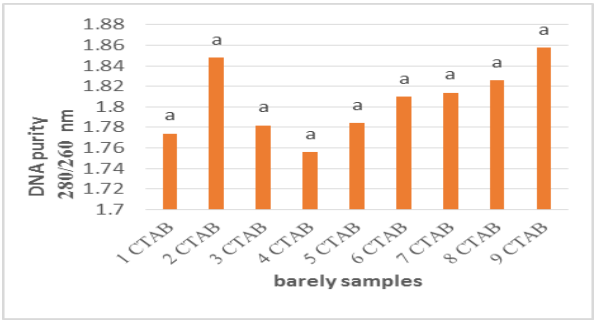

Figure (2). effect of CTAB method on purity of extracted DNA from barley seeds.

3.2.2. SDS (DNA Extraction Buffer) Assessment.

Successful molecular studies are depend on the quality of extracted DNA, while several DNA extraction methods and protocols have been involved in order to obtain high quality DNA. Plant DNA extraction methods have been modified by scientists time to time to achieve better results. SDS buffer has been used for plant DNA extraction since many years ago, and there are some studies reported that use of SDS for DNA extraction gives positive results and produce DNA with suitable quality. Furthermore, SDS methods have been successfully used for DNA extraction from microbial communities (Zhongtang and Mark, 2004). According to our obtained result figure (3), we observed that SDS method was not able to extract genomic DNA with high quality and purity from barley crop. The results of spectrophotometer analysis proved that the genomic DNA has been extracted through use of SDS but the purity level of extracted DNA through SDS was low, making this method not suitable for DNA extraction from barely plant. SDS had overall better A260/A280 ratio rich to 1.648 which mean that use of SDS method in this study was not successful.

The results showed also that among SDS samples there were no significant differences have been found in terms of DNA purity. Our results were in agreement with Behrooz *et a*l., (2012), the researcher found DNA extraction with SDS method had not good quality and quantity

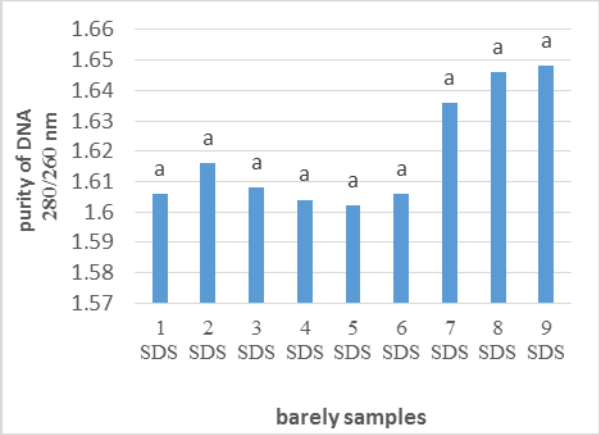

Figure (3). effect of SDS method on purity of extracted DNA from barely seeds

3.2.3. Comparison between CTAB and SDS (DNA Extraction Buffers).

As well known that high quality with satiable quantity are critical factors for successful PCR applications. The quality of DNA extractions highly infected by many compounds that inhibit DNA amplification such as polysaccharides, lipids and polyphenols (Daniel *et al*., 2017).

The comparative assessment of electrophoreses has been done to both used DNA extraction protocols (CTAB and SDS). As being reported two different DNA, extraction protocols were used to evaluate and select the best protocol for DNA extraction from cereal plants through use of seeds of barley crop. The first protocol was based on use of CTAB and second one was based on use of SDS buffer. The result figure (4) of the experiment showed that use of CTAB buffer for DNA extraction tend to be clearly better than use of SDS buffer. Furthermore the results of spectrophotometer analysis explained that the purity degree of extracted DNA through use of CTAB was is in good levels, which proved that CTAB method suitable for DNA extraction from barley plant .CTAB had overall better A260/A280 ratio (1.756-1.858), while SDS had ratio of (1.602-1.648). The results also proved that among the CTAB treatments there were no significant differences, also the same in SDS treatments there were no significant differences among the tested samples, which proved that the differences were found between the extraction methods which are CTAB and SDS but among the samples for each method there were no differences. This obtained results were in agreement with Daniel *et al*., (2017), the spectrophotometric analysis of their study resulted that CTAB extraction method had better results and batter DNA purity than SDS method, while CTAB method had ratio of (1.767 - 2.146).

Furthermore Behrooz *et al.*, (2012) found Murray and Thompson with use of 2% CTAB had the best results compared with other tested methods with SDS , while these results are very close and agree with what we had resulted in our experiment.

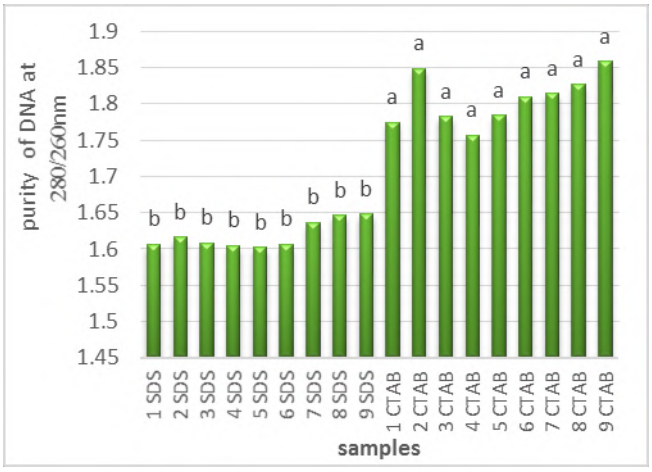

Figure (4). effect of CTAB and SDS on purity of extracted DNA.

4 Conclusions

Agriculture production become the main source for human food but more researches still needed to increase crop productivity .Plant productivity improvement is depend on plant genetic engineering and plant molecular studies .Extraction of plant DNA with high quality and suitable quantity is very important stage in molecular studies in order to produce scientific research which may help to improve plant yield .Several methods have been used for plant DNA extraction since DNA can be extracted and some of extraction methods have been modified to obtain high quality DNA. The result of this study proved that use of 2% CTAB gave the best results, and it can be successfully used for DNA extraction from cereal crops specially barely crop compared with SDS method. SDS method was not suitable for extracting DNA from barely crop.

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Changes in some Hematological Parameters of Chelon Labrosus (Risso,1826) in Umm Hufayn - Eastern Libya During Different Seasons

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A B S T R A C T

This study aimed to investigate the effects of seasonal variation on some Hematological Parameters of *Chelon labrosus* (Risso, 1826) in Umm Hufayn lagoon - eastern Libya, Mediterranean coast, and surface water temperature and salinity. The results revealed that the surface water temperature recorded (11 ±1.73) 0C between December to February and (23±0.00)0C between June to August, salinity recorded (4.67±0.88)% between December to February and (23±1.15)% between June and August, on the other hand, seasonal variation affected hematological parameters whereas (WBCs) reached to high during the autumn season and recorded the lowest level during the summer, also (RBCs) reached to high during the autumn and the lowest levels during the spring. Furthermore, (HCT) reached high levels during the autumn and the lowest levels during the summer, otherwise recorded (MCV) at high levels during the spring and the lowest levels during the summer, and (MCH) reached high levels during the spring and lowest levels during the summer, while (MCHC) recorded the high levels during the winter and lowest levels during the autumn and finally (PLT) reached to high levels during the spring and lowest value during the autumn.

1 Introduction

Temperature plays an important role in various aspects of the life history, ecology, and physiology of ectotherms (Angilletta *et al.*, 2002). Growth rates (Arnold and Peterson, 1989; Avery, 1994; Litzgus and Brooks, 1998a), reproduction (Schwarzkopf and Shine, 1991; Litzgus and Brooks, 1998b; Rock and Cree, 2003), seasonal activity patterns and habitat use (Webb and Shine, 1998; Whitaker and Shine, 2002), and geographic distribution (Castonguay *et al.*, 1999) are all influenced by environmental temperatures, for example, variation in environment temperature can affect many reproduction and life history traits, including metabolism and different activity. The aim of this study examined the effect of seasonal variations on some hematological parameters of *Chelon Labrosus* (Risso,1826) in Umm Hufayn - Eastern Libya.

2 Materials and Methods

2.1 Study Area

Umm Hufayn is a relatively small lagoon located about 80 km east of Derna in the direction of Tobruk (Fig.1). It is connected to the open sea through a gate sized about 0.5 Km, through it seawater enters the lagoon on tide times. Underground springs at the inner side of the lagoon discharge water (Abd AL Hamid *et al.*, 2017).

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Figure (1). Umm Hufayn lagoon Eastern Libya.

2.2 Characteristics of Surface Water of Um Hufain Iagoon

Temperature measurements (°C): In situ, water temperatures were measured by using an ordinary thermometer.

2.3 Salinity (S%):

Salinity was determined by measuring the electrical conductivity using an inductive Salinometer (Beckman; model RS-10).

Collection of fish and blood samples: A total of forty fish adults of *Chelon Labrosus* (Mugilidae) were collected during each season (autumn, winter, spring, and summer season) and blood was rapidly drawn from the caudal vessel or heart of each fish (figure 2&3) according to Hrubec *et al*., 1997 method and blood was taken sent to a medical laboratory for determination of some hematological parameters.

Figur.2. blood taking from cadual peduncle.

Figure (3). Blood taking from heart puncture.

Statistical Analysis

Data were presented as means ± standard error (SE). The statistical analysis was performed with multi-variant analysis of variance (ANOVA) using the SPSS (version 15) software package for Windows comparing the multi-variations between the groups. F-test was calculated and considered statistically significant at $p < 0.05$.

3 Results

Characteristics of Surface Water of Um Hufain lagoon (Mean±SE):

In Um Hufain lagoon surface water temperature ranged from (11 ±1.73) between December to February to (23 ±0.00)0C between June to august and salinity from (4.67±0.88) between December to February and (23± 1.15) % between June and August .

Table (1). Characteristics of surface water of Um Hufain lagoon (Mean±SE):

Season / Parameter	Summer (Jun.-Aug.)	Autumn (Sep.-Nov.)	Winter (Dec.-Feb.)	Spring (Mar. – May)
Temperature ^{0}C	23 (0)	18 (0)	11 (1.73)	14 (1.15)
Salinity %	23 (1.15)	12 (.58)	4.67 (.88)	9 (1.732)

Effect of Seasonal Variation on some Hematological Parameters: -

Parameters content in sampled were recorded in Table (2). The seasonal variation of WBC_S: - It can be seen that the highest WBC_S value (185.32±18.27) was estimated during autumn, while the lowest value (171.44± 6.12) was obtained during summer.

The Seasonal Variation of RBCs: -

The highest RBCs count (2.75± 0.25) was recorded during the autumn, and the lowest value (1.21± 0.41) was recorded during the spring season.

The Seasonal Variation of HGB: -

The highest value (11.17±0.51) was recorded during the autumn, moreover, the lowest value (5.86±1.56) was shown during the summer season.

The Seasonal Variation of HCT: -

The highest value (40.77±3.03) was recorded during the autumn, furthermore, the lowest value (19.96±6.04) was shown during the summer season.

The Seasonal Variation of MCV: -

The highest value (171.10±4.80) was recorded during the spring, and the lowest value (122.70±6.15) was shown during the summer season.

The Seasonal Variation of MCH: -

The highest value (36.04±1.57) was recorded during the winter, and the lowest value (27.65±1.41) was shown during the autumn season.

The Seasonal Variation of MCHC: -

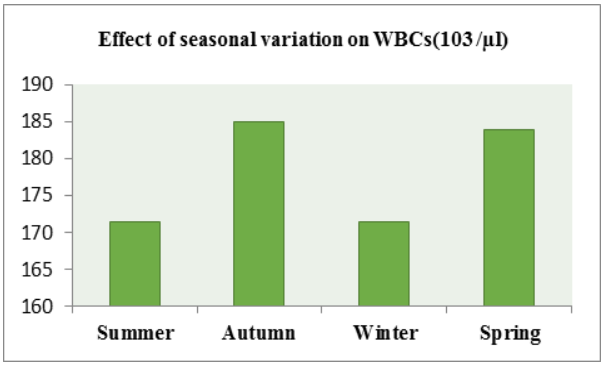
The highest value (36.04±1.57) was estimated during winter, one the other hand, the lowest value (27.65±1.41) was shown during the autumn season.

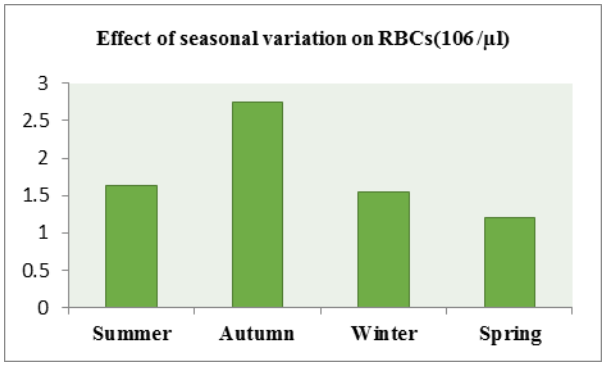
The Seasonal Variation of PLT: -

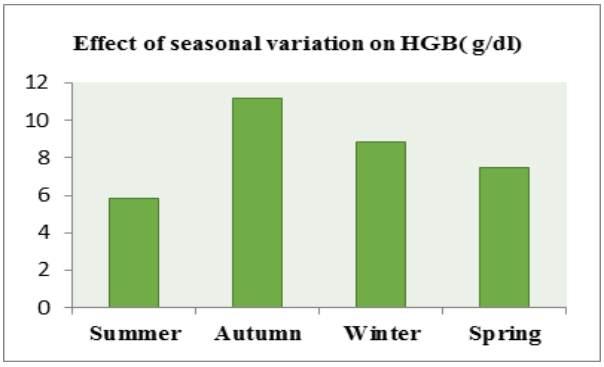
The highest value (283.00±276.00) was recorded during the spring, and the lowest value (20.25±3.09) was shown during the autumn season.

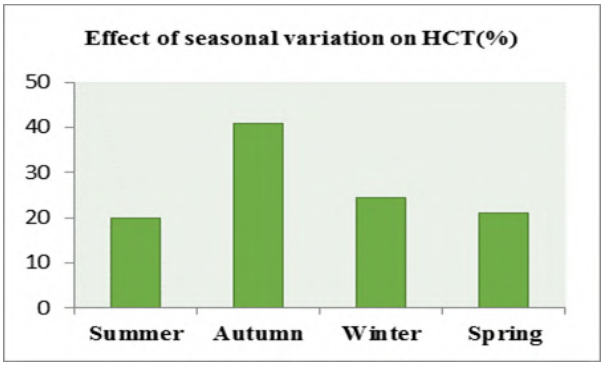
Table (2). Effect of seasonal variation on some on hematological parameters:

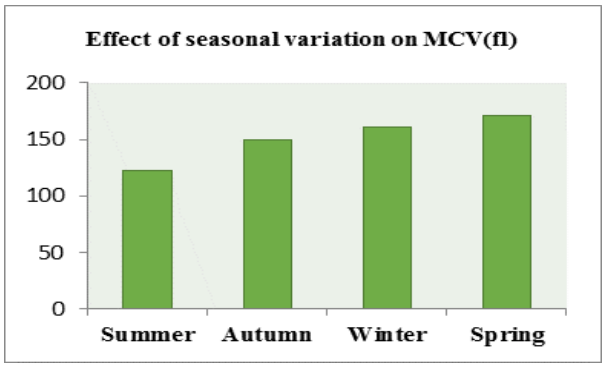
Parameters	Summer	Autumn	Winter	Spring
WBCs (103 /µl)	171.44± 6.12^{a}	185.32± 18.27^{a}	171.50± 13.85^{a}	183.85± 32.85^{a}
RBCs (106 /µl)	1.63± 0.45^{ab}	2.75± 0.25^{b}	1.55± 0.22^{ab}	1.21± 0.41^{a}
HGB (g/dl)	5.86±1.56^{a}	11.17±0.51^{b}	8.86±1.14^{ab}	7.45±2.65^{ab}
HCT (%)	19.96±6.04^{a}	40.77±3.03^{b}	24.52±2.80^{a}	20.90±7.60^{a}
MCV (fl)	122.70±6.15^{a}	149.97±8.50^{b}	160.64±4.85^{b}	171.10±4.80^{b}
MCH (Pg)	36.50±1.68^{a}	41.70±4.20^{a}	57.78±2.78^{b}	61.15±1.15^{b}
MCHC (g/dl)	29.98±1.84^{a}	27.65±1.41^{a}	36.04±1.57^{b}	35.75±0.35^{b}
PLT (103 /µl)	22.60±17.44^{a}	20.25±3.09^{a}	88.60±36.74^{ab}	283.00±276.00^{b}

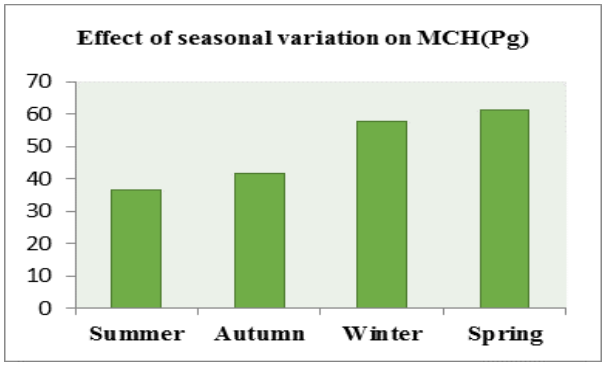


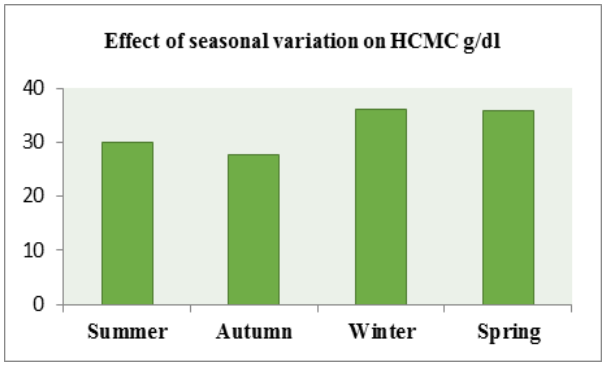


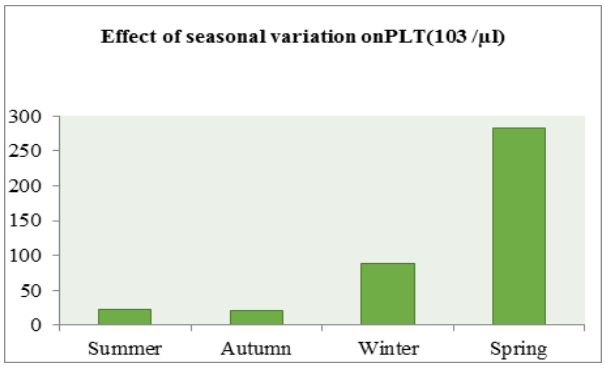












4 Discussion

Show significant variation in different seasons, especially between warm and cold seasons like water temperature, dissolved oxygen, turbidity, and electrical conductivity, and unlike Forghally *et al*., (1973 mentioned that the results showed that there is no significant difference in relation to gender except in hematocrit and WBCs of *Alburnoides eichwaldii*. it has a higher value in females. Forghally *et al*., (1973) Reported that the temperature of the aquatic environment is important for ensuring survival, distribution, and normal metabolism of fish, failure to adapt to temperature fluctuations is generally ascribed to the inability of fish to respond physiologically with resultant mortality, which is related to changes in the metabolic pathways.

Olaoluwa *et al*. (2015) was indicated to effect to a seasonal variation on some physiological parameters and mentioned that some live enzymes were increased during the autumn season.

Aboudbous *et al*. (2017) observed that there is a significant increase in liver enzymes in the autumn season compared to the other seasons in blue tuna in Misurata – Libya.

Bhat (2017) in his study on '*Schizothorax niger* found that the effects of the independent variables e.g., sex, weight, and length values were determined seasonally.

The analysis revealed that the highest number of leukocytes was found in spring and the lowest number was found in winter, hemoglobin and hematocrit values were highest during the months of summer and lowest during winter. It was also seen that males were having greater values for hemoglobin and hematocrit than females, whereas total leukocyte count in females was higher than in males. It was also found that there was a positive correlation between length/weight and hemoglobin and hematocrit values whereas a negative correlation between length or weight and total leukocyte counts.

Abdalhafid *et al*., (2021). Reported that, the levels of GOT and GPT of *Chelon Labrosus* were markedly increased during the autumn compared with the winter while ALP levels almost remained stable during four seasons.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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