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Preliminary Study of SurfactinProduction by Malaysian Local Isolates of *Bacillus Subtilis*

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Abstract: Surfactin is one of the most powerful lipopeptidebiosurfactants produced by various strains of *Bacillus subtilis*. It has exceptional surface activity, with antiviral, antibacterial, and antitumor properties. The four local isolates, which were named *Bacillus subtilis*1M, 3M, 7M, and 8M were provided by the School of Biosciences and Biotechnology, Faculty of Science and Technology, University of Kebangsaan, Malaysia. In this study, fermentation on shaker flasks was carried out to assess the ability of four local isolates of *Bacillus subtilis* strains to produce surfactin by using Cooper's media formulation, and comparing their production with a commercial strain of *Bacillus subtilis* ATCC 21332, which was obtained from the American Type Culture Collection. High-Performance Liquid Chromatography (HPLC) was used for surfactin identification and surfactin concentration measurements. Results obtained show the four local isolates have the ability to produce surfactin. The *Bacillus subtilis*3M strain showed the highest amount of surfactin production with 117 (3) mg/L, while the *Bacillus subtilis*1M strain produced the lowest amount with 65 (5.4) mg/L. In addition, the production of *Bacillus subtilis* ATCC 21332 strain was found at 101(4) mg/L under the same fermentation conditions.

دراسـة أوليـة لإنتـاج مـادة مضـادة للتـوتر السطحي *(Surfactin)* قـوي للغايـة نـتج مـن العزلات المحليـة الماليزيـة مـن البكتيريـا العصوية الرقيقة

المستخلص: Surfactin هو واحد من أقوى المواد الخافضة للتوتر السطحي الدهني الت ي تنتجه ا سلالات مختلفة من *Bacillus subtilis*. له نشاط سطح استثنائي فع ال، م ع خص ائص مض ادة للفيروسات، والبكتيريا، ومضادة للأورام. تم توفير العزلات المحلية الأربعة التي تم تس ميتها باس م 1M *Bacillus subtilis* و 3 M و 7 M و 8 M من قبل كلية العلوم البيولوجية والتكنولوجيا الحيوية، كلية العلوم والتكنولوجيا، جامعة كيبانغسان ماليزيا. في هذه الدراسة تم إجراء التخمي ر عل ى دورق شاكر لتقييم قدرة أربع عزلات محلية من سلالات العصيات الرقيقة على إنتاج سيرفاكتين باس تخدام تركيبة وسط كوبر، ومقارنة إنتاجها بالسلالة التجارية من العصوية الرقيقة ATCC 21332، والت ي تم الحصول عليها من سلالات العص وية الرقيق ة. مجموع ة الثقاف ة الأمريكي ة. ت م اس تخدام الكروماتوغرافيا السائلة عالية الأداء (HPLC) لتحديد سيرفاكتين، وقياس ات تركي ز س يرفاكتين. أظهرت النتائج المتحصل عليها أن العزلات المحلية الأربع لديها الق درة عل ى إنت اج Surfactin. أظهرت سلالة 3M *Bacillus subtilis* أعلى كمية إنتاج للسيرفاكتين مع 117 (3) مجم / لتر، بينما أنتجت سلالة 1M *Bacillus subtilis* أقل كمية مع 65 (5.4) مجم / لتر. بالإضافة إلى ذلك، ت م العثور على إنتاج سلالة 21332 ATCC *Bacillus subtilis* عند 101 (4) ملجم / لتر تحت نفس ظروف التخمير.

الكلمات المفتاحية :

بكتريا عصويه رقيقه؛

إنتاج surfactin؛

كروم اتوجرافي س ائل ع الي الأداء(HPLC)؛

مقياس الطيف الضوئي الحيوي.

INTRODUCTION

Bacillus subtilis, one of the most studied gram-positive bacteria (Li et al., 2010), is a sporulating rod bacteria that thrives in the soiland is non-pathogenic to human beings (Zweers et al., 2008). The ability of *Bacillus subtilis* to produce a lipopeptide has been

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documented for over 50 years (Waewthongrak et al., 2014), and the research on its antibacterial effectsis still ongoing (Fahle et al., 2022). The antibacterial properties of surfactinaredue to the ability of *Bacillus subtilis* to produce a variety of antibacterial agents(Cheng et al., 2018), which includes a broad spectrum of potent biosurfactants lipopeptides (Sumi et al., 2015).

The target spectrum of potent biosurfactants lipopeptidesproduct in this study is surfactin, known as one of the most effective surface activeagents available (Sousa et al., 2014). It has the ability to reduce the surface tension of water from 72 to 27 mN m^{-1} at a trace concentration as low as 0.005% (Amit & Rukhsar, 2013). This number is significantly lower than most biosurfactant surface tension data, as reported by (Al-Bahry et al., 2013). Surfactinis a cyclic lipopeptide consisting of a heptapeptideheadgroup with the sequence of L-Glu, L-Leu, D-Leu, L-Val, L-Asp, D-Leu, and L-Leu, closed to a lactone ring by a C$_{13-15}$ β-hydroxy fatty acid (Isa et al., 2007). In addition to high surface activity, surfactinhasseveral other attractive properties (Pereira et al., 2013), which include fibrin-clotting inhibition, antibiotic, antiviral, hemolytic, and antitumor properties (Chen et al., 2008). Due to these properties, surfactin was found to have the potential to be applied in biotechnology and medicine. This prospect makes surfactin production and application very attractive economically (Jiao et al., 2017) .Surfactin utilization for various applications in terms of critical micelle concentration (CMC) and minimum surface tension will increase the high cost of capital investment (Ríos et al., 2017). Furthermore, surfactinisnot widely utilized in industry due to high production costs associated with using expensive substrates and strains (dos Santos et al., 2010), suggested that several factors, such as microbes, the microbial growth substrate or feedstock processing, and by-product recovery, may influence biosurfactant production costs. During the last four decades,more research has been

carried out to minimize the cost of surfactin production by improving its yield.(Zhi et al., 2017), managed to produce surfactin of 50-100 mg/L after 24 hours of culture. Jajor 2016 used a mineral salts' medium as fermentation media and achieved a surfactinyield of 780 mg/L with continuous product removal and metal cation addition.

(Mulligan et al., 1989), found an ultraviolet mutant of *Bacillus subtilis* ATCC 21332, which produced over three times more surfactin (1124 mg/L).In 1997, Senand Swaminathano ptimized the fermentation medium and obtained a maximum surfactin production of 760 mg/L. Another discovery (Wei & Chu, 2002) used an inorganic salt-enriched medium accompanied by appropriate pH control and managed to produce a surfactin yield of nearly 3500 mg/L, which seemed ready for commercialized applications. These latter efforts made it possible to reduce the costofsurfactin production. This study aimed to assess the ability of four local *Bacillus subtilis* strains, namely (1M, 3M, 7M, and 8M) to produce surfactin and compare their production with a commercial strain of *Bacillus subtilis* ATCC 21332 obtained from the American Type Culture Collection.

MATERIALS AND METHODS

Local isolates of *Bacillus subtilis*strains, namely 1M, 3M, 7M, and 8M, were obtained from the Biosciences and Biotechnology lab, Faculty of Science and Technology, University Kebangsaan Malaysia. The commercial strain of *Bacillus subtilis* ATCC 21332 was obtained from the American Type Culture Collection. At 4°C, bacterialstrains were maintained on nutrient agar (NA). Tow loopfuls of colonies were inoculated in 100 ml of Cooper's medium, composed of 4% (w/v) glucose and mineral salt medium (Jajor et al., 2016) in 200 ml Erlenmeyer flasks and wereincubated in an incubator shaker at 200 rpm for 24 h at 30°C. 0.5 ml of the seed culture was then inoculated into 200 ml of Cooper

media in 500 ml Erlenmeyer flasks (Wei & Chu, 2002).

Determination of Bacterial Growth: The determination of bacterial concentration was referred to the method proposed by (Amit & Rukhsar, 2013). Cellular growth was expressed in terms of optical density at 600 nm (OD_{600nm}), measured by a Bio-spectrophotometer (Eppendorf, Germany).

Quantitative Analysis of Surfactin: To determine the concentration of surfactin, culture samples were withdrawn aseptically and centrifuged at 10 000 × g for 10 min and then filtered through a 0.45 µm Nylon membrane filter. The surfactin concentration was measured by using High-Performance Liquid Chromatography (HPLC) (Agilent Technologies, 1200 Series, USA) equipped with a C-18 column (Agilent Zorbax Eclipse C18, 250 mm × 4.6 mm, 5µm), and detected at 205 nm with a Variable Wavelength Detector (VWD). The system was run in isocratic mode at a flow rate of 1.5 mL/min with a mobile phase of 3.8 m Mtrifluoroacetic acid (TFA) in 80% acetonitrile. As a standard, surfactin from *Bacillus subtilis* with 98% purity from Sigma was used.

Statistical Analyses: The collected data were subjected to a mean analysis, a standard deviation of the mean value, and a level of significance determination using the Student's t-test. The differences between measurements were considered significant at the level of ($P<$ 0.05).

RESULTS AND DISCUSSION

Several *Bacillus subtilis* strains are inherently defective in surfactin biosynthesis due to a frame-shift mutation in the *sfp* gene (Abushady et al., 2005) .*Bacillus subtilis* strains were grown using a chemically defined mineral salt (MS) medium. In 1981 the medium was designed to promote surfactin production and was proposed by Cooper (Jajor et al., 2016). Environmental factors play a significant role in the yield and characteristics of the produced surfactin. In order to obtain large quantities of surfactin it is necessary to optimize the process conditions because the production of surfactin may be induced by changes in pH, incubation temperature, aeration, or agitation speed. Results obtained in this study showthe four local isolates of*Bacillus subtilis*namely (1M, 3M, 7M, and 8M), have the ability to produce surfactin at 30°C, 200 rpm for 168 hours in a mineral medium containing 4% (w/v) glucose.

Time Course of Surfactin Production and Cell Growth: Figure1(A) and Figure1 (B) show the growth curve and surfactin production of *B. subtilis*1M, 3M, 7M, 8M, and a commercial strain of ATCC 21332 using Cooper medium, which was composed of 4% (w/v) glucose and mineral salt medium (MSM). Production of surfactin for *Bacillus subtilis*3M and 7M started at the log phase of fermentation and continues until 144 hours of fermentation. Maximum surfactin production in both strains was achieved in 96 hoursof fermentation in the range of 117 (±3)mg/L - 86 (±11) mg/L. Maximum cell growth was achieved in 72 hours of fermentation. Referring to Figure1 (B), maximum surfactin production was achieved early in the exponential phase.For *Bacillus subtilis*8M, surfactin production started at the log phase of fermentation and continued until 144 hours of fermentation, as shown in Figure 3. Maximum surfactin production was achieved at around 72 hours of fermentation, with a surfactin yield of 105 (±12) mg/L. Referring to Figure1 (A), the highest bacterial growth was at 72 hours of fermentation and was similar to results obtained by (Abdel-Mawgoud et al., 2008) for the production of surfactin by *Bacillus subtilis* Isolate BS5. Surfactinproduction started after 24 hours and wascontinues for144 hoursfor*Bacillus subtilis*1M. The highest concentration of surfactin with 65 (±7) mg/L was achieved at 72 hours of fermentation, as shown in Figure 1(A),and cell growth remains almost stationary from 48 to 144 hours of

fermentation. Referring to Figure 1(B), maximum surfactin production was achieved during the stationary phase. Surfactin production for *Bacillus subtilis* ATCC 21332 startsat the log phase of fermentation and continues until 144 hours. As shown in Figure 1(A), the highest surfactin production was at 96 hours of fermentation with $101(\pm4)$ mg/L.Cell growth remains almost stationary from 48 to 144 hours of fermentation. Referring to Figure 1(B), the highest surfactin production was during the stationary phase. Surfactin production rises during the first 96 hours of incubation and falls after 120 hours. The amount produced was found to be quite similar to the previous work done by (Wei & Chu, 2002), with the highest amount of surfactin of 100 mg/L. Surfactin production was reported to begin at the late log phase and the early stationary phase of bacterial growth when nutrients in culture media become scarce (Kinsinger et al., 2005). Overall, the results obtained show *Bacillus subtilis*3M produced the highest amount of surfactin, whereas *Bacillus subtilis*1M produced the lowest amount of surfactin. In addition, the production of *Bacillus subtilis* ATCC 21332 was found quite similar to *Bacillus subtilis*3M under the same fermentation conditions. Statistical analysis shows the surfactin production of *Bacillus subtilis* ATCC 21332 strain was significantly higher ($P\leq0.01$) in comparison to *Bacillus subtilis*1M, whereas there was no significant difference between *Bacillus subtilis* ATCC 21332 and *Bacillus subtilis*3M ($P\geq0.05$).

Chromatographic Characterization: The High-Performance Liquid Chromatography (HPLC) assay used in this research work for surfactin identification and quantification was highly sensitive and reproducible. The identification and quantification of surfactin are complex since surfactin has a number of isoforms (Wei & Chu, 2002).
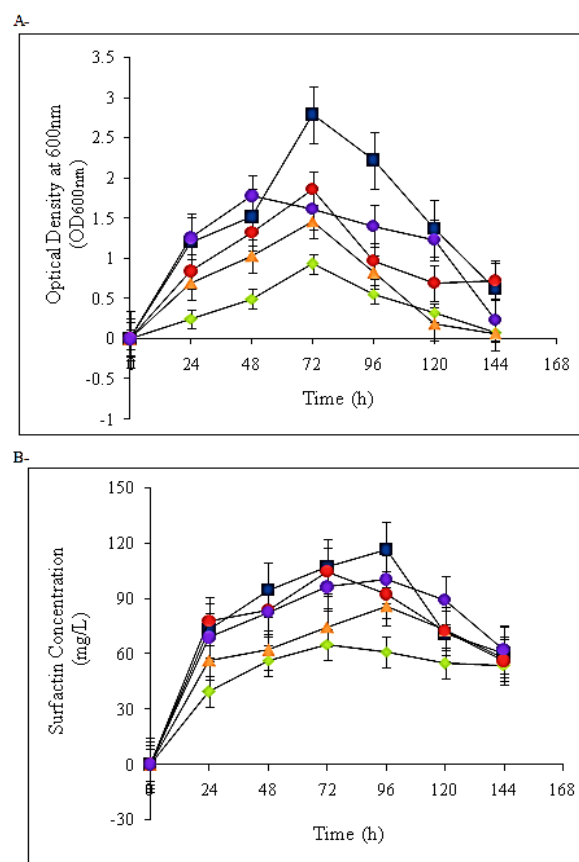
Figure (1). Time course of bacterial growth (A) and surfactin concentration (B):*Bacillussubtilis* 1M (♦);*Bacillussubtilis* 3M (■);*Bacillussubtilis*7M (▲);*Bacillussubtilis*8M (●);*Bacillussubtilis*ATCC21332 (●)

Previous studies by (Wei et al., 2003), demonstrated that surfactin has six isoforms, while others stated that so far, at least nine different surfactin isoforms have been identified (Abdel-Mawgoud et al., 2008). These isoforms differ in the length of the -hydroxy fatty acid chain, which is typically C13 to C15 (Vater et al., 2002).Therefore, HPLC analysis was performed in this study on crude fermentation broth to identify different surfactin isoforms that may be present. The surfactin standard chromatogram as shown in Figure 2, shows eleven different isoforms, which were eluted between 8 and 38 min. Of these isoforms, six were major namely, peaks 1, 2, 4, 5, 7, and 8, and five were minor namely, peaks 3, 6, 9, 10, and 11. In addition, the figure shows that all surfactin isoforms were well separated except for isoforms numbers 7

and 8, which differed in their retention times by less than 0.5 min, so they appeared somewhat merged. Surfactin chromatogram in Figure 3shows *Bacillus subtilis* 3M produced nine different surfactin isoforms and overall showsthe closest similarity to surfactin standard isoforms in Figure 2. The surfactin isoforms in Figure 3(designated by letters) show very similar retention times compared to the surfactin standard in Figure 2(designated by numbers), except for peaks8 and 9 in the surfactin standard, which were undetected inthesurfactin chromatogram of *Bacillus subtilis* 3M.

ferent isoforms were not the same in both the local isolates and *Bacillus subtilis* ATCC 21332, which may be related to strain variations. (Campos et al., 2013) stated that surfactin consists of a family of lipopeptides with similar chemical structures, i.e., isoforms, which are slightly different in their physicochemical properties due to variations in the chain length and branching of its hydroxy fatty acid component as well as substitutions of the amino acid components of the peptide ring. These differences depend on the strain variation and the nutritional and environmental conditions (Abdel-Mawgoud et al., 2008).

Figure (2). HPLC chromatogram of surfactin standard from Sigma
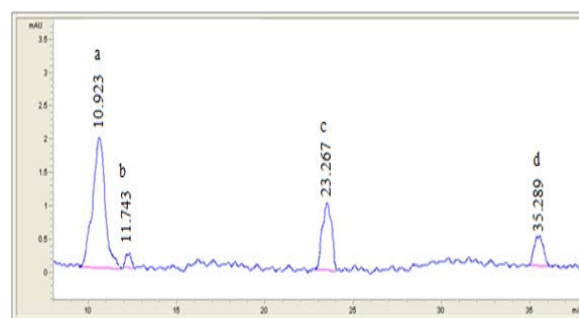
Figure (4). HPLC chromatogram of Bacillus subtilis 1M

Figure (3). HPLC chromatogram of *Bacillus subtilis*3M

Bacillus subtilis 1M produced the least number of surfactin isoforms with only four, whereas *Bacillus subtilis* ATCC 21332as shown in Figure 4, *Bacillus subtilis* 8M, and 7M in Figure 6, Figure 5, and Figure 7, respectively produced in the range of six to seven different surfactin isoforms. It appeared that the number of surfactin isoforms produced and the relative abundance of the dif-

Figure (5). HPLC chromatogram of Bacillus subtilis 8M
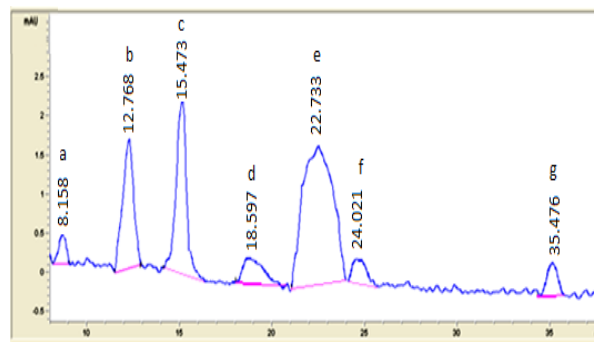
Figure (6). HPLC chromatogram of *Bacillus subtilis*ATCC21332
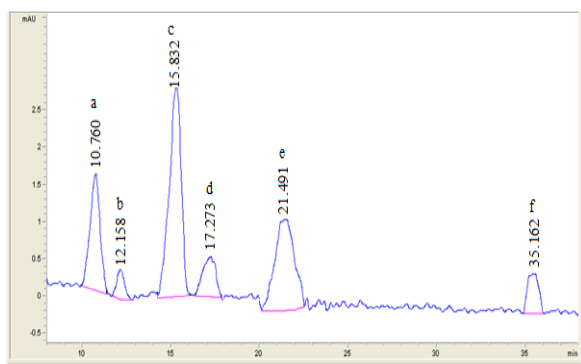
Figure (7). HPLC chromatogram of Bacillus *subtilis* 7M

CONCLUSION

The main objective of this research is to study the production of surfactin by using local isolates of *Bacillus subtilis*. This study shows the four local *Bacillus subtilis* isolates namely (1M, 3M, 7M, and 8M), have the ability to produce surfactin at 30ºC in a mineral medium containing 4% (w/v) glucose. Surfactin-HPLC chromatogram of four local isolates of *Bacillus subtilis* overall shows a similar profile of surfactin isoforms in comparison to the surfactin standard and surfactin produced by the ATCC 21332 strain, only differing slightly in terms of abundance of individual peak retention times and the number of isoforms. In addition, the *Bacillus subtilis* 3M strain has the highest capacity to produce surfactin under facultative aerobic conditions, with a maximum production of 117 mg/L, and the *Bacillus subtilis* 1M strain produced the lowest amount of surfactin at 65 mg/L, at the same fermentation condition.

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Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

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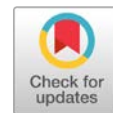
Congo Red Removal From Aqueous Solutions by Ion ExchangerAmberliteLA-2

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Abstract: Amberlite LA-2 was used as an ion exchanger to remove Congo red dye from aqueous solution. The effect of the initial concentration of dye and the initial concentration of Amberlite LA-2 on the efficiency of dye removal from the aqueous phase to the organic phase at different contact times, temperatures and pH values was studied. this research aims to study the potential of using Amberlite LA-2 as an extracting material for Congo red dye removal. UV-visible Spectrophotometer were used to assay the dye concentration in the aqueous solution before and after removal. Distribution coefficients (K_D), loading capacity (Z), and extraction efficiency (E%) were computed using experimental data. The maximum extraction efficiency of 99.69 % was obtained with a loading capacity of 384.366 mol/kg. The obtained results also indicated that the removal of dye increases with the increase in the concentration of Amberlite LA-2. The best removal was obtained in a neutral medium (pH = 7), a temperature of 20 ^{o}C. A mechanism of extraction by Amberlite LA-2 was also proposed.

إزالة صبغة الكونجو الأحمر من المحاليل المائية بواسطة المبادل الأيوني‏Amberlite LA-2

المستخلص : في هذه الدراسة، تم استخدام Amberlite LA-2 مبادلا أيونيا لإزالة صبغة الكونغو الأحمر من المحلول المائي، وذلك بدراسة تأثير التركيز الابتدائي للصبغة، والتركيز الابتدائي لمادة Amberlite LA-2 على كفاءة إزالة الصبغة من الطور المائي إلى الطور العضوي في أزمنة، ودرجات حرارة، وقيم أس هيدروجيني مختلفة. استخدمت أطياف امتصاص الأشعة المرئية، وفوق البنفسجية UV-VIS لتقييم تركيز الصبغة في الوسط المائي قبل الإزالة وبعدها. تم استخدام النتائج التجريبية للاستخلاص لحساب معاملات التوزيع (K_D)، وسعة التحميل (Z)K وكفاءة الاستخلاص (E %)تم الحصول على أقصى كفاءة استخلاص بنسبة% 99.69، وقدرة تحميل 384.366 مول/ كجم$^{-1}$. كما أشارت النتائج المتحصل عليها إلى أن إزالة صبغة الكونغو الأحمر تزداد مع زيادة تركيز مادة Amberlite LA-2 . حيث أمكن الحصول على أفضل إزالة في وسط متعادل (pH =7) ، ودرجة حرارة C^o 20. كما اقترحت آلية لعمل المبادل الأيوني Amberlite LA-2 مزيلا لصبغة الكونغو الأحمر من الوسط المائي.

الكلمات المفتاحية :

صـبغة الكونغـو الحمـراء؛ اسـتخلاص سـائل–سـائل؛ Amberlite LA-2؛ معاملات التوزيع؛ معاملات التحميل؛ كفاءة الاستخلاص.

INTRODUCTION

Environmental pollution is a serious problem facing humans in modern life. These problems have emerged as a result of human technological, industrial and civilized progress. Thus, the problem of aqueous solution pollution has received great attention from researchers (Hassaan et al., 2017). Organic matter constitutes an important part of the components of industrial wastewater. Organic pollutants have high risk in terms of their long-term impact, as some of them cause cancerous diseases (Padhi, 2012). Dyes are among the

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many organic materials polluting water sources. They are used in the textile industries, in printing, in photographic colors, and as additives in the oil industries, as well as in other wide fields (Faust & Aly, 2013). Approximately 10-15 % of these dyes are lost as waste in the water, which is thrown into water sources or soil, causing major problems for plants, animals, and humans (Asthana, 2001; Faust & Aly, 2013). Therefore, finding ways to remove different pollutants is very important to meet the increasing demand for water with increasing population density. Wastewater-containing dyes can be treated by physical, chemical, and biological methods, such as coagulation, fusion, oxidation, membrane filtration, ion exchange, and photo catalytic degradation (Al-Sakkaf et al., 2020) Liquid–liquid extraction is one of the most important of these techniques due to its high efficiency in this field and the simplicity of the technology used for this purpose compared to other methods (Al-Abbasi et al., 2010; Uday et al., 2016).

Congo Red (CR), is an anionic azodye (Hassaan et al., 2017; Padhi, 2012). Congo red dye with the formula $C_{32}H_{22}N_6Na_2O_6S_2$ (Figure 1) is considered a very dangerous dye for various living organisms, and exposure to this dye may lead to human cancer. This dye is sensitive to the change in the pH value, as its red color is stable in the pH range (5-10), and its colour changes to blue in the acidic medium (pH < 5) (Kloetzer et al., 2019), Congo red dye has a strong absorption band of max 498 nm in the visible spectrum, giving it a red color. Synthetic dyes such as Congo Red are difficult to decompose due to their chemical, thermal and optical stability. Congo red dye as sodium salt form has a high solubility in water, which makes the process of treating or removing this dye from polluted water very difficult (Sayğılı, 2015). The use of water-insoluble acid-base and liquid reagents as liquid ion exchangers was first proposed by (Smith et al., 1937). Since then, there has been growing interest in the use of bases and acids with high molecular weights

as extracts (Werner, 1974). The most important properties of a useful liquid exchanger are low solubility, high selectivity, ability to regenerate in cyclic processes, stability to common reagents under normal conditions, and low cost (Khopkar, 2007; Kunin & Winger, 1962).
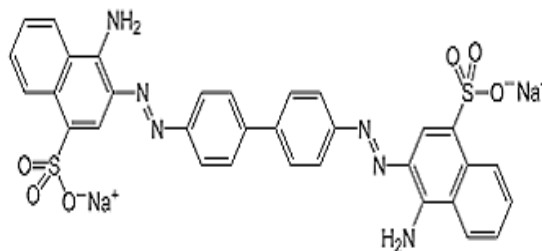
Figure: (1). Chemical structure of Congo red dye

Amberlite LA-2 is chemically named *N-Lauryl (trialkylmethyl)*, and it is a high molecular weight liquid that is soluble in most common nonpolar organic solvents. Hydrometallurgy is one of the main applications of amberlite LA-2 in mineralogy. It has been mainly used in the recovery and purification of uranium from low-concentration samples, as well as the separation of iron, cobalt, and nickel in hydrochloric acid solutions (Lee & Wang, 1995). Moreover, Amberlite LA-2 was used to remove and purify many organic acids, including L(+) tartaric acid (Asçı & İnci, 2009), formic acid (Uslu et al., 2009), propionic acid (Kloetzer et al., 2019), picric acid (Uslu, 2016), malic acid (Uslu & Kırbaşlar, 2010), fumaric acid (Khopkar, 2007; Kloetzer et al., 2019), glycolic acid (Asçı & İnci, 2009), and levulinic acid & malic acid (Uslu & Kırbaşlar, 2013). Removal of Congo Red dye from aqueous solution using Amberlite IRA-400 was also investigated (Sinha et al., 2018). In this study, a maximum loading efficiency (99.99%) of congo red dye was obtained at the conditions pH 4.5, Temp. 303 K, contact time 180 min (Sinha et al., 2018).

In a recently published paper by our group, Amberlite LA-2 was used to remove the Methyl Orange Dye, the maximum extraction

value of about (97.65%) was obtained (Al-abbasi & Abdulljaoad, 2022; Alabbasi et al., 2022; Alabbasi & Suliman, 2022). Thus, this research aims to study the potential of using Amberlite LA-2 as an extracting material for Congo red dye removal. Optimization of the ideal conditions for the extraction process as the initial dye concentration, Amberlite LA-2 concentration, contact time, pH, and temperature. Moreover, the mechanics of the extraction process of Congo red dye with an anion exchanger in aqueous solutions were studied.

MATERIALS AND METHODS

Chemicals and Instrumentations: The chemicals used are all of a high degree of purity: Congo Red dye (T-Baker Lab chemicals,100 %), Amberlite LA-2 (BDH, 99 %), dichloromethane (Chemsolute, 98.5 %), sodium hydroxide (Fluka, 98 %), hydrochloric acid (Chemsolute, 37 %) and sodium carbonate (Fluka , 99.8 %). pH device (Thermo) and VU-Visible Spectrophotometer (Shimadzu UV2450 spectrometer.).

Preparation of Standard Solutions: Stock standard Congo red dye solutions (100 ppm) were prepared by dissolving about 10 mg in 100 mL of deionized water. Amberlite LA-2 solution with different concentrations of 0.011, 0.022, and 0.04 M was prepared by taking volumes of 0.25, 0.5, and 1 mL, respectively, in a 50 mL stander flask, and the volume was completed with dichloromethane solvent and placed in an airtight bottle before use.

Standard calibration curve of congo red: The best wavelength at which the absorption of the Congo red dye solution occurred was determined by measuring the absorbance of the Congo red dye solution with a concentration of 20 ppm in a range of wavelengths (400-800 nm), where the highest absorption of the dye solution was at 498 nm. To determine the standard calibration curve of Congo red, standard solutions of 0, 20, 40, 60, and 80 ppm were prepared to form a series of five

flasks. The absorbance was measured for all flasks at the maximum wavelength of the dye (498 nm) (Vogel, 2000). The relationship between the dye concentration in each flask and the corresponding absorbance was drawn, so a straight line was obtained, and its slope equation was used to measure the concentration of the dye in the aqueous phase at the end of each extraction experiment. The equation for a straight line was $y = 0.0303 x - 0.0007$

Extraction experiments: In tightly capped bottles, two equal volumes (10 mL) of the previously prepared organic and aqueous layers are taken in a 1:1 ratio. The organic layer (Org) consists of an Amberlite LA-2 solution dissolved in dichloromethane (DCM). The aqueous layer (aq.) consists of aqueous solutions of Congo red dye. The bottles with the mixture were placed in a shaking water bath (200 rpm). The bottles were shaken for the define time, and then the aqueous layer was separated from the organic layer using a separating funnel. Immediately, 3 mL was withdrawn from the aqueous layer to measure the absorbance at the λ_{max} of Congo Red (498 nm). To obtain the best conditions for the removal process, the effect of the initial concentration of Congo red dye (20, 40, 60, 80 ppm) and the initial concentration of Amberlite LA-2 (0.044, 0.022, 0.011 M), as well as the effect of temperature (20, 30, 40 °C) and time (15, 30, 45, 60 min), were studied. Moreover, the effect of pH on the extraction process was studied at different pH values (1,3,5,9,11,13). The distribution coefficient (K_D) and percentage of the extracted dye (%E) were calculated.

RESULTS AND DISCUSSION

Congo red was extracted from an aqueous solution using LA-2 in DCM as an extractant. In this section, the effects of the initial dye concentration, equilibration time, temperature effect, and LA-2 concentration were studied, and the results are presented in Table 1.

Theoretical Approach: Liquid–liquid extraction is a physical separation method that aims to remove or separate a certain substance from another substance that has been mixed (Blumberg, 1988) It is defined as the separation of a compound from a mixture utilizing a liquid solvent. This method has proven to be very useful in recovering compounds from mixtures of materials, and it is a method that depends on the solubility of materials in two immiscible solutions, water and organic nonpolar solvents (Blumberg, 1988). The principle of extraction depends on the factor of the distribution of materials between two immiscible liquids (Uslu & Kırbaşlar, 2013). The extraction of Congo red dye with Amberlite LA-2 can be described by the following reaction:

$$(R_3NH^+) + X^- \leftrightharpoons ((R_3NH^+X^-) \qquad (1)$$
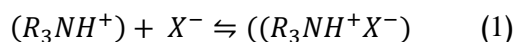
The distribution coefficient (K_D) is the ratio between the concentration of a substance distributed between two immiscible solvents (Seader et al., 2016). The distribution coefficient gives information about the distribution of the dye in the aqueous and organic phases. If the distribution coefficient values K_D are greater than one, the dye concentration in the organic phase is greater than the dye concentration in the aqueous phase, and if the distribution coefficient is less than one, the dye concentration in the organic phase is less than its concentration in the aqueous phase.

$$K_D = \frac{[Dye]_{org}}{[Dye]_{aq}} \qquad (1)$$

where $[Dye]_{org}$ is the dye concentration in the organic layer and $[Dye]_{aq}$ is the dye concentration in the aqueous layer. The removal percentage, (E%), is the percentage of solute that moves from one phase to another at equilibrium. It is expressed as the removal efficiency or removal percentage (E%) as in the following equation:

$$E\% = \left(1 - \frac{[Dye]_{aq}}{[Dye]_{0,aq}}\right) X\ 100 \qquad (2)$$

where $[Dye]_{0,aq}$ is the initial concentration of dye in the aqueous layer. Loading capacity, (Z) indicates the ratio of the concentration of dye in the organic phase at equilibrium to the initial concentration of Amberlite LA-2. The loading capacity (Z) can be expressed by the equation below:

$$Z = \frac{[Dye]_{org}}{[R_2NH]_0} \qquad (3)$$

where $[R_2NH]_0$ is the primary concentration of benzoyl thiourea in the organic phase. The larger this number is, the better the material is for the dye extraction process.

Extraction of the congo red: Anionic congo red was extracted from an aqueous solution using Amberlite LA-2 in DCM as an extractant. In this section, the effects of the initial dye concentration, equilibration time, temperature effect, and LA-2 concentration were studied, and the results are presented in Table 1. From Table (1), it is noticed that all K_D values were greater than one, which indicates the preference of the dye for the organic phase over the aqueous phase. It has been noted that the Z value increases as the concentration of Amberlite LA-2 decreases.

To verify the role of the organic solvent on the extraction process, the extraction processes in the absence of Amberlite AL-2 was carried out. Among the results of the experiments shown in the table (1) it was noted that, the12.528, 31.418, 27.446 and 21.667 ppm of the red Congo dye was extracted from the aqueous phase to the organic phase when the initial dye concentration were 20, 40, 60 and 80 ppm, respectively. This might be explained by the partial solubility of Congo red dyes in the DCM solvent.

From the results of the experiments shown in Table (1) in the absence of Amberlite LA-2, the E % decreases with an increase in the initial dye concentration, as it decreases from 62.64 % to 27.087 % when the initial dye

concentration is increased from 20 to 80 ppm. This is attributed to the saturation of the sol-

vent with the increase in dye concentration.

Table:(1). Characteristic performance data obtained for Congo Red dye by Amberlite LA-2

C_{LA-2} (M)	C_{CR} (ppm)	t (min)	C_{aq}	C_{org}	K_D	Z	E%
0	20	30	7.471947	12.528	1.676678	/	62.64026
	40		28.58218	31.418	1.0992	/	52.363
	60		32.55446	27.446	0.843066	/	45.74257
	80		58.3303	21.667	0.371508	/	27.08746
0.0443	20	15	2.448	17.552	7.1699	0.477359	87.76
		30	1.795	18.205	10.142	0.118	92.356
		45	1.696	18.304	10.79245	0.497811	97.36
		60	3.1155	16.88	5.4181	0.459082	97.14
	40	15	3.0577	36.94	12.081	1.004651	91.025
		30	2.455	37.545	15.2933	1.021105	93.86
		45	1.55	38.449	24.8058	1.045691	98
		60	1.99	59.007	29.6518	1.604803	97.92
	60	15	1.583	58.42	36.9046	1.588838	91.52
		30	1.2	58.799	48.9992	1.599146	96.12
		45	1.069	58.93	55.1263	1.602709	98.22
		60	1.135	38.865	34.2335	1.057005	98.33
	80	15	2.2885	77.71	33.9567	2.113465	84.4
		30	1.66	78.34	47.19277	2.130599	96.68
		45	1.333	78.67	59.017	2.139574	97.162
		60	1.333	78.67	59.017	2.139574	98.33
0.022	20	15	1.246	18.754	15.0514	2.054107	93.77
		30	2.4	17.6	7.33333	1.927711	94.92
		45	1.736	18.264	10.538	2.000438	95.78
		60	0.712	19.288	27.0899	2.112596	97
	40	15	2.032	37.968	18.685	4.158598	88
		30	2.288	37.712	16.482	4.130559	94.28
		45	2.116	37.884	17.9036	4.149398	95.85
		60	1.74	38.26	21.988	4.19058	96.87
	60	15	2.532	57.468	22.6967	6.294414	91.32
		30	2.49	57.51	23.0964	6.299014	94.71
		45	2.532	57.468	22.6967	6.294414	95.78
		60	1.818	58.182	32.0033	6.372618	96.85
	80	15	2.4	77.6	32.333	8.499455	96.44
		30	2.504	77.496	30.9489	8.488059	95.56
		45	2.52	77.48	30.746	8.486309	96.97
		60	1.632	78.368	48.0196	8.583573	97.96
0.011	20	15	2.59	17.4	6.718	7.62322	87.05
		30	2.82	17.18	6.0922	7.526835	93.12
		45	2.62	17.38	6.6336	7.614458	96.073
		60	2.521	17.48	6.9334	7.658269	99.08
	40	15	2.75	37.25	13.5454	16.31983	85.9
		30	2.75	37.25	13.5454	16.31983	93.12
		45	2.59	37.4	14.44	16.38555	95.63
		60	2.455	37.5	15.275	16.42935	96.97
	60	15	2.36	57.6	24.407	25.23549	86.9
		30	2.62	57.38	21.9008	25.1391	93.5
		45	0.363	59.64	164.298	26.12925	99.395
		60	0.277	59.72	215.5957	26.16429	99.69
	80	15	0.7393	79.26	107.2095	34.72508	87.39
		30	2.422	77.58	31.769	33.98905	93.9
		45	0.244	79.756	326.869	34.94239	99.54
		60	0.54	79.46	147.148	34.81271	99.3

Table:(2). Effect of temperature on the extraction of Congo red dye by AmbeliteLA-2

C_{LA-2} (M)	C_{CR} (ppm)	T (°C)	C_{aq}	C_{org}	K_D	Z	E%
0.022	40	20	2.288	37.712	16.4825	2.065277	94.28
		25	2.43	37.57	15.4609	2.0575	93.93
		30	0.352	39.648	112.6363	2.1713	99.12
		40	2.884	37.116	12.8696	2.032636	92.79
	60	20	2.49	57.51	23.0963	3.149505	95.85
		25	2.349	57.651	24.5427	3.157227	96.08
		30	1.35	58.65	43.4444	3.211936	97.75
		40	3.908	56.09	14.3526	3.071741	93.48

Table:(3). The results obtained when extracting Congo red dye by AmbeliteLA-2 under the pH effect.

C_{LA-2} (M)	C_{CR} (ppm)	t(min)	pH	C_{aq}	C_{org}	K_D	Z	E%
0.022	60ppm	30	1.99	4.238	55.762	13.159	2.534636	92.937
			3.8	5.063	54.937	10.851	2.497136	91.562
			5	5.3036	54.696	10.313	2.486182	91.16
			7	2.49	57.51	23.0964	2.614091	94.71
			9.2	1.003	58.997	58.803	2.681682	98.328
			11.23	2.653	57.347	21.612	2.606682	95.578
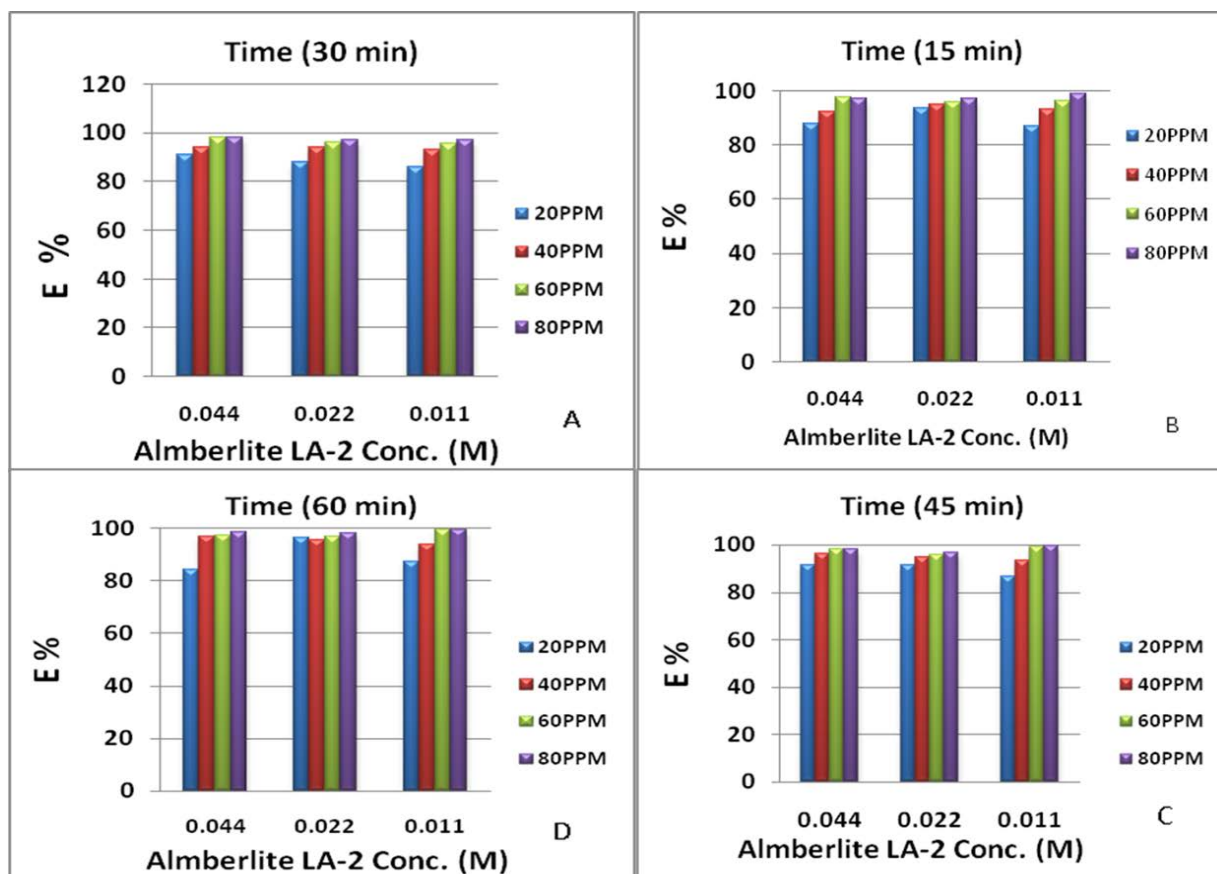
Figure (2). Effect of initial concentration of CR dye, Amberlite LA-2, and contact time on the extraction process
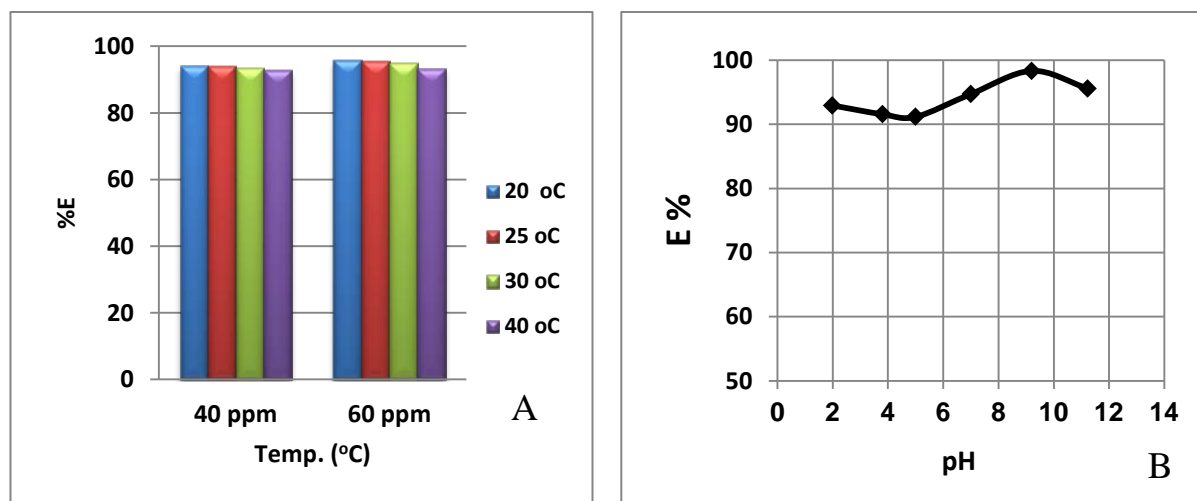
Figure (3). Effect A) temperature B) pH on the extraction congo red dye by Amberlite LA-2

Figures (2 A, B, C, and D) show that E % is an insignificant change when the Amberlite concentration increases from 0.011 to 0.044 M at a constant day concentration and specific time as an example at a day concentration of 80 ppm, the E% is 98.33 %, 97.96 % and 99.3 % for 0.044 M.022 M, and 0.011 M. However, Figure (2) shows that E % increases by increasing the concentration of the initial dye. The results showed that the dye extracted from the aqueous phase to the organic phase increases with time, and the quantitative transfer of the dye takes place after 60 min, this was in good agreements with our previous study on removing of methyl orange by the Amberlite ion-exchanger (Alabbasi et al., 2022).

Figure (3) shows that E% decreases with increasing temperature, which is attributed to the physical bonds between the dye molecules and Amberlite being weakened and its transfer to the organic layer being reduced. Moreover, the possibility of solvent evaporation as it has a low boiling point of 45 °C.

Dye extraction mechanism: In this study, significant extraction of Congo red by Amberlite LA-2 was observed, and the extraction mechanism is suggested as in our previous study (AL-abbasi et al., 2022) as follows: First, CR is dissolved in the aqueous phase, and then the sodium sulfonate groups (R - SO_3Na) are ionized as in equation (2):

$$R\text{-}SO_3Na + H_2O \rightarrow R\text{-}SO_3^- + Na^+ \qquad (2)$$

Second, the interaction of the dye with Amberlite LA-2 is induced by the electrostatic attraction between the Amberlite LA-2 cations ($R2NH_2^+$) and the negative dye anions, as shown in equation (3):

$$R_2NH_2^+ + R\text{-}SO_3^- \rightarrow [R_2NH_2][O_3SR] \qquad (3)$$
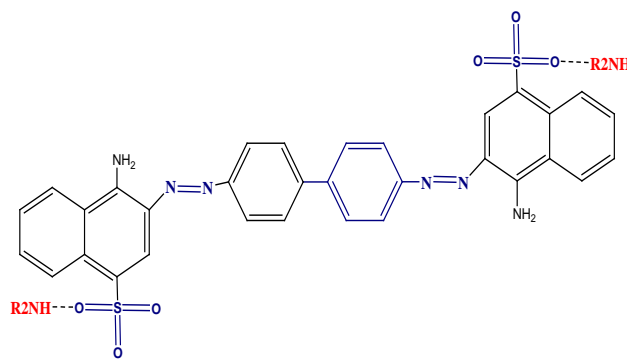
Figure (4). The complex formed by the electrostatic attraction between the positive Amberlite ion and the negative dye ion

CONCLUSION

In this paper, Congo red dye was successfully extracted from an aqueous solution by liquid–liquid extraction using Amberlite LA-2. The values of the distribution coefficients were also determined and these values were greater than 1 which indicates that the pigment distri-

bution in the organic layer is higher than in the aqueous layer. The values of distribution modulus and loading capacity increase with increasing initial dye concentration and decrease with increasing concentration of the substance (Amberlite LA-2). It was noticed that by increasing the value of the acidity function pH, the value of the distribution coefficient and the loading capacity increased, and therefore the extraction efficiency up to 7 pH started decreasing with the increase of the baseline. Increasing the temperature leads to a decrease in the distribution coefficient and extraction efficiency values.

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Morphological , Anatomical and Chemical Studies of *Salvia Rosmarinus* Spenn. Growing in Al- Marj (Libya)

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Abstract: *Salvia rosmarinus* Spenn (synonym: *Rosmarinus officinalis* L.) is economically and medically important and grows naturally in Al-Marj (Libya). Macro, micro morphological and chemical characteristics were studied. Leaf venation, leaf anatomy and chemical compounds of essential oils were carried out according to traditional methods. The objective of the present study is to provide detailed description of the characteristics of this species. Morphological charactersistics of leaves, calyx, corolla, stamens are useful for sectional and specific delimitations in *Salvia*. The leaf architecture characters of primary, secondary, tertiary and quaternary veins are good diagnostic markers for the identification and classification of species. Anatomical characters such as glandular and non- glandular trichomes , mesophyll structure, presence of hypodermis and structure of vascular bundles have been found to have taxonomic value. Cineol (32.38%) is main component of essential oil, followed by Camphor, β-pinene, borneol, Caryophyllene, α-terpineol and α-pinene. Chemical data in essential oils carried taxonomic value of *Salvia* species.

دراسات مورفولوجية و تشريحية و كيميائية لـ . *S. rosmarinus* **Spenn** النامية فى المرج (ليبيا)

المستخلص: النوع *S. rosmarinus* Spenn (المرادف: *Rosmarinus officinalis* L) هو نبات مهم اقتصاديًا وطبياً، وينمو بشكل طبيعي في المرج (ليبيا). تمت دراسة الصفات الكبيرة، والدقيقة المورفولوجية، والكيميائية لنوعين. تم إجراء تعرق الأوراق، وتشريح الأوراق، وتحليل المركبات الكيميائية للزيوت الطيارة وفقًا للطرق التقليدية. الهدف من هذه الدراسة تقديم وصف مفصل لهذا النوع. وجد أن الصفات المورفولوجية، كالأوراق، الكأس، التويج، والأسدية صفات مفيدة في تحديد الأنواع على مستوى النوع، والقسم. تمييز الأنواع: تعد الصفات الهندسية لتعرق الأوراق كعروق الأول، الثاني، الثالث، والرابع علامات تشخيصية جيدة لتحديد الأنواع. تعد الصفات التشريحية كالشعيرات الغدية، والغير غدية، بنية النسيج الأوسط للورقة، وجود طبقة تحت البشرة، وتركيب الحزم الوعائية صفات ذات قيمة تصنيفية. السنيول Cineol هو المكون الرئيسي للزيوت الأساسية، يليه الكافور، بيتا-بينين، بورنيول، كاريوفيلين، ألفا ترينول وألفا بينين. أيضا البيانات الكيميائية في الزيوت الأساسية تحمل قيمة تصنيفية لأنواع جنس *Salvia*.

INTRODUCTION

The genus *Salvia* L. (Lamiaceae), comprises approximately 1000 species widely distributed (Walker & Sytsma, 2007). According to (Jafri & El-gadi, 1985) Flora of Libya comprises 10 *Salvia* L. species, out of which 3 are cultivated. Recently*, Salvia rosmarinus* Spenn., also known as rosemary, the most used name, *Rosmarinus officinalis L.*, has to be considered a synonym of the actual name, because molecular investigations evidenced

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as *Rosmarinus* L. is nested in *Salvia* L.(Drew et al., 2017).

Salvia L. can be distinguished from the other Lamiaceae genera by unique stamina architecture, having only two stamens which are separated by a significantly elongated connective tissue (Hu et al., 2018; Kriebel et al., 2019; Will & Claßen-Bockhoff, 2017). Based on the calyx, corolla and stamen morphology, Benthams first created an infrageneric classification of *Salvia* L. (Hu et al., 2018). Molecular studies have demonstrated that *Salvia* is not monophyletic (Will & Claßen-Bockhoff, 2017). Regarding classifications of the genus *Salvia* L., different opinions are proposed, one option is to treat the five embedded genera (*Dorystaecha Meriandra*, *Perovskia*, *Rosmarinus*, and *Zhumeria*) as subgenera and maintain *Salvia* in a broad sense (Drew et al., 2017; Kriebel et al., 2019) and the other is to split *Salvia* L. into six smaller genera (Will & Claßen-Bockhoff, 2017).

Salvia species are usually composed of perennial, herbaceous, semi-shrubby or shrubby plants and leaves, calyx and trichomes on the flower are important characteristics used to distinguish species (Davis et al., 1982). Also in the revision studies on Salvia; morphological features such as stamen type, verticillaster number, calyx shape, corolla shape, corolla length, length of the corolla tube, whether or not annulus or upper lip is flat or helmet shaped, are distinctive characters in the determination of species (Doğan et al., 2008; Kahraman et al., 2009b).

Leaf characters are considered as important morphological features for taxonomic investigations (Swaminathan et al., 2012). These include leaf architectural characters, venation patterns (Pulan & Buot Jr, 2014). Although leaves are generally plastic, mostly to environmental conditions, and are sometimes neglected in taxonomy, the leaf architecture and venation patterns have long been considered useful for the taxonomy of many different dicotyledonous families, including, Rubiaceae (Banaticla & Buot Jr, 2004), Solanaceae (Inamdar, 1978), Apocynaceae (Mohan & Inamdar, 1982), and even monocotyledonous families (Inamdar et al., 1983; Obico et al., 2007).

Some studies indicated that anatomical studies is a good tool for grouping *Salvia* species (Celep & Dirmenci, 2017). Recently, several anatomical investigations on *Salvia* species have been done (Bagherpour et al., 2010; Buyukkartal et al., 2011; Celep et al., 2014; Kahraman, Celep, & Dogan, 2010; Kahraman, Celep, & Doğan, 2010; Kahraman & Dogan, 2010; Polat et al., 2017; Polat et al., 2010). Studies on the anatomy of this species in Libya are limited.

Various *Salvia* L. species are used in traditional medicine worldwide, for food flavoring, in cosmetics, perfumery and pharmaceutical industry (Dweck, 2000), due to the production of the essential oils which are proved to exhibit considerable biological activities (Alimpić et al., 2015; Alimpić Aradski et al., 2015). *S. rosmarinus* is a rich source of essential oil (Loizzo et al., 2013). The essential oil of *S. rosmarinus* was demonstrated to possess antibacterial, antioxidant, antifungal, and anti-inflammatory properties (Kačániová et al., 2017).

S. rosmarinus grow naturally in Al-Marj and has economic and medical importance. There are not enough studies on this species in Libya, so the aim of this work is to study the morphological, anatomical characteristics and chemical compounds of essential oil, and provide new set of characters to description this species.

MATERIALS AND METHODS

Fresh materials of *S. rosmarinus* were collected from farm on Al-Marg city (Bata). The plant samples were identified using the Libyan flora (Jafri & El-gadi, 1985). Morphological characteristics of studied species, flower, stem, leaf morphology and lamina architecture were determined from fresh materials.

Leaf architectural investigation: Fresh leaves of studied species were accomplished by soaking the fresh samples in a strong household bleach solution (sodium hypochloride less than 5%, sodium hydroxide less than 5% and water) until it turns white (took 24 hours), then transferred into water. Then, the cleared leaves were photomicrographed using (LM) and mobile camera (Galaxy A10s, 8.0 Mega Pixels). The description of leaf veins was carried out by Manual of Leaf Architecture(Ash, 1999).

Micro morphological investigation: The leaves of the studied species were prepared using hand microtome at 10-20 µm. Then were double stained using safranin and light green and mounted in Canada balsam according to (Johansen, 1940) then, examined using light microscope. Photomicrographs were taken using digital camera (Canon power-shot A720, 8.0 mega pixels), the magnification power was expressed by (x).

Essential oils extraction: Fresh leaves of the examined species were submitted for 2 h to water- distillation using a Clevenger distillation apparatus (Clevenger-type) (Su et al., 2012).

Gas-chromatography–mass spectrometry (GC-MS) analysis: Quantitative and qualitative analysis of the essential oil was done using a GC-MS (Model GC-2010 plus, SHIMAD24, Japan) at Faculty of Pharmacy, (ASU),Cairo, Egypt, equipped with a Rtx-5 MS(Cross bound 5% diphenyl/95% dimethyl polysiloxane capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The major components of oils recognized by National Institute of Standards Technology (NIST) V.11 GC–MS library, established by (Adams, 2007) and previous studies on *S. rosmarinus* and different species of Salvia. The relative concentration of each compound in essential oils counted based on the peak area integrated by the analysis program (Su et al., 2012).

RESULTS

S. rosmarinus Spenn., Handb. Angew. Bot. 2: 447 (1835) (IPNI). Replaced synonym: *Rosmarinus officinalis* L., Sp. Pl. 1: 23 (1753). Common name: Kleel. Collection date: 30-1-2022.

Macro-Morphological characteristics (Fig1&2): Evergreen aromatic shrub, up to 85cm tall. Stem square, grey and glabrous. Leaves 5x0.2cm, opposite, sessile. Lamina simple, linear, shiny green, rough on upper surface and white, tomentose on lower surface, revolute margin , acute apex and attenuate base.

Leaf venation pinnate. Secondary vein category festooned brochidromous with spacing irregular and angle one pair acute on base**.** The interseconderies weak. Tertiary vein arise at obtuse angle to primary vein with sinus course and is regular polygonal reticulate category. Quaternary vein regular polygonal reticulate category. Areolation well developed. The marginal ultimate vein are looped. Inflorescence verticillaster (verticals 2-8 flowered). Bract ovate. Calyx 0.5cm, 3-lobed, campanulate, brown-green, tomentose. Corolla 1.2cm, upper lip bifid and lower lip 3-lobed, pale purple-pale blue. Stamens 2, anther one- celled .Style bifid.

Figure (1): *S. rosmarinus*

Figure:(2). a-Leaf morphology. **b&c-** Floral morphology. d-Stem morphology

Micro-morphological characteristics (Lamina anatomy Fig3): Epidermis is uniserate and covered by a thick cuticle on the adaxial surface and a thin cuticle on the abaxial. The epidermial cells are radially to tangential on both surfaces. The non-glandular and glandular trichomes are on the abaxial surface only. The non-glandular trichomes (dendritic trichomes) consist of one cell, followed by a bifurcated series of cells. Several types of glandular trichoms are distinguished.

One type of peltate and three types of capitates. Peltate type has a secretory head with 3-4 cells, a basal cell and large stalk. One capitate type exhibits a unicellular secretory head, abasal cell and a unicellular stalk. A second capitate type has a unicellular secretory head, a basal cell and a bicellular stalk. The third capitate has a unicellular secretory head, a basal cell and a tricellular stalk. A hypodermis occurs under the adaxial epidermis. The midrib contain rounded single vascular bundle surrounded by 2-3 rows of polyhedral parenchyma. The leaf is dorsiventral of 2-3 raws elongated rectangular palisade tissue and 1-2 raws of spongy tissue.

Chemical compounds of essential oil (Table1): Thirty six components was identified in essential oil of *S. rosmarinus*. The main component was cineol (32.38%)**.** Essential oil

was dominated by oxygenated and non- oxygenated monoterpenes (83.53%). Monoterpenes that were present in high concentrations were cineol (32.38%), Camphor (12.32%), β-pinene (6.49%), borneol (5.84%), α-terpineol (4.04%) and α-pinene (3.20%). Sesquiterpenes were present in low concentration in *S. rosmarinus*. Caryophyllene is one of non- oxygenated sesquiterpenes and found in a good amount of about (4,80%) in oil.
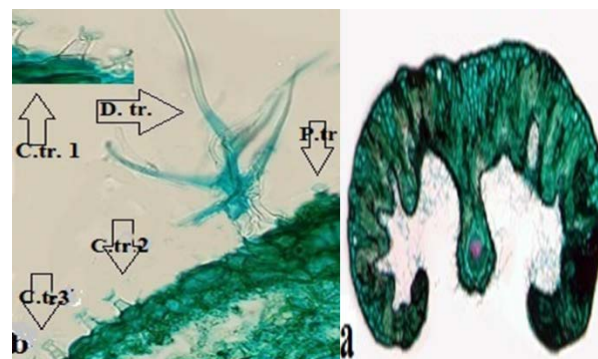
Figure(3): a. Micromorphology. **b.** Types of trichomes **C.tr.1:** The First type of capitate. **C.tr.2:** The second type of capitate.**C.tr.3**: The third type of capitates. **D.tr.:** Dendritic trichome

Table (1). Chemical composition of essential oil of *S. rosmerinus*.

Coumpound	R.Time	*S. rosmerinus*
o-Xylene	5.637	0.19
Heptane,2,4-dimethyl	6.865	0.16
Tricyclo[2.2.1.0(2,6)heptane, 1,7,7-trimrthyl-	7.340	0.58
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- (alpha-Thujene)	7.504	0.32
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (alpha-Pinene)	7.693	3.20
Camphene	8.046	8.50
2,4,4-Trimethyl-lhexene	8.133	0.33
Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)- beta-Pinene)	8.851	6.49
.beta.-Myrcene	9.344	1.61
Decane	9.888	0.20
alpha-Terpinene	10.080	0.74
o-Cymene	10.181	1.04
Eucalyptol (Cineole)	10.437	32.38
trans-.beta.-Ocimene	10.723	0.25
.gamma.-Terpinene	11.346	1.01
5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	11.448	0.22
(+)-4-Carene (Terpinolene)	12.264	0.42
Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha- (beta-Terpineol)	12.364	0.12
Linalool	12.515	0.18
Undecane	13.022	0.33
(+)-2-Bornanone (Camphor)	13.477	12.32
Pinocarvone	14.021	1.30
Bicyclo[2.2.1]heptane-2-ol, 1,7,7-trimethyl-, (lS-endo)- (Borneol)	14.391	5.84
3-Cyclohexene-1-ol,4methyl-1-(1-methylethyl)-, (R)-	14.789	1.60
alpha.-Terpineol	15.145	4.04
Bicyclo[2.2.1]hep-3-en-2-one, 4,6,6-trimethyl-,(1S) (Verbenone)	15.320	0.65
Endo-Borneol (Borneol)	15.548	0.72
Dodecane	16.083	0.15
Bicyclo[2.2.1]heptane-2-ol, 1,7,7-trimethyl-, (lS-endo)- Bornyl acetate)(	18.005	0.29
Benzen,1,2-dimethoxy-4-pr0penyl-(Z) (Methyleugenol)	21.903	0.61
Caryophyllene	22.048	4.80
Humulene	22.916	0.30
Caryophyllene oxide	25.925	1.13
4a(2H)-Naphtalenol,1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S,4R,4aS,8aR)-	26.899	0.37
Tau-candol (gamma-Muurolene)	27.310	0.55
Androstan-17-one,3-ethyl-3-hydroxy-,(5.alpha)-	27.655	0.36

DISCUSSION

In present study, most of the morphological characteristics agreed with the description recorded in the Flora of Libya (Jafri & El-gadi, 1985). Some of the characteristics dif-fered, namely the size of the leaves and the calyx, and other characteristics not mentioned by Jafri, 1985, which are the number of verti-cils and bract shape. Morphological attributes such as bract shape and calyx shape have been recorded in other species of *Salvia* (Kaplan & Çakir, 2019). (Walker et al., 2015) also observed that the stamen morphology of the genus *Rosmarinus* remarkably similar to some species of *Salvia.*

A study of leaf venation for the *Salvia* genus is limited. There is a rich diversity of vena-

tion patterns in dicotyledonous plants, leaf venation can be classified into some characters that may have taxonomic value for the identification and classification of species (Hickey, 1973). In this work, the characteristics of primary, secondary, tertiary and quaternary veins were studied. The primary vein category is pinnate. This result is in agree with (Alimpić Aradski et al., 2021) indicated that primary vein of some species of *Salvia* is pinnate.

In *S. rosmarinus*, the non-glandular and glandular trichomes are on the abaxial surface of leaves. The non-glandular trichomes are unicellular dendroid form. Several types of glandular trichoms are distinguished, Peltate type and three types of capitates. These results in accordance with (Boix et al., 2011). Trihomes have often been used in plant taxonomy (Atalay et al., 2016; Gul, Ahmad, Zafar, Bahadur, Celep, et al., 2019; Gul, Ahmad, Zafar, Bahadur, Sultana, et al., 2019; Wagner, 1991). Also our results showed the presence of a hypodermis and this is in harmony with (Boix et al., 2011; KiliÇ & KiliÇ, 2022), found this layer in four species of *Salvia*. The structure of vascular bundles in the leaf anatomy of Salvia species can be used as a very useful marker for distinguishing the species. *S. blepharochlaena* has one vascular bundles in the middle region and two small bundles on the sides (Ozkan & Soy, 2007), while S. halophila has two large bundles in the middle in the midrib (Kaya et al., 2007). According to our result *S. rosmarinus* has single vascular bundle in the midrib surrounded by parenchymatic tissue (Fig3a). This result in agreement with (Kahraman et al., 2009b), who observed these characteristics of *S. glutinosa*. The leaves of *S. indica* (Kahraman et al., 2009a) are bifacial, however those of *S. halophila* (Kaya et al., 2007) are monofacial. Our result in harmony with (Kaya et al., 2007).

The results obtained in this research showed that peltate, capitates glandular hairs are present on the abaxial surface of S. rosmarinus

(Fig3b). Capitate trichomes are commonly composed of basal cell, 1-2 stalk cells and 1-2 cells forming a round or pear-shaped secretory head (Fahn, 1988; Werker et al., 1985). Plant species that contain glandular hairs produce relatively large amounts of bioactive compounds which include highly concentrated phytochemicals with biological activities of interest to many industries (Dyubeni & Buwa, 2012).

In our study, the essential oil of *S. rosmarinus* were analyzed by GC-MS chromatography. 36 compounds were detected. The main volatiles were oxygenated and non-oxygenated monoterpenes. The cineole, camphor, α-terpineol, borneol, α-pinene, β-pinene, and caryophyllene are the most abundant compounds of essential oils. The studies of (Leporini et al., 2020) indicated that oxygenated and non-oxygenated monoterpenes are the main volatiles of *S. rosmarinus* at two different coasts of Italy, and main compounds were cineole, α-pinene, camphor, and caryophyllene. (Leporini et al., 2020) found β-pinene of low concentation but borneol was not detected. In a study at Al-Jabal Al-Akhdar (Libya) by (El-Ageeli et al., 2020)recorded that main component of essential oil of *S. rosmarinus* is camphor this result disagree with our results. Cineol, α-pinene, β-pinene and caryophyllene were detected by (El-Ageeli et al., 2020) but in different concentrations. The variation in the essential oils components could be attributed to geographical origin, seasonal maturity, genetic variation, growth stages, part of plant utilized and postharvest drying and storage which may influence the essential oil composition (Anwar et al., 2009; Hussain et al., 2008; Marotti et al., 1994).

(Giweli et al., 2013) indicated that cineol is the main component of essential oil and compounds such as camphor, β-pinene, myrcene, α-pinene, caryophyllene and α-terpineol were in high concentrations when studying the species of *Salvia*. These compounds were discovered in our current study. (Coassini Lokar

& Moneghini, 1990) indicated that the chemical data are useful for determining the degree of affinity between species in the genus *Salvia*.

CONCLUSION

In conclusion, The morphological characteristics of *S. rosmarinus* show some differences with those reported in the flora of Libya (Jafri & El-gadi, 1985). Also some morphological characters of this species are absent in the flora of Libya. Macro & micro morphological characteristics are a very useful key for the identification and classification of species. The cineole, camphor, α-terpineol, borneol, α-pinene, β-pinene, and caryophyllene are the most abundant compounds of essential oils of *S. rosmarinus*. Further study might include studying *S. rosmarinus* with a large number of species and studying the antibacterial activity of the essential oils of plants.

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How to Move from Agile to Agility in Software Organizations

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Abstract: Agility adoption in software development organizations is considered a strong solution to managing a rapidly changing, uncertain, and unsteady workplace. Especially, as the objective of Agility is to control changes that may happen. So, moving from Agile to Agility increases the organizations' ability in swiftly and effectively react to unexpected variations in market requests. Agile refers to a mindset emphasizing teamwork, frequent value delivery, and the ability to deal with functional changes. The distinction between Agile and Agility needs to be understood in order to prevent misunderstandings, because Agility is recognized as one of the most important attributes of an organization against market turbulence. Through systematic mapping, this research explores the transition from Agile to Agility in software development companies. Systematic mapping is a technique for gathering, collating, and presenting research evidence. Eight research questions were identified, and to provide answers to these questions, several research papers have been explored in electronic databases. Eventually, 33 research papers were inspected, and answers to all research questions were provided. The results that have been achieved by this research proved that Agile and Agility differ in terms of definitions, attributes, numbers of dimensions, and the dimensions themselves.

كيفية الانتقال من Agile إلى Agility في مؤسسات البرمجيات

الكلمـــات المفتاحيـــة :

Agility , Agile ،
نموذج . أبعاد

المستخلص : يعتبر اعتماد Agility في مؤسسات تطوير البرمجيات الحل المناسب لإدارة التغيرات السريعة والغير مستقرة في مكان العمل خصوصا أن الهدف من Agility هو السيطرة على التغيرات التي يمكن أن تحدث. لذلك فإن الانتقال من Agile الى Agility يزيد من قدرة المؤسسات على الاستجابة السريعة والفعالة للتغيرات المفاجئة في متطلبات السوق. يشير Agile إلى طريقة التفكير التي تشجع على العمل الجمـاعي، و التسليم المتكرر، والقدرة على التعامل مع التغييرات الوظيفية. يجب فهم الفرق الجوهري بين Agile و Agility لإزالة أي لبس بينهما، لأن Agility معترف بها كواحدة من أهم سمات التنظيم في مواجهة اضطرابات السوق باستخدام الخرائط البحثية. يستكشف هذا البحث الانتقال من Agile إلىAgility في شركات تطوير البرمجيات. يعد رسم الخرائط المنهجية أسلوبًا لجمع الأدلة البحثية ومقارنتها وعرضها. تم تحديد ثمانية أسئلة بحثية، ولإيجاد إجابات عن هذه الأسئلة تم التحري وإجراء دراسة أولية في عدة أوراق بحثية تم نشرها في قواعد بيانات إلكترونية. في نهاية المطاف، تم فحص 33 ورقة علمية وتقديم إجابات عن جميع أسئلة البحث. اثبتت النتائج التي تم تحقيقها من خلال هذا البحث أن Agile و Agility تختلفان من حيث التعريفات، السمات ،عدد أبعاد كل منهما، بل ان لكل منهما أبعاد مختلفة عن الآخر .

INTRODUCTION

Agile associations implant a common objective and utilize new information to give choice privileges to the groups nearest to the data. In addition, Agile associations can preferably merge speed and adaptability with steadiness and effectiveness. So Agile development has taken broad steps somewhat recently, significantly further developing programming delivery and establishing more acceptable workplaces in many associations (Highsmith, 2013). These days, companies work in an exceptionally tempestuous climate adapting to a heightened speed of progress.

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Changes in business climate and doubt have entered administration studies and exploration for quite a while (Sherehiy et al., 2007). How to deal with the acceleration to adapt to expanding dynamism and disturbance of the workplace has been the main determinant of an association's prosperity or lack of success for a few decades. These aggressive and stressful conditions power companies to combine new business esteem, increment functional proficiency, and recognize and promptly react to actual organizational dangers. In reality, continuing with process association continues to be a struggle, as it requests the high ability of quick adaptation (Triaa et al., 2016). However, numerous academics and counselors have been looking for fruitful ways to assist companies to succeed in this quickly changing, doubtful, and unstable workplace. One of the best and most current methods of continuity and achievement of organizations is Agility, considered an unavoidable aspect of the present forward-looking companies. The objective of Agility is to dominate changes that may happen. Throughout the most recent twenty years, corporates have concentrated on developing the Agility of their business processes over two aspects: organizational and technological levels (Triaa et al., 2016).

Agility is identified as one of the most significant attributes of an organization in lasting against market turbulence. The idea was first presented by the specialists of the Iacocca organization of Lehigh University (USA) in the mid-1990s and got significant consideration from that point forward. (Bottani, 2009; Sherehiy et al., 2007; Yusuf et al., 1999). Agility is characterized as the capacity of associations to react powerfully and swiftly to sudden variations in market requests (Bottani, 2009; Harraf et al., 2015). Such a reaction is directed to satisfying diverse client needs relating to item specification, value, quality, amount, and delivery (Lyytinen & Rose, 2005).

Organizational Agility is firmly obligated to notions of adaptability and elasticity and these concepts are sometimes interchangeably utilized to signify the endeavors made by an association for dealing with dynamic and unpredictable changes in the market. In other words, adaptability and flexibility comprise two principle attributes crucial for the evolution of associations toward accomplishing Agility. The most significant level of development is reflected in the type of organizational Agility, which contains the two ideas of adaptability and flexibility (Sherehiy et al., 2007).

In the way to illustrate the difference between Agile and Agility (Park & Cho, 2022) explained that the Agile methodology is not dependent on academic theories, but rather, it is a result of how different technologies and tools are designed, used, and organized in the software development business. When it comes to the creation of competitive behavior and chances for innovation, Agility is the capacity of an organization to react rapidly and effectively to changes in the market, supply, and demand. Innovation is one aspect of organizational performance that Agility is known to enhance. Moving from Agile to Agility increases the ability of organizations to react effectively and swiftly to surprising variations in market requests. Business organizations need to adopt Agility to deal with unexpected changes in the market. Still, some organizations may have doubts about adopting Agility. For this reason, a systematic study was carried out to answer some questions pertaining to Agile and Agility movement. This systematic study attempts to evaluate, combine, and present the current finding. The structure of this paper is introduced as follows: Section II illustrates related research; Section III presents the research method; Section IV reports the results of the study; Section V contains the discussion; Section VI contains threats to validity; Section VII holds the conclusion, limitations, and future work.

RELATED WORKS

Businesses now run in a worldwide and speedily changing environment. They have to respond to new chances, changing economic conditions, and the emergence of vying services. As software is used in practically all business operations, new software is produced swiftly to take advantage of new opportunities and adapt to competition pressure. These requirements confirm the need to use Agile software, were Agile methods are incremental development methods and based on iterative delivery of software to customers in which the increments are short, and new system releases are routinely generated and made available to clients every two or three weeks. Customers are involved in the development process to obtain quick feedback on changing requirements. They minimize documentation by using informal communication rather than formal meetings with written documents (Sommerville, 2015).

Agile is primarily intended to serve dynamic and small team sizes, which are typically collocated in one location. These characteristics make Agile methodologies suitable for use in managing small and medium-sized enterprises (SMEs), where the number of members is limited, and the nature of their projects is typically dynamic and flexible (Bin-Hezam et al., 2018). However, with Agile software development, teams can quickly adapt to changes in requirements without affecting release dates. Not only that, but it also aids in the reduction of technical debt, the improvement of customer satisfaction, and the delivery of a higher-quality product. Due to rapid changes in business organizations as well as the need to respond to these changes, the trend towards "Agility" has become important to keep pace with this evolution. The attributes of Agile supported some business companies and factories to apply Agility in their work.
The idea of adjusting to unanticipated changes has led to the evolution of some concepts in business strategies and is referred to as the concept of Agility. Agility is quickly becoming a key business driver for all organizations, as well as a critical factor in a company's ability to survive and thrive in uncertain and volatile markets (Ganguly et al., 2009). Many studies have been conducted regarding Agile development, Agility measurement, and the effectiveness of Agile methods (Gandomani & Nafchi, 2014). But there are not many studies about the transition from Agile to Agility.

"Agile" does not equate to "Agility". Following the Agile manifesto in small projects can lead to Agility (VanderMeer, 2008).

Customer and employee satisfaction is the goal of an Agile enterprise. An enterprise essentially owns a set of capacities that allow it to respond appropriately to changes in its business environment. However, the business conditions in which many companies find themselves are characterized by volatile and irregular demand due to the growing urgency to pursue Agility.

Agility can thus be defined as an enterprise's ability to respond quickly to changes in business and customer demands. To be truly Agile, a company should have a number of distinct Agile enablers (Dahmardeh & Banihashemi, 2010).

A conceptual model was proposed by (Dahmardeh & Banihashemi, 2010; Zhang & Sharifi, 2000) for implementing Agility (as Figure 1 shows). It consists of three major stages: 1. Determining a company's Agility needs and current Agility level; 2. Determining the Agility functionalities required for the company to become Agile; 3. Identifying business practices and tools that could bring about recognized capabilities for the company. This model improves the fact that companies which need to achieve Agility must be Agile.
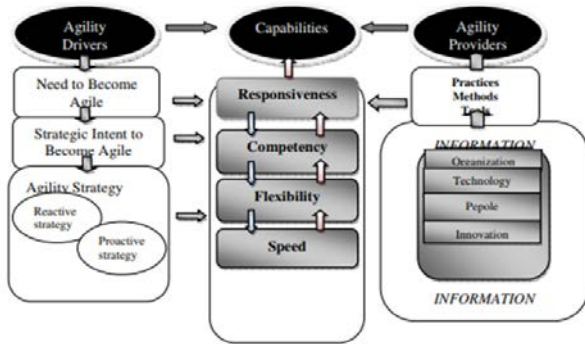
Figure: (1). Conceptual model for implementing Agility (Zhang and Sharifi, 2000)

Many software engineering researchers have focused on measuring the Agility of software companies. Although a few Agility assessment models have been proposed, they have significant drawbacks, such as being inconsistent with the Agile approach, having less flexibility, having limited scope and application, and so on. (Gandomani & Nafchi, 2014) proposed a model in their paper that has none of the disadvantages of the previous models. To create an assessment model, they used 44 Agile practices and their values in achieving Agility in software companies. The proposed model can simply compute a company's Agility based on the practices that the company has adopted.

In terms of business management and cultural lenses, (Karvonen et al., 2018) introduced the definition of enterprise Agility for analyzing large-scale Agile transformation. They summarized their findings, stating that there are numerous proportions associated with enterprise Agility, as well as numerous ways to transform. Agile transformation may focus on operational, strategic, or cultural aspects of Agility; however, holistic transformation to enterprise Agility requires a sophisticated and unique interplay of all of these elements. They addressed contemporary challenges associated with a large organization's transformation to 'enterprise Agility' in the higher education domain. Enterprise Agility transformation is difficult because it necessitates the application of numerous considerations at the same time.

The author of (Sidky, 2017) presented a chapter to introduce a transformation approach for achieving sustainable organizational Agility. He presents the organization's ecosystem, which plays a key role in the culture of an organization and subsequently in its Agility. Next, a couple of common Agile transformation approaches were explored while highlighting sustainability challenges with both. Then, the Culture-led Transformation was presented, which focuses on changing organizational habits in a staged approach leading to sustainable changes.

The Agile Manifesto and Agile Principles are usually used to identify "Agile" and "Agility". But, to understand how they can scale Agile Software Development and achieve Agility, they took a look at the available definitions, especially from sources that look at Agility from a viewpoint that it is more than just one team. They attributed that to the different perceptions that people have of "Agile" and "Agility" making deployment of Agile Methods very hard. The conclusion of this study showed that people really do mean different things when they are talking about Agile Software Development (Laanti et al., 2013).

MATERIALS AND METHODS

RESEARCH QUESTIONS

RQ1. Which journals and conferences are more focused on Agile and Agility?
RQ2. What is the yearly distribution of articles?
RQ3. What is the country-wise distribution?
RQ4. How many researchers have a long-term interest in Agile and Agility?
RQ5. What are the attributes of Agility?
RQ6. Is Agile and Agility the same?
RQ7. Do we need a practical model to achieve organizational Agility?
RQ8. What are the dimensions that each of Agile and Agility have?

RESEARCH METHODOLOGY

A systematic mapping was used in this paper to determine the kind and the extent of the obtainable research papers to answer the research questions as it is shown in figure 2.
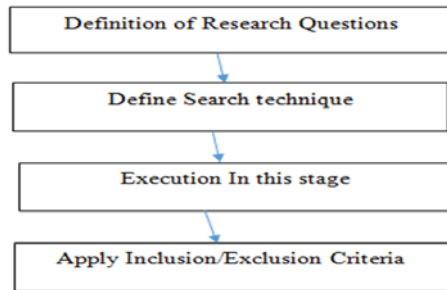
Figure: (2). Research Methodology

1. Definition of Research Questions

Eight research questions (RQs) were set up to decide the premier researches that investigate if Agile is the same as Agility which are:

RQ1. Which journals and conferences are more focused on Agile and Agility?

RQ2. What is the yearly distribution of articles?

RQ3. What is the country-wise distribution?

RQ4. How many researchers have a long-term interest in Agile and Agility?

RQ5. What are the attributes of Agility?

RQ6. Is Agile and Agility the same?

RQ7. Do we need a practical model to achieve organizational Agility?

RQ8. What are the dimensions that each of Agile and Agility have?

2. Define Search technique

A number of keywords and their equivalent words were identified to look for pertinent papers in electronic databases: "Agile" and "Agility". A logical operator AND was utilized to make a combination of the essential terms. The timeframe was set between 1999 and 2021 when this SM was conducted. During this research, five electronic databases were chosen, as they are the most well-known scientific search engines for article locating and publication. The chosen databases as displayed in table 1.

Table: (1). Selected databases.

Source	Location
IEEE xplore	http://ieeexplore.ieee.org
ACM digital	http://portal.acm.org
Springer ink	http://www.springer.com
Science direct	https://www.sciencedirect.com
Google scholar	https://scholar.google.com
Research gate	https://www.researchgate.net

3. Execution In this stage

Different electronic databases were looked through, utilizing the search string. At first, around 149 preliminary studies on Agile and Agility were observed.

4. Apply Inclusion/Exclusion Criteria

As a rule, each title, abstract, and conclusion were investigated to distinguish articles that focus on Agile and Agility. Some studies were excluded based on the accompanying rules:

• Studies not presented in English.

• Studies not accessible in full-text.

• Studies not related to the subject

• Literature review studies

The number of papers initially acquired and later included in this research concentrates after applying the exclusion criteria can be shown in table 2.

Table: (2). Articles related to Agile and Agility

Database	Obtained	Included
IEEE xplore	30	10
ACM digital	43	4
Springer ink	32	10
Science direct	12	3
Google scholar	19	3
Research gate	13	3
Total	**149**	**33**

RESULTS

RQ1. Which journals and conferences are more focused on Agile and Agility?

The most studied papers in the current research are conference papers at 52%. The remaining papers are journal publications at 39%, and others (Books & Workshops) at 9.1%, as displayed in figure 3.
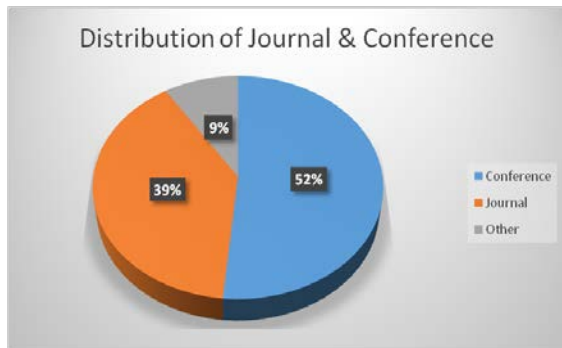
Figure (3). Distribution of Journal & Conference

There are three papers published in the same conference, which is the ***International Conference on Agile Software Development*** conference*,* and the other three conferences published two papers. These conferences are: ***Conference on Extreme Programming and Agile Methods***, ***Agile Conference***, and ***International Conference on Software Engineering***. The rest of the conferences published ***one*** paper, as shown in Table 3.

Table: (3). Conference focused on Agile and Agility

Conference Name	Number of Papers
International Conference on Agile Software Development	3
International Conference on Software Engineering	2
Agile Conference	2
Conference on Extreme Programming and Agile Methods	2
European Conference on Software Process Improvement	1
International Conference on System Science, Engineering Design and Manufacturing Informatization	1
IEEE International Conference on Management of Innovation and Technology	1
Euromicro Conference on Software Engineering and Advanced Applications (SEAA)	1
Malaysian Software Engineering Conference (MySEC)	1
Proceedings of ISSM2000. Ninth International Symposium on Semiconductor Manufacturing	1
International Working Conference on Transfer and Diffusion of IT	1
IFIP International Working Conference on Business Agility and Information Technology Diffusion	1
AIS SIGSAND Symposium on Research in Systems Analysis and Design	1

In terms of journal publications, there are about 2 different papers published in the same journal which is IEEE Software journal. The rest of the journals published one paper related to the study topic as shown in Table 4.

Table: (4). Journals focused on Agile and Agility

Journals Name	Number of Papers
IEEE Software	2
IEEE Transactions on Engineering Management	1
Technological Forecasting and Social Change	1
Public Relations Review	1
Production Economics	1
European Journal of Economics, Finance and Administrative Sciences	1
Review of Managerial Science	1
Information Systems Frontiers	1
Research in Engineering Design	1
Technovation	1
Research Journal of Applied Sciences, Engineering and Technology	1
Academy of Management Annual Meeting Proceedings	1

RQ2*. What is the yearly distribution of articles?*

The papers included in this study were published between 1999 and 2021. The highest number of papers was published in 2018 at 4% in comparison with other years, as shown in figure 4. In the meantime, the most minimal number in this regard, was between 2001- 2003 and 2011-2012.
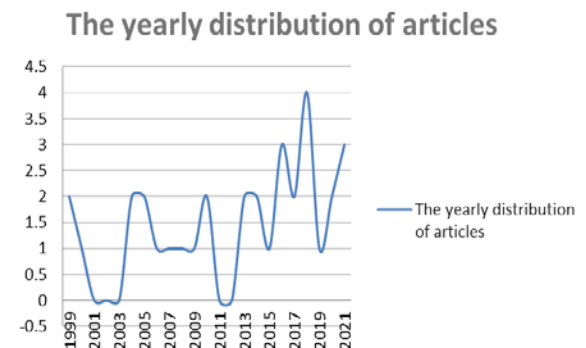
Figure. (4): Number of papers per year

RQ3*. What is the country-wise distribution?*

The primary authors of the papers included in the current study are from thirteen distinct nations, as displayed in table 5. The vast majority of the lead authors are from the U.S.A and Portugal, followed by the United Kingdom as nations having the highest number of authors in this field of research.

Table: (5). Country wise distribution

Country	Number of Journals and Conference Publications
USA	6
Portugal	2
United Kingdom	1
Canada	1
India	1
Ireland	1
China	1
Singapore	1
Austria	1
Malaysia	1
Japan	1
Argentina	1

RQ4. *How many researchers have a long-term interest in Agile and Agility?*

Long-term researchers are important, as they have a greater chance to follow the area of study in more depth, enhancing their results.

RQ5.*What are the attributes of Agility?*

In attempting to define the main attributes of Agility, there was an initial analysis of Agile organizations and unification of exploration on Agility that has prompted ten tentative attributes to serve as principal attributes of Agility. These ten attributes are: a culture of innovation, empowerment, tolerance for ambiguity, vision, change management, organizational communication, market analysis and response, operations management, structural fluidity, and the

development of a learning organization (Harraf et al., 2015).

RQ6. *Is Agile and Agility the same?*

Agility can be identified in software development as the capability of a software developer to feel and respond to advanced technical and business chances to remain inventive and competitive in an unstable and rapidly changing business climate (Lyytinen & Rose, 2005). While Agile is a perspective that centers on cooperation, recurrent conveyance of value, and the capacity to manage functionality changes. It comprises rules, values, methods, and practices. At the end of the day, Agile is a number of tools and strategies that assist us with accomplishing Agility (Laanti et al. 2013)**.**

RQ7. Do we need a practical model to achieve organizational Agility?

One of the primary integrated systems to accomplish Agility is presented by (Gunasekaran, 1998), which shows how the fundamental capabilities of Agile industrialization, for example, collaboration, worth- based pricing methodologies, investments in individuals and data, and organizational changes, ought to be confirmed and integrated with proper lithe empowering agents to evolve an adaptable association (Gunasekaran, 1998; Sharifi & Zhang, 2001; Zhang & Sharifi, 2000) evolved a 3-step method to implement Agility in industrialization associations, which ties Agility drivers (i.e., changes or tensions from the business climate that lead organizations to embrace the Agility model) to four fundamental Agile attributes, to be specific, responsiveness, capability, elasticity, and speed (Sharifi & Zhang, 2001; Zhang & Sharifi, 2000).

In addition, one of the studies that propose a technique to accomplish organizational Agility is led by (Bottani, 2009). The Author has evolved a model that links Agile attributes

to Agile enablers utilizing the quality function deployment (QFD) method and fuzzy logic (Bottani, 2009).

RQ8.What are the dimensions that each of Agile and Agility have?

Each of Agile and Agility has different numbers of dimensions and different dimensions. In Agile there are six dimensions which are: Organization, Talent, Technology enablers, Planning and performance management, Ways of working, and Risk and compliance (Siegel, 2020). While, there are four dimensions of business Agility which are: The degree of Agility, Research & development, Agility, Transformation Agility, and Operation Agility (Gagnon & Hadaya, 2018).

DISCUSSION

To obtain the research publications on the transformation from Agile to Agility, the study explores six electronic databases IEEE Xplore, ACM Digital, Springer, Science Direct, Google Scholar, and Research Gate. Initially, there were 149 research studies on Agile and Agility. However, only 33 research publications on the subject topic were retrieved. The title, abstract, and conclusion of each paper were examined to filter the results and acquire the publications that focused more on the research topic. A substantial number of publications were eliminated because they were not presented in English, were not publicly available as a full text, or were unrelated to the research subject (transformation from Agile to Agility). Following that, a spreadsheet was created to extract the properties related to the research questions. Generally, as can be seen from table 2, the study discovers that 60.6% of the results were accessed through IEEE Xplore and Springer, while the remaining papers were retrieved from the other databases. Figure 2 shows that about half of the articles examined in the present study (52%) are conference papers, while the (39%) are journal publications and the remainder are books and workshops at (9.1%).

Apparently, as shown in table 3, the (International Conference on Agile Software Development) is the most focused conference on transforming from Agile to Agility, with three conference papers published. Meanwhile, there are three conferences (International Conference on Software Engineering), (Agile Conference) and (Conference of Extreme Programming and Agile Methods), and each one of them published two papers related to the topic of research. IEEE, on the other hand, is the most focused in terms of publishing journal papers, where two research papers have been published in the research relating to transforming to Agility from Agile.

The study includes papers published between 1999 and 2021. It can be seen from Figure 3 that in the period 2016 - 2018, the number of published papers increased by 4% when compared to previous years. Meanwhile, the periods with the lowest numbers in this regard were 2001- 2003 and 2011-2012. Which means there was an increase. Interestingly, the great majority of the present study's lead authors are from the United States of America, 6 out of 18 authors, which can be seen in table 5. That is one-third of the lead authors all over the world. Despite the necessity of a long-term researcher with a comprehensive understanding of the field and its limitations, our research shows that there is no dedicated author in the field of Agile to Agility transition.

Following an initial analysis of Agile businesses and organizations as well as the consolidation of Agility research, the investigation resulted in ten tentative attributes to serve as the main attributes of Agility. These ten characteristics are: innovation culture, empowerment, ambiguity tolerance, vision, change management, organizational communication, market analysis and response, operations

management, structural fluidity, and the formation of a learning organization.

(VanderMeer, 2008) found that Agile is not equivalent to Agility while doing a detailed theory-based investigation of the "Agile manifesto" and developing the "Agility principle". In response to question 8, the finding shows that Agile and Agility differ in terms of the number of dimensions and the dimensions themselves. It has been found that: degree of Agility, research and development Agility, transformation Agility and operation Agility are the four dimensions of business Agility (Gagnon & Hadaya, 2018), while Agile has six dimensions which are: Organization, Talent, technology enabler, planning and performance management, ways of working, and risk and compliance (Siegel, 2020).

The study addressed the subject of whether or not a practical model for organizational Agility was required. It was shown that many studies suggest and evaluate practical models during their research. (Gunasekaran, 1998) presents one of the key integrated systems for achieving Agility. In addition, a three steps model for implementing Agility in industrialized organizations has been developed by (Gandomani & Nafchi, 2014; Sharifi & Zhang, 2001; Zhang & Sharifi, 2000) create an assessment model that can simply be used to compute a company's Agility based on 44 Agile practices and their values that have been implemented in the companies' software.

THREATS TO VALIDITY

The validity issues are fundamentally in the papers' picked cycle. Especially, the issue identified with the chance of losing relevant papers. To ensure the totality of our paper archive, the most known scholarly web indexes, including IEEE Xplore, Research gate, and so forth, are chosen. In addition, different mixes of the subject of interest and

their equivalent words identified with Agile and Agility are utilized.

CONCLUSION, LIMITATIONS, AND FUTURE WORK

In recent years, the software development industry has seen a significant increase in the use of Agile methods. Almost all software companies claim to be ''Agile'' on some level and employ Agile practices in their software development processes. With globalization, technology, and outsourcing all contributing to uncertainty and unpredictability in all sectors, an organization's ability to adapt to unexpected change is critical to achieving and maintaining a competitive advantage. This concept of adapting to unforeseen changes has resulted in the evolution of one of the most recent concepts in business strategies, which is known as the concept of ''Agility''.
As a result, transitioning from Agile to Agility improves an organization's ability to respond quickly and effectively to unexpected variants in business requests.

Our search shows there are no researchers who have a long-term interest in the transformation from Agile toward Agility. This, we believe, is due to the confusion that Agile and Agility are synonyms, which has been discussed in our research and we found that they are not. The findings clearly show that Agile and Agility differ in terms of definitions, attributes, number of dimensions, and the dimensions in themselves.

During the search process, the most popular electronic databases were searched (IEEE Xplore, ACM Digital, Springer, Science Direct, Google Scholar, and Research Gate). However, we might have missed some related papers published in these databases during the elimination process either because of the constraints imposed by some of them, such as the paper's full-text accessibility, or because they were not written in English . In terms of future work, examining the success and failure factors of adopting Agility in software

organizations would be interesting. As a result, these factors can be used to measure the success of software organizations that have adopted Agility. Also, further research needs to be done to know if Agility can work well with large organizations.

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Fluoride Concentration in Both Tap Water and Drinking Bottled Water (Commercial) in Baninah Area -Benghazi, Northeastern Libya

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Abstract: Drinking bottled water has become both popular and increasingly controversial. as a result of the use of these techniques, some of the necessary elements in water are exposed to remove it such as fluoride. For this reason, the present study aimed to determine the fluoride content in both Tap Water and some types of Bottled water (Commercial) in the Baninah area -Benghazi. Six different types of bottled water from the local market, and three sampling point of tap water (water network supply) were collected for analysis of pH, electrical conductivity (EC), total dissolved solids (TDS), and fluoride for all samples. The results revealed a decrease in total dissolved salts in some types of bottled water compared to the Libyan standard specifications for the quality of drinking water. While the Fluoride content in bottled water and tap water showed fluoride concentrations lower than 1.5 mg/l according to the WHO standards and Libyan standards for drinking water. This requires a medical study to know the other sources that compensate for the lack of fluoride in the water (tap water network supply - bottled water).

تركيز الفلوريد في كل من مياه الصنبور ومياه الشرب المعبأة (التجارية) في منطقة بنينة – بنغازي ، شمال شرق ليبيا

الكلمـــات المفتاحيـــة :

الفلوريد ،

مياه الصنبور ،

المياه المعبأة ،

بنينة .

المستخلص : أصبح شرب المياه المعبأة شائعًا ومثيرًا للجدل على نحو متزايد. نتيجة لاستخدام هذه التقنيات ، تتعرض بعض العناصر الضرورية في الماء لإزالتها مثل الفلورايد. لهذا السبب هدفت الدراسة الحالية إلى تحديد محتوى الفلورايد في كل من مياه الحنفية وبعض أنواع المياه المعبأة (التجارية) في منطقة بنينة – بنغازي. تم جمع ستة أنواع مختلفة من المياه المعبأة في زجاجات من السوق المحلي ، وثلاث نقاط أخذ عينات من مياه الصنبور (إمدادات شبكة المياه) لتحليل الأس الهيدروجيني، والتوصيل الكهربائي (EC) ، والمواد الصلبة الذائبة الكلية (TDS) ، والفلورايد لجميع العينات. أظهرت النتائج انخفاض إجمالي الأملاح الذائبة في بعض أنواع المياه المعبأة مقارنة بالمواصفات القياسية الليبية لجودة مياه الشرب. بينما أظهر محتوى الفلوريد في المياه المعبأة ومياه الصنبور تركيزات فلوريد أقل من 1.5 ملجم / لتر وفقًا لمعايير منظمة الصحة العالمية والمعايير الليبية لمياه الشرب. وهذا يتطلب دراسة طبية لمعرفة المصادر الأخرى التي تعوض نقص الفلورايد في المياه (إمدادات شبكة مياه الحنفية – المياه المعبأة).

INTRODUCTION

Global demands for trusted drinking water are widening and the inability to meet supplies is important to increasing water conflicts (Sivakumar, 2011). Usual groundwater and surface water are the essential drinking water sources, whereas desalination of brackish and seawater is playing an increasing role. Bottling water began in 1820 in the United States and was fairly popular in the Nineteenth Century. Whilst the 1980s, bottled water became a 'craze' and products rapidly sprang up to meet the demand. (Nutt and Wilson, 2010; Wehr, 2011). Drinking bottled water has become both continuously

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accepted and continuously argumentative (Askari et al., 2021). In comparison with other countries, Libyan markets aren't an outlandish case. Despite the improved quality of drinking water in water distribution systems, the use of bottled water is progressively expanding (Collins and Wright, 2014; Hu et al., 2011). Some people believe, especially those living in locations where tap water quality standards have been violated of bottled water is safer or tastes better for this reason people choose to pay for a good (bottled water) (Bach et al., 2012). The growing consumption of bottled water is due to Perceptions of the safety, taste, and convenience of bottled water. Users are more doubtless to believe that bottled water is safer and tastes greater if tap water or they live in states with more widespread violations of water quality standards (Viscusi et al., 2015).

Fluoride is the ionic form of the element fluorine. Fluoride is found everywhere in the air, water, soil and foodstuff. In the 1930s the first study linked the Natural fluoride concentration of drinking water with decreased caries occurrence in teeth (O Mullane et al., 2016). Geological information may supply info proper for estimating doubtless drinking water fluoride concentrations, utilizing information on the distribution of rock types to point to doubtless fluorine, calcium, and sodium exposures from which the population fluoride vulnerability could be derived. Correct geological information of this nature isn't accessible at the global level (Fewtrell et al., 2006). Groundwater is the main source of water in the study area, as it is fed from the Sidi Mansour field and some wells in the Baninah field. In addition, the local population uses bottled water for drinking. Drinking water generally contributes the most to daily fluoride intake (Murray., 1986). The World Health Organization specifies a maximum acceptable concentration of fluoride in drinking water of 1.5 mg/L (Organization, 2004). For example, high or low levels of

certain ions, such as fluoride, chloride, magnesium and calcium, can lead to various diseases, hardness and other health problems due to water drinking consumption (Sengupta, 2013). Fluoride levels in groundwater vary depending on the minerals in the rocks and ores through which the water flows (Fawell et al., 2006; Vural and Gundogdu, 2020). High levels of fluoride in groundwater can cause dental fluorosis (staining of teeth). On the other hand, fluoride in drinking water is deficient. Because fluoride enters the body primarily from drinking water sources, the geochemical state of fluoride in a given area or neighborhood is extremely important for studying the incidence of dental disease in that area (Dissanayake, 1991). Reverse osmosis (RO) helps remove fluoride and other chemical contaminants from drinking water supplies because their molecules are larger than water molecules. On the other hand, the beneficial effects of fluoride in reducing dental caries may require supplementation when drinking water is exposed to water purification systems that reduce fluoride to well below optimal levels (Khairnar et al., 2018). This study attempted to identify Fluoride Concentration in both Tap Water and Some Types of Bottled Water (Commercial) in the Baninah area -Benghazi.

MATERIALS AND METHODS

Study Area: Baninah is a town east of Benghazi in Libya, about 15 km east of the city centre. The study area is located in the area coordination of latitude 32.08.4570 and longitude 20.25.5113 in the Baninah area, Northeastern Libya. The study area depends on groundwater for the main water supply in the public water network as a source of domestic water. In addition, the residents of the area depend on bottled water as a source of drinking.
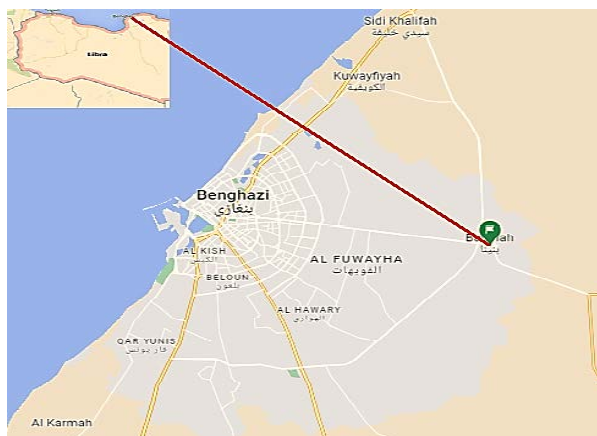
Figure: (1) The sampling location at Baninah Village.

Sampling and Analysis.: A total of 9 water samples were collected as follows: 6 different types of bottled water (local market), and 3 samples from tap water (a water distribution network). Tap water (water network supply) was collected in August 2022 from 3 points sampling in the Baninah region. The information about the location of these points sampling (coordinates) of the tap water (water network supply) is given in table (1).

Table (1). Coordinates the point sampling (water network supply).

Points sampling	Latitude	Longitude
Tap water (Number 1)	32.07 748	20.25 980
Tap water (Number 2)	32.08 610	20.25 998
Tap water (Number 3)	32.08 742	20.25 226

Each sample was analyzed three times. conductivity and the Total dissolved solids(TDS) by using Microprocessor HI 9835 (HANNA) meter were measured while the Fluoride concentration of water samples was measured using the SPADES method using DR 3900 spectrophotometer. according to the standard method(APHA, 1999). All the samples were analyzed in the laboratory of the agriculture faculty at Benghazi university.

RESULTS AND DISCUSSION

Groundwater is the primary source of drinking water in this area and some people are using bottled water for drinking. Tables (2

and 3) illustrate four parameters determined for 3 taps of water and determined for 6 Types of Bottled Water (Commercial) in the Baninah area -Benghazi which was the most used in the study area.

Table: (2) Mean of conductivity EC, pH, T.D.S and F at some Taps Water in Baninah Village.

Location	T.D.S	EC	pH	F
Tap Water in Baninah Village (Number 1)	1344	2320	7.95	1.03
Tap Water in Baninah Village (Number 2)	1410	2429	7.97	0.63
Tap Water in Baninah Village (Number 3)	1385	2386	8.02	0.62
Permissible level (mg/L)	1000	-	6.5-8.5	1.5

All the Parameters Value are Expressed in mg/l; Except pH, and Conductivity EC (μS cm-1).

Table: (3) Mean of conductivity EC, pH, T.D.S and F in Some Types of Bottled Water (Commercial) in the Baninah area -Benghazi.

Location	T.D.S	EC	pH	F
Bottled Water (Aseel)	102	176	7.49	< 0.01
Bottled Water (Areen)	63	107	7.43	< 0.01
Bottled Water (Aquafine)	78	135	7.42	< 0.01
Bottled Water (Alain)	102	176	7.32	< 0.01
Bottled Water (Pure)	72	124	7.30	0.07
Bottled Water (Kharoba)	96	165	7.6	0.04
Permissible level (mg/L)	100	-	6.5-7.5	1.5

All the Parameters Value are Expressed in mg/l; Except pH, and Conductivity EC (μS cm-1).

The pH values : The pH of water is a crucial factor in determining different types of uses, including drinking, bathing, cooking, washing, and agriculture, among others (WHO, 2011). The pH values of the Tap water in Baninah village in the study area ranged from 7.97 to 8.03 with an average of 7.98 (Table 2). This shows that the Tap water in Baninah village in the study area is mainly alkaline. According to (WHO, 2011), the safe limit of the pH lies between 6.5 and 8.5 pH. It is indi-

cating the neutral nature of pH throughout the area of study. This water is suitable for drinking, and this is confirmed. On the whole, Groundwater quality catchment is affected by geology with very little anthropogenic influence(Zakaria et al., 2021). The geology of the catchment region and the water's ability to act as a buffer generally have an impact on the pH of groundwater(Weber Jr and Stumm, 1963).

Electrical conductivity (EC): Electrical conductivity (EC) is related to the concentration of ions in the water. The EC is an excellent index of the amounts of TDS in water, with a high EC indicating a high level of TDS in the water. The higher value of EC is due to the high dissolved solids which may subscribe to the conductivity(Srinivas et al., 2000). Table (2) shows the high amount of Electric Conductivity (EC) in the water supply in the study area (2429 μS/ cm) in the tap water of the study area. These conductive ions come from dissolved salts and inorganic substances such as alkalis, chlorides, sulfides, and carbonate compounds.

Total Dissolved Solids (TDS): Table (2) and figure (2) show the high amount of total dissolved solids (TDS) in the water supply in the study area was (1410 mg/l). which is compared to WHO and Libyan standards (WHO, 2011). The type and quantity of dissolved salts originate from the dissolving or weathering of the soil's minerals, such as gypsum, lime, and other slowly dissolved ones(Joshi et al., 2009). In another meaning, TDS in drinking water originate from natural sources, urban runoff, industrial wastewater, and sewage(WHO, 2011). This increase in the concentration of total dissolved salts and the low quality of tap water is reflected in the volume of bottled water consumption. Not surprisingly, the consumption of bottled water is sometimes higher in communities with serious problems with tap water(Colburn and Kavouras, 2021; Doria, 2006).

Figure (2) The mean of total dissolved solids (T.D.S mg/l) compared with Permissible level (mg/L) in some Taps of Water at Baninah Village.

Table (3) and Figure (3) show the total dissolved solids (TDS) in bottled water. Where the highest amount of dissolved salts was recorded at 102 mg/litres of Alain Company, and the lowest amount of salts recorded in Areen Company's sample was 63, which was less than the permissible limits in the Libyan specification for bottled water (Center, l. N. 2008). Minerals are therefore necessary for good taste and to make up for the deficiency in the public's health (Islam et al., 2016). However, if there aren't any minerals in the water, the body won't get what it needs, and there won't be any flavor.

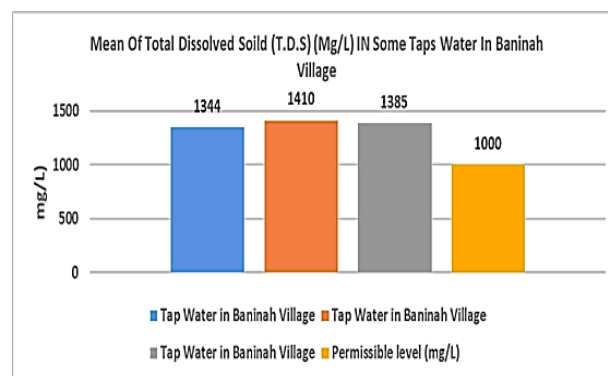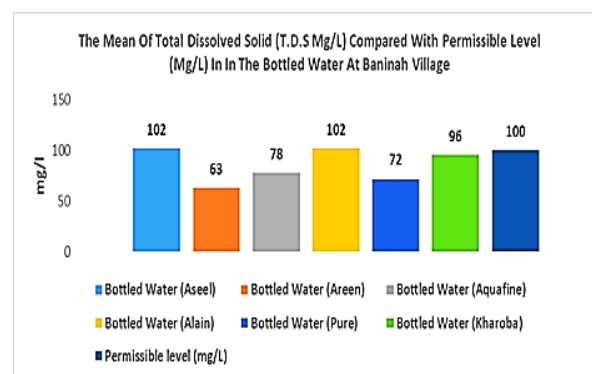**Figure (3)** the mean of total dissolved solids (T.D.S mg/l) compared with the Permissible level (mg/L) in the bottled water at Baninah Village.

Fluoride Concentration: In this study, Fluoride concentrations were determined in three points of tap water. Figure 4 shows The result of the samples analyzed in the study area. The highest concentration of fluoride in Tap water was (1.03 mg/L) in Baninah vil-

lage. In addition, the average fluoride was 0.76 mg/L in tap water. Naturally, fluoride in drinking water is known to reduce dental caries among consumers, but excessive fluoride intake can cause skeletal and dental fluorosis that may either benefit or harm the health of consumers. (Guissouma et al., 2017; Sham et al., 2021; Vishali et al., 2020). The permissible limits of fluoride (1.5 mg/L) are set by the WHO and Libyan standards (WHO, 2011).
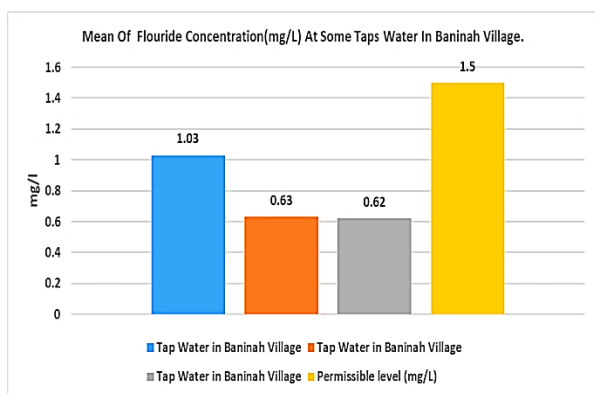
Figure (4) the mean Fluoride Concentration F (mg/l) compared with the Permissible level (mg/L) in some Taps Water at Baninah Village.

The lowest Fluoride Concentration observed in the bottled water as Aseel, Areen, Aquafin, and ALAIN was (< 0.01 mg/L) while the rest of the bottled water was 0.07 and 0.04 in bottled water as Pure and kharoba respectively. that shown in Figure (5).
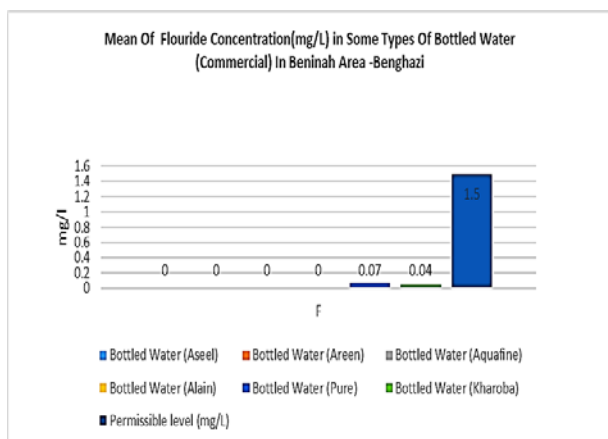
Figure (5) the mean Fluoride Concentration F (mg/l) compared with the Permissible level (mg/L) in the bottled water at Baninah Village.

CONCLUSION

The objective of this study was to determine Fluoride Concentration in both Tap Water and Some Types of Bottled Water (Commercial) in the Baninah area -Benghazi. The main conclusions follow.

• To conclude, six Types of Bottled Water (Commercial) in the Baninah area -Benghazi were investigated in terms of fluoride parameters and compared with the WHO guidelines for drinking water quality.

• The decrease in total dissolved salts in some types of bottled water compared to the Libyan standard specifications for the quality of drinking water(Standardization, 2008), and this in turn leads to serious health problems when you continue to rely on it completely.

• Fluoride measurements in bottled water showed that some of the samples had fluoride levels below 0.01 mg/L in bottled water ASEEL, AREEN, AQUAFINE, and ALAIN. whereas bottled water KHAROBA and PURE showed that the samples had fluoride levels of 0.04 mg/l and 0.07 mg/l respectively. Again, the lowest fluoride levels were in Bottled Water (Commercial) in the study area.

• Reverse osmosis system is an effective method of de-fluoridation. Given the beneficial effect of fluorides in reducing tooth decay, when drinking water is exposed to water purification systems that significantly reduce fluoride to a suboptimal level, it may be necessary to use fluoride supplementation if necessary.

• In tap water, fluoride concentration in the public drinking water supply (tap water) was found to be in the range of 0.62–1.03 ppm, which is far below the recommended levels of Libyan Standard (1.5 ppm) (Center, L. N. 1992; WHO, 2011).

• The increase in total dissolved salts in the public drinking water supply (tap water) compared to the Libyan standard specifica-

tions for the quality of drinking water.

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Monitoring the Reproduction and Development of Eggs in the Sea Hare *Aplysia fasciata* under Laboratory Conditions

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Abstract: This study looks at spawning patterns, egg mass shape, and embryo development to learn more about the reproductive biology of the A. fasciata species that lives in Libya's coastal waters. In *June* 2022, eight mature *Aplysia fasciata* collected from Tajura coast and kept into aquarium contain aerated seawater and marine algae (*Ulva lactuca*). After five days, a mating couple was seen in the aquarium, and, a mass of fertilized eggs were produced. The clusters eggs were transfer to new aquarium. After few hours, the eggs started development to embryo and at 10 day the egg masses colour changed to a brownish as the embryo developed to the trochophore stage and started to rotate inside capsules. After 16 day of spawning the trochophore broke the egg capsules to hatch as free swimming larvae (the veliger stage). Unfortunately, after 25 day of fertilized eggs all larvae died as veliger. Probably, the result of inadequate conditions for rearing or lack an availability of appropriate food. Whereas *A. fasciata* has never been recorded before in the Libyan waters.

مراقبة تكاثر وتطور بيض أرنب البحر *Aplysia fasciata* في ظل ظروف المختبر

الكلمــات المفتاحيــة :
أرنب البحر
Aplysia fasciata،
التكاثر ،
يرقة التروكوفور ، يرقة
الفيلجر،
ليبيا.

المستخلص: في يونيو 2022 ، جمعت ثمانية أفراد ناضجة من *Aplysia fasciata* من شاطئ تاجوراء، ووضعت في حوض يحتوي على ماء البحر، وغذيت بالطحالب البحرية الطازجة (*Ulva lactuca*) مع التهوية المستمرة. بعد خمسة أيام، شوهد زوج منها في حالة تزاوج ونتج عن ذلك كتلة من البيض المخصب. وتم نقل عناقيد البيض إلى حوض آخر. بعد ساعات قليلة من إنتاج البيض المخصب، بدأ البيض في التطور ليصبح جنينًا. بعد 10 أيام تغير لون كتل البيض إلى اللون البني مع تطور الجنين إلى مرحلة التروكوفور ، وبدأ في الدوران داخل الكبسولات. كسرت التروكوفور كبسولات البيض بعد 16 يومًا من الإخصاب، لتفقس على شكل يرقات تسبح حرة (تسمى مرحلة الفيلجر). ولكن لسوء الحظ، بعد 25 يومًا ماتت جميع اليرقات على شكل يرقات فيلجر. ربما يكون ذلك نتيجة لظروف غير ملائمة للتربية، أو عدم توافر الغذاء. حيث لم يتم تسجيل *A. fasciata* من قبل في المياه الليبية.

INTRODUCTION

The sea hares, members of the Aplysidae family, are important grazers (Kajino et al., 2022). The genus Aplysia, often known as sea hares, is made up of 50 species that are found all over the world and graze primarily in the tidal and subtidal zones. Grouping under the following categories: Class Gastropoda, Subclass Heterobranchia, Infraclass Euthyneura, Subterclass Tectipleura, Order Aplysiida, Superfamily Aplysioidea, Family Aplysiidae, Genus Aplysia, and Species *Aplysia fasciata*. The benthic herbivorous Opisthobranchia, Aplysia, is found primarily in tropical and subtropical marine regions. (Beeman, 1968; Klussmann-Kolb, 2004; Lee et al., 2014). The *Aplysia* sp. it is a member of the Opisthobran-

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chia subclass, and the he reproduction, metamorphosis, growth, fecundity, and seasonal abundance of several aplysiid species have all been studied in various parts of the world (Kempf, 1981; Lee et al., 2011; Nimbs et al., 2017; Switzer-Dunlap & Hadfield, 1977; Yusa, 1996). Most gastropod exhibit gonochorism, and some clades as Opisthobranchia and Pulmonata are typically simultaneous hermaphrodites (Lee et al. 2014). The *Aplysia* sp. uses a variety of mechanisms, including inks, to avoid predators (Derby & Aggio, 2011). These organisms create eggs and sperm concurrently, but they often do not self-fertilize. Instead, they cross fertilize by copulating with another individual (Berry et al., 1994; Hadfield et al., 1984; Klussmann-Kolb, 2004; Kress & Schmekel, 1992; Painter et al., 1985; Plaut et al., 1995). The female role includes producing gelatinous egg masses in series and storing all sperm for transfer to another animal and the male is responsible for inducing the creation and transfer of aut sperm (Carefoot, 1987) and (Yusa, 1994).

In order to protect their fertilized eggs from severe environmental changes like salinity, temperature, desiccation, and water flow, many intertidal gastropod species encase their eggs in capsular egg masses (Lee et al., 2014; Przeslawski, 2004; Przeslawski & Benkendorff, 2005; Rawlings, 1999). Many marine invertebrate groups commonly encapsulate fertilized eggs, and the composition and shape of egg mass vary between species (Klussmann-Kolb & Wägele, 2001; Lee et al., 2014; Przeslawski, 2004; Przeslawski & Benkendorff, 2005) Egg capsules come in a variety of sizes and designs that range from millimeters to centimeters in length, from flat, hemispherical disks to towering, upright vases (Switzer-Dunlap & Hadfield, 1977).

Sea hare distribution in the Western Atlantic, which stretches from New Jersey to Brazil, and the Eastern Atlantic, which includes the Mediterranean Sea and the coast of West Africa (Valdés et al., 2013). Some individuals assume that *A. brasiliana*, a species found along the Atlantic coast of the Americas, is simply *A. fasciata* with a different regional colour pattern. Spread along the French Atlantic coast, in the Mediterranean, in West Africa, and on occasion, in the southern British Isles (Susswein et al., 1993). *Aplysia punctata* species found along the Catalan coast (NE Spain, Western Mediterranean Sea) exhibit unique color variants that differ in size, mating style, egg mass, and larval colour (Gonzalez et al., 2022).

Genus Aplysia's body colour is black and the parapodia, feet, and cephalic tentacles have a red border. The body occasionally has a few pale patches. The parapodial lobes are considerably apart from one another both anteriorly and laterally. Can grow to be 40 centimetres long (Bebbington & Hughes, 1973; Beeman, 1968; Klussmann-Kolb, 2004). The reproduction, metamorphosis, growth, fecundity, and seasonal abundance of several aplysiid species have all been studied in various parts of the world (Kempf, 1981; Kriegstein et al., 1974; Lee et al., 2011; Yusa, 1996). However, little is known about the reproductive traits and embryogenesis within egg masses of *A. fasciata*.

This study looks at spawning patterns, egg mass shape, and embryo development to learn more about the reproductive biology of the *A. fasciata* species that lives in Libya's coastal waters. One of the least researched species of Libyan fauna as *Aplysia* sp. There are reports of the spotted sea hare *A. dactylomela* in four different places in the maritime borders of Libya (Rizgalla et al., 2019). Whereas *A. fasciata* has never been recorded before in the Libyan waters.

MATERIALS AND METHODS

Adults of *A. fasciata* specimens were collected alive on June, 2022 from Tajura shore at Marine Research Centre, east of Tripoli, Libya (32°.89'61.31"N 13°.34'95.66"E) and transferred in seawater tanksto laboratory. Eight adult of *A. fasciata* were incubated in aquarium under control conditions (salinity

37 ppt ±1, pH 7.8, water temperature 21°C ±1, and aeration). Water of the aquaria was changed daily. *A. fasciata* were fed daily with fresh greenalgae (*Ulva lactuca*) based on (Gonzalez et al., 2022).

After five days, a mating was seen between couple of *A. fasciata* in the aquarium and, a mass of fertilized eggs was produced. The Egg clusters were removed from old aquariums to new one under the same condition as above (Ghory et al., 2020). After the eggs hatched, the larvae removed and placed in 1L flasks contain filtered seawater and microalgae (*Dunaliella salina*) as food. Leica binocular microscopes (Leica Microscope MZ9.5 With Tilting Binocular Head and Dual Illuminated Stand) and a digital camera (an Olympus TG4 compact camera and it is an automatic stacking feature). Were used to track the development and hatching eggs every day.

RESULTS

The body of *A. fasciata* was black colour, and the foot are very soft and flabby. Head and neck Wide and short. The well developed, flat, leaf-like cephalic tentacles. Tiny and distinct eyes; Foot is thin, thick, squishy, but hardens upon contraction, with well-defined edges, rounded front edges, and a short, blunt tail (Fig.1 A and B). A secretion from the ink gland that is dark purple, *A. fasciata* feeding on macroalgae of other surfaces. Egg masses appear as a long (15 cm) and yellowish masses, with jelly capsules (Fig.2 A and B). Morphological feature of eggs after fertilized were longs as noodle (Fig.1 F). Each capsule carried 18 to 25 egg, and there were 60 capsules per one centimetre (Fig.2 B and C). The masses were bright yellow after spawning (Fig.3 A). And this colour remained the same until embryos reached trochophore stage (Fig.3 B).

A. fasciata produced fertilized eggs through internal fertilization through copulation (Fig.1

C and D). After spawning, the egg masses were yellow in colour (Fig. 1), but as they developed, their colour changed (Fig.2 and 3). Embryogenesis at spawning, the fertilized eggs were spherical and had a diameter of around 80 µm (Fig.2 A). There was an unequal spiral cleavage during the cell division. About 12 hour after spawning, the first cleavage and development of a compact 2 cell embryo occurred (Fig.2 E). The cleavage of the two blastomeres was not synchronized during the second cleavage (Fig.2 E). After spawning, it takes around 18 hour for a compact 3 cell embryo to form (Fig.2 F). The four little blastomeres were split from the animal pole of each large blastomere during the third cleavage, which happened spirally. The first quartet was called by these tiny blastomeres. After spawning, it takes around 24 hour for a compact 8 cell embryo to form, and takes around 6 hour more to reach a 16 cell embryo (Fig.2 G).

At 10th day after spawning, the colour of the egg masses changed to a brownish colour as the embryos progressed from the trochophore stage to the veliger stage. The embryo reached the trochophore stage and started rotating inside the egg capsule (Fig.3 A and B), and the trochophore larva develops to the veliger stage than broke the egg capsule and hatched as free swimming larva (Fig.3 C). They entered the veliger stage three more days later. The veliger between 12 and 15 day after hatch develop the prevelum in larva than becomes the velum at 16-17 day (Fig.3 D). Hatching larva has shell foot stick out and the velum covered with cilia; and there is inside digestive gland surrounded by jelly matrix (Fig.3 E and F). After 25th day sadly, all larva died as veliger perphases.
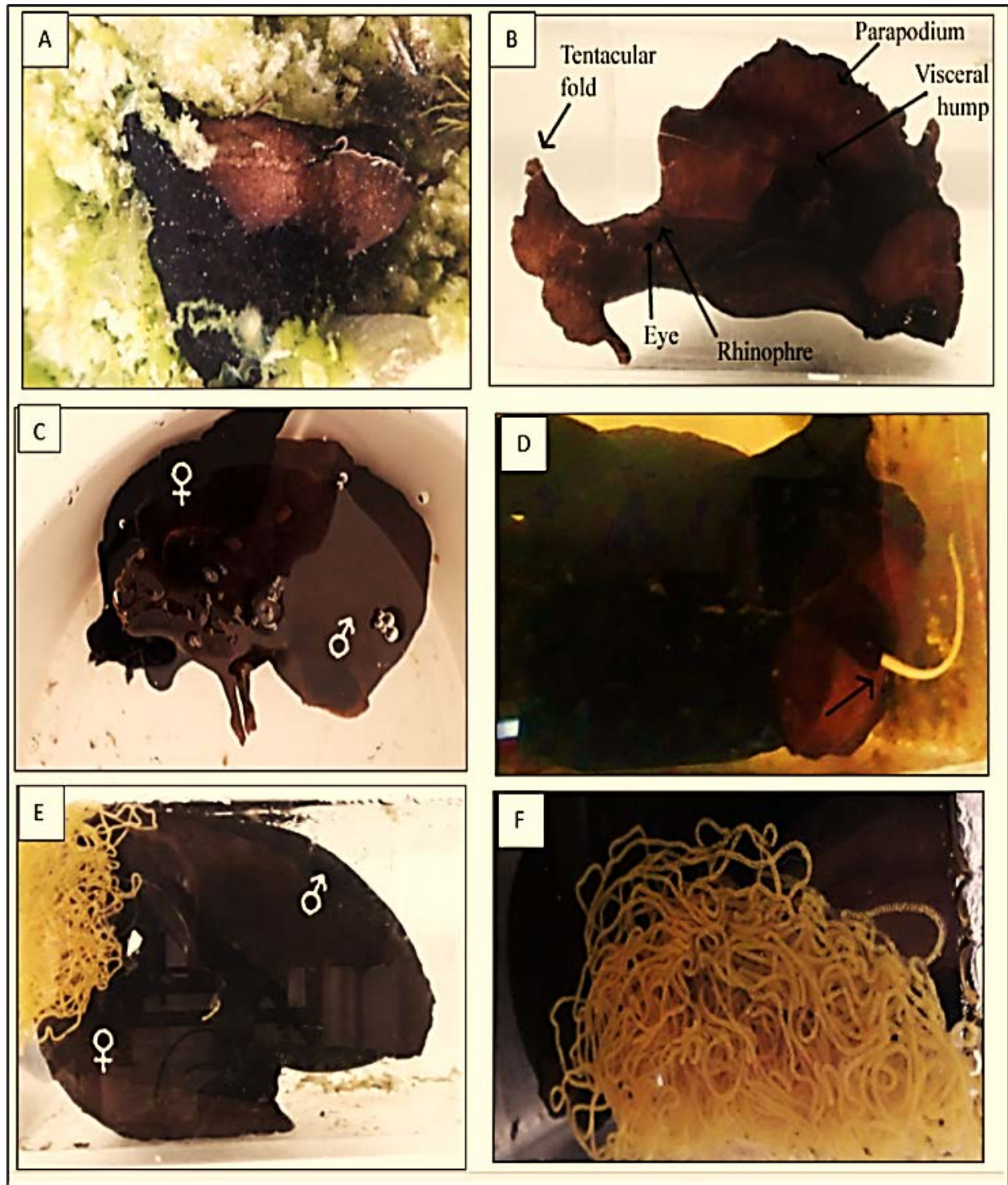
Fig (1). Morphological feature of *A. fasciata* (a, b), pair (male and female) (c), Penis of male (d), Egg masses after spawning (e) and masses of threads containing fertilized eggs (f).
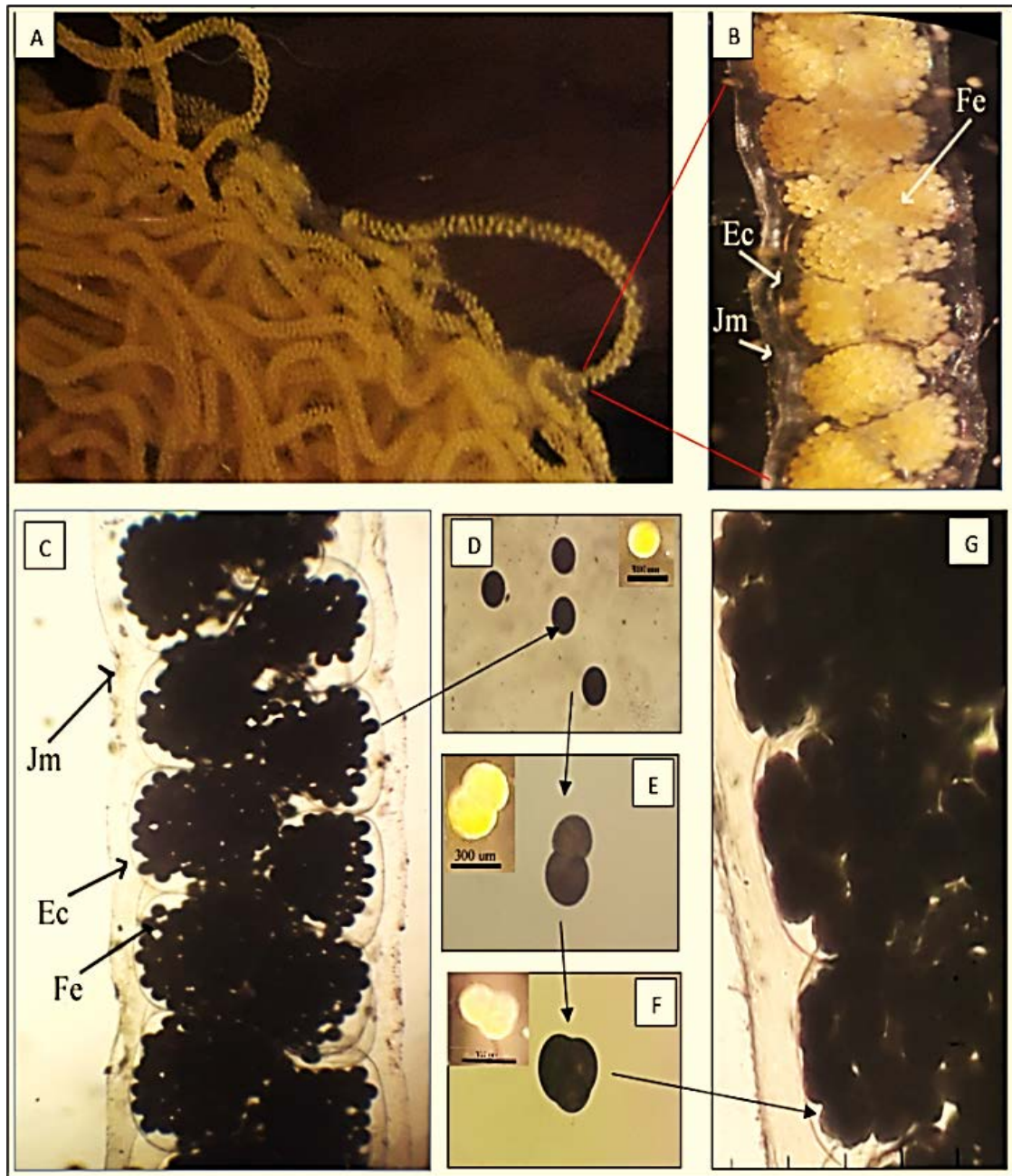
Fig. (2). Development of egg masses of *A. fasciata* in tank (A), formation of coupling chain and egg masses just after spawning (B), Change in colour of egg masses over developmental stage (C), Early developmental stage of *A. fasciata* fertilized egg (D), 2-cell stage embryo talks about 12hr after spawning (E), Beginning of 3 to 4 cell stage (F) and 8-cell and 16 cell ended with Trochophore stage (G). Scale bars indicate 300 μm. [Ec= Egg capsule, Jm= Jelly matrix and Fe = Fertilized egg / Scale bars indicate 10 cm].
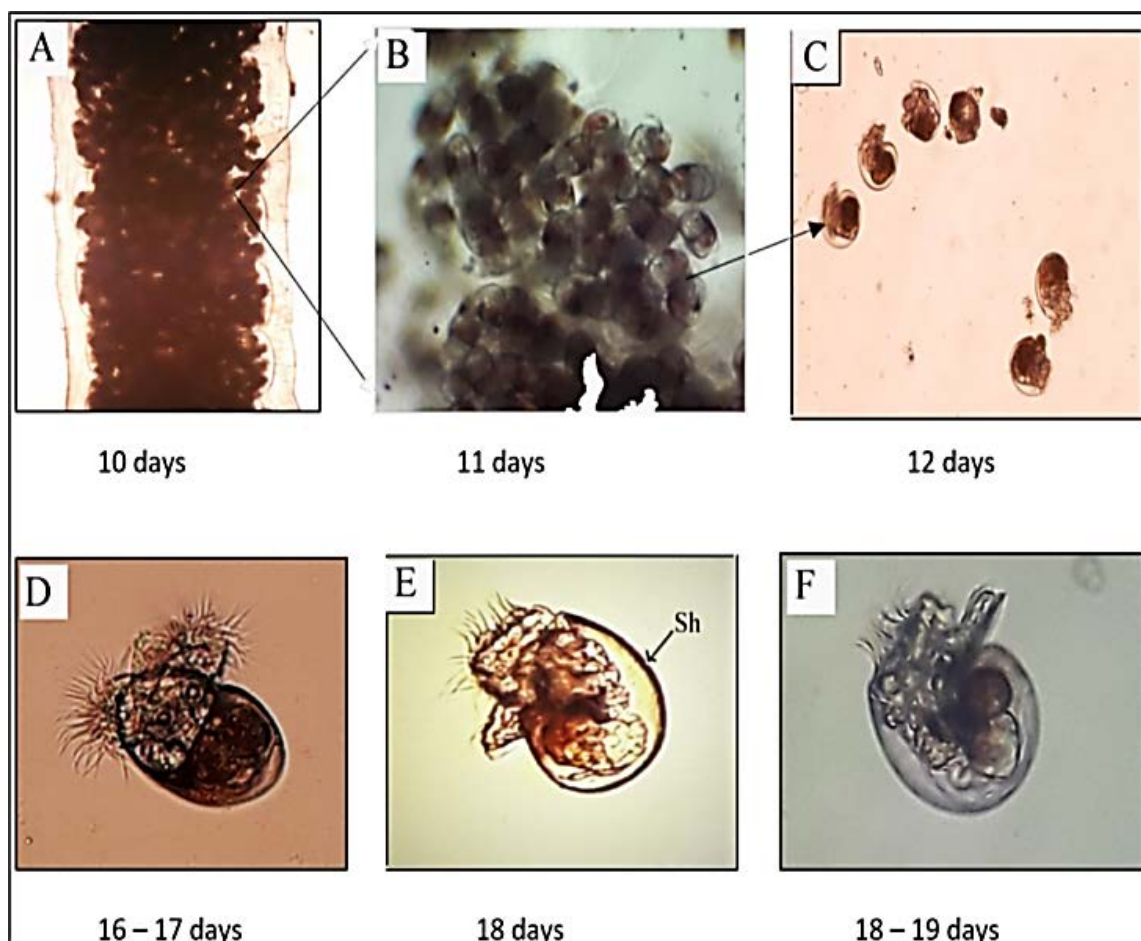
Fig. (3). Early developmental stage of *A. fasciata* (A), trochophore stage (B), veliger stage (C), and (D, E and F) hatching larva. [CI= cilia; FO= foot; JM= jelly matrix; LA= larva; PR= prevelum; SH= shell; VE= velum. Scale bars indicate 20 μm (A) and 500 μm (B to F).

DISCUSSION

The body of *A. fasciata* was black colour, and the foot are very soft and flabby and the Head and neck Wide and short. The well developed, flat, leaf-like cephalic tentacles. Tiny and distinct eyes; Foot is thin, thick, squishy, but hardens upon contraction, with well-defined edges, rounded front edges, and a short, blunt tail (Fig.1 A and B). A secretion from the ink gland that is dark purple. The first study of *A. fasciata* was made on March 12, 2022, when discovered adult animal of this species in the intertidal zone of the Tajura shore. This animal was kept in a laboratory with filtered seawater that was changed daily and was fed by green algae *U. lactuca* on a daily with continuously aerated. After a few days, spawned egg masses seen in the aquarium, *A. fasciata's*

egg mass structure and egg development were observed under a light microscope, but no eggs were developing. Although the majority of opisthobranchia, including aplysiids, are simultaneous hermaphrodites, an adult animal has both a functioning female reproductive system and a functional male reproductive system. However, it produces egg masses through internal cross fertilization during copulation (Hadfield et al., 1984). According to the direction of facing, the varieties of copulation in opisthobranchia are either unilateral or reciprocal. In reciprocal copulation, two animals face one other in opposing directions; insert their penises into one another's common genital apertures, and exchange sperm (Eyster, 1986; Kandel, 1979; Lalli & Conover, 1973; Yusa, 1996), *Aplysia* spp. is the principal species that participate in unilat-

eral copulation. If there are more than two individuals present, a coupling chain is frequently created (Blankenship et al., 1983). This study *A. fasciata* participated in unilateral copulation with chain creation as part of it is mating behaviour. In chain copulation, the first animal assumed the role of a female while the second animal served as a sperm donor male to a female.

The characteristic of egg mass at spawning, the fertilized eggs were spherical and had a diameter of around 80 µm (Fig.1 F). The eggs embedded in jelly capsules that formed a cylindrical string (Fig.2 A and B). Each capsule carried 18 to 25 eggs, and there were 60 capsules per centimetre (Fig.2 B and C). The egg masses were a bright yellow colour after spawning (Fig.3 A). The colour of this egg mass remained the same until embryos development to trochophore stage (Fig.3 B). Many marine invertebrates commonly encapsulate their eggs within benthic egg capsules. Different opisthobranch has different egg masses structures and compositions. The Aplysiid's fertilized eggs are contained in capsules that are placed between layers of Mucopolysaccharide jelly to create a cylindrical string known as an egg masses (Przeslawski, 2004). The shape and structure of the egg mass of different Aplysiid species are very similar, but the proportion of capsules to egg mass and the quantity of eggs in each capsule differ between them. According to (Bridges, 1975 ; Lee et al., 2014; Switzer-Dunlap & Hadfield, 1977). There is an inverse link between size and egg count per capsule, however for many species with smaller eggs, the relationship is unclear. Additionally, the quantity of eggs per capsule increased with increasing animal body size as in *A. californica*, *A. brasiliana* (Capo et al., 2009).

At spawning, the spherical embryos and fertilized eggs had a diameter of around 80 µm (Fig.2 A). There was an irregular spiral cleavage during the cell division. The most of molluscs have been through spiral holoblastic cleavage, and egg size and temperature effect how an embryo develops. In opisthobranchia, the hatching size similarly rises with increasing embryonic time, and the egg diameter positively correlates with the size of the hatched veliger larvae (Hadfield et al., 1984). In a study of the growth of four Aplysiid species, it was shown that, at the same temperature, the embryonic periods vary between species with smaller and larger eggs, being shorter for the former and longer for the latter (Switzer-Dunlap & Hadfield, 1977). About 12 hour after spawning, the first cleavage and development of a compact 2 cell embryo occurred (Fig. 2E). The two blastomeres in the second cleavage did not split at the same time; the smaller blastomere split five hour after the development of the 2 cell embryo (Fig.2 E). After spawning, it takes around 18 hour for a compact 3 cell embryo to form (Fig.2 F). The four little blastomeres were split from the animal pole of each large blastomere during the third cleavage, which happened spirally. The first quartet was called by these little blastomeres. After spawning, it takes around 24 hour for a compact 8 cell embryo to form, and takes around 6 hour more to reach a 16 cell embryo (Fig.2 G).

A. fasciata larva produced fertilized eggs through internal fertilization through copulation (Fig.1 C and D). The mass of fertilized eggs was laid out in the shape of a string and were yellow colour (Fig. 1), but as they developed their colour changed to a brownish colour as the embryos development from the trochophore stage to the veliger stage (Fig.2 and 3). At 10th day after spawning, the colour of the egg masses changed to a brownish colour as the embryos progressed from the trochophore stage to the veliger stage. The embryo reached the trochophore stage and started rotating inside the egg capsule (Fig.3 A and B), and the trochophore larva develops to the veliger stage than broke the egg capsule and hatched as free swimming larva (Fig.3 C). They entered the veliger stage three more days later. The veliger between 12 and 15 day after hatch develop the prevelum in larva than becomes the velum at 16-17 day (Fig.3 D).

Hatching larva has shell foot stick out and the velum covered with cilia; and there is inside digestive gland surrounded by jelly matrix (Fig.3 E and F). After 20th day sadly, all larva died as veliger per phase.

Although the particular time from egg laying to hatching varies between Aplysiid species, the range does not, it is typically around 16 days. The fertilized eggs of *A. fasciata* species underwent spiral cleavage, like those of other gastropod, but with unequal cell division. Within the range of other Aplysiids, the eggs hatched after 10 days spawning (Reverol et al., 2004). Unfortunately all larvae died as veliger stage, probably as a result of the unfavourable conditions of their rearing, such as contaminated water or lack of suitable food produce, as well as their longer developmental time to adult stage, suggesting that these stages may be especially sensitive to environmental changes.

CONCLUSION

Aplysia fasciata is one of the species, it has appeared recently in western coast of Libya and that has received less research. Therefore, this research focused on the spawning behaviour, number of eggs per capsule, egg masses structure, and egg growth and development. Moreover, the results of present study showed preliminary information about the reproductive of the *A. fasciata*, but unfortunately, all larvae died as veliger stage, possibly as a result of the unfavourable conditions in which they have been grown.

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ETHICS

There are no ethical issues of this manuscript.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: Abdulfattah Mohamed designed the experiment, applied it in the laboratory, and collected the data. Najla Mohamed followed up the experiment in the laboratory, analyzed the results, and wrote the manuscript.

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Endogenous Salicylic acid Estimation in Wheat leaves treated with Salicylic acid and infected with *Alternaria triticina*

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Abstract: This study was aime to measure the accumulation of endogenous salicylic acid, as indicator for systemic acquired re-sistance of wheat plants of "Utique" variety. The plant was sprayed with salicylic acid (SA) or water as control, at the five-leaf stage, later infected with leaf blight caused by *Alternaria triticina*. Leaf samples were removed after 10, 20, and 30 days of inoculation to test their endogenous content of salicylic acid as it is the primary internal signal indicating the emergence of systemic acquired resistance in plants, by the spectrophotometer measuring. Endogenous SA values were ascending dramatically from 10 days to 20 days and maximum with 30th day significantly, while control plants exhibit lower values in all day periods, insignif-icantly. SA treatments proved reduction in disease incidence after 10 days with 54% and after 20 days with 64%, while after 30 days the reduction recorded high percent-age of 80%. In comparison between the time intervals, disease severity was clearly reduction and reached to 83% after 10 days of inoculation then decreased to 72% for both 20th and maintained its stability on the 30th day of inoculation by 72%. This study was proved the reduction of wheat leaf blight incidence and disease severity as a result of treatment by using 1Millimolar (mM) of SA was leading to accumulation of significant levels of endogenous SA as indicator for internal induced resistance in plant.

تقدير حمض الساليسيلك الداخلي المنشأ في أوراق القمح المعاملة خارجيا بمنشط الساليسيلك والمصابة

بـ *Alternaria triticina*

المستخلص : : تم رش نباتات القمح صنف "Utique" في مرحلة الخمسة ورقات بحمض الساليسيلك (1ميللي مول) أو الماء كشاهد، ثم أُجريت العدوى لاحقًا بمرض لفحة أوراق القمح المُتسبب عن *Alternaria triticina*. ازيلت عينات من الأوراق المُصابة بعد 10 و 20 و 30 يوماً من العدوى بلقاح الفطر لتقدير محتواها الداخلي من حمض الساليسيليك باعتباره الإشارة الداخلية الأولية التي تدل على نشوء المُقاومة الجهازيـة المُكتسبة في النباتات، وذلك عن طريق قياس امتصاص الأشعة فوق البنفسجية عند 540 نانومتر بواسطة المطياف الضوئي Spectrophotometer. سجلت النتائج تزايد قيم SA الداخلية المنشأ بشكل ملحوظ بعد 10 أيام إلى 20 يوم حتى بلغت الحد الأقصى في اليوم الـ30 من العدوى، بينما أظهرت نباتات الشاهد قيمًا أقل في جميع فترات القياس. أثبتت المعاملة بـ SA انخفاض في معدل ظهور المرض بعد 10 أيام بنسبة 54٪ وبعد 20 يومًا 64٪ ، في حين سجل الانخفاض نسبة عالية بلغت 80٪ بعد 30 يومًا من العدوى بلقاح الفطر ، وعند قياس شدة المرض في نفس الفترات الزمنية اتضح أن شدة المرض انخفضت بنسبة 83٪ بعد 10 أيام من التلقيح وبنسبة 72٪ عند اليوم العشرين وحافظت على ثباتها 72% حتى اليوم الثلاثين من التلقيح أثبتت هذه الدراسة انخفاض معدل ظهور المرض وشدته نتيجة المعاملة بـ 1 ميللي مول من SA مما أدى إلى تراكم مستويات كبيرة من SA الداخلي وهو دليل استحثاث المقاومة في النبات.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops under Poaceae family grown throughout the world includ-ing Libya. It is one of the most important winter crops which are sensitive to tempera-ture. In 2019 the world production of wheat

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was reached 765 million tons, making it the second most-produced cereal after maize (1.1 billion tons) (Faostat, 2019). In Libya, the average yield and total production of wheat have been estimated at 5510 tones on average area, 5312 hectares (Faostat, 2019). It is being a favorite food for millions of people which contains large amounts of carbohydrates and protein. There are several restrictions limiting the potential yield of wheat. Among them, foliar blight has recently emerged as a major concern throughout the world (Dubin & Ginkel, 1991).

The disease initially appears as small and irregularly scattered chlorotic lesions on the leaves in late December. As the disease advances, several spots are merge and cover the whole or part of the leaf showing a blighted appearance. Heavily infected fields show a burnt appearance (Kakraliya et al., 2017). *Alternaria triticina* is the causal agent of wheat leaf blight, and a small number of related cereals (Prasada & Prabhu, 1962). Infection occurs through seed-borne transmission, planting into infected soil, or from infested crop residues, where rain splash or leaves direct contact with the soil leads to infection. *A. triticina* is unable to infect young wheat seedlings under four weeks of age, with symptoms not seen until plants were reached seven weeks of age. Susceptibility was increased with plant age (Murray, 2009).

Following inoculation with *A. triticina*, phenol content increased in resistant leaves and decreased in susceptible ones. Free amino acids, especially those involved in aromatic metabolism, are raised markedly in resistant cultivars. In resistant cultivars, disease progress stopped after 5 days and changes in phenol and nitrogen content persisted for up to 10 days. Phenolic compounds accumulated more rapidly in resistant cultivars than in susceptible cultivars as a result of infection (Chalkley, 2012).

Systemic acquired resistance (SAR) is an inducible form of plant defense that confers broad-spectrum immunity to secondary infections beyond the initial infection site (Wang et al., 2018; Yasuda, 2007) summarized that in incompatible interactions between plants and pathogenic microorganisms, plants recognize the virulence gene products of individual pathogens using specific receptors, the R gene products. This interaction causes, at the infection site, a burst of reactive oxygen species (ROS), the rapid induction of a hypersensitive response (HR) involving controlled cell death, and the expression of pathogenesis-related (PR) genes. Following these events in the infected leaves, the uninoculated leaves exhibit an increased level of PR gene expression and usually develop long-lasting enhanced resistance to further attacks by pathogens, termed systemic acquired resistance SAR (Yasuda et al., 2008). Activation of SAR needs accumulation of the endogenous signaling molecule salicylic acid (SA). Exogenous application of SA is sufficient to trigger SAR and the concurrent induction of defense-associated genes (van den Burg & Takken, 2009). SAR can be induced by either pathogen infection or treatment with salicylic acid (SA) or its functional analogs 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH), which is associated with transcriptional activation of pathogenesis-related (PR) genes (Fu & Dong, 2013).

(Malamy et al., 1990) reported that the first evidence that SA is an endogenous signal molecule in plant defense came from studies with pathogen-challenged tobacco and cucumber plants. The detection of SA levels were increased in systemic leaves and in the phloem led many researchers to believe that SA might be a systemic signal for SAR (Ghanbari et al., 2015). Because *A. triticina*, is a seed-borne pathogen, and causes major damage to susceptible wheat varieties under wet or dry conditions. It is necessary to rely on stimulating systemic plant resistance through treatment with chemical inducers,

so this study was conducted to measure the accumulation of endogenous salicylic acid, as indicator for systemic acquired resistance.

MATERIALS AND METHODS

Wheat variety source: Seeds of wheat variety "Utique" F1 were obtained from Central Mutual Company of Seeds (Cosem), Manouba – Tunisia.

Experimental design: The experiment was conducted in complete randomized block design, by planting two basins with an area of 60 × 30 cm, at a rate of 300 wheat seeds in each basin, and irrigated by flooding method. The irrigation was continued once every five days.

Exogenous SA preparation and spray: Salicylic acid (SA) Sigma-Aldrich chemie-France was purchased from Sigma Co. branch, Cairo. The shoot system at the five-leaf stage plants in the first block was sprayed with Salicylic acid (1mM), while the other block was sprayed with sterile water.

Fungal isolate: *Alternaria triticina* isolate was obtained from fresh infection of wild wheat plants grown near the research station of the faculty of Agriculture farm. Isolation was carried out on fresh wheat leaves, showing early blight symptoms, carefully washed in running tap water, then surface sterilized with 10% sodium hypochlorite. The infected parts were cut into small pieces by using a sterilized scalpel at the zone of infection, then washed several times in sterile distilled water and dried between two sterilized filter papers, and transferred to the surface of Potato Dextrose Agar (PDA) medium in Petri dishes and incubated at 25 °C for 4-7 days to enhancing sporulation. Wheat plants of Utique variety (60 days old) which treated with SA or H_2O before, were inoculated with fungal spore suspension. A modified technique of (Fritz, 2005) was applied through this experiment. Fungal spores of 10 day-old cultures were harvested by gentle brushing to separate the spores from the mycelium. The spore suspension was quantified using a haemocytometer to 10^4 spores/ml. Plant leaves were inoculated by spraying the spore suspension until run-off. To ensure good spore germination, the plants were covered with transparent plastic bags for 24 hours to increase the relative humidity.

Plant extraction: Plant extraction was done according to the modified methodology of (Warrier et al., 2013). Leaf samples of wheat were cutting and grounded in 70% ethanol solvent to ensure the solubility of SA from tissues in the presence of interfering substances. Samples were mixed well in the solvent followed by centrifugation at 10,000g for 10 min. The supernatant was kept in ice for SA measurement.

Calibration curve of SA: The free phenolic hydroxyl group present in salicylic acid reacts with ferric chloride and forms a violet-colored mixture i.e., ferric salicylate which is proportional to the concentration of salicylic acid. The calibration curve of SA was constructed according to the method of (Venkataswamy, 2018). Ferric chloride reagent is prepared by adding 1 gm of $FeCl_3$ to 100 ml of 1% hydrochloric acid (HCL). Stock solution 1: Stock solution of salicylic acid (1mg/ml) is prepared by dissolving 100 mg of salicylic acid in a few ml of methanol and completed to 100 ml with distilled water in a volumetric flask. Stock solution 2: 10 ml of this stock solution 1 is diluted with 100 ml distilled water to get 100 µg/ml of salicylic acid solutions. Dilutions: The respective samples (1ml, 2ml, 3ml, 4ml, 5ml, and 6ml) each transferred in a test tube, reagent and distilled water were added to make a total volume of 10 ml to produce 10µg/ml, 20µg/ml, 30µg/ml, 40 µg/ml, 50µg/ml, and 60µg/ml.

Endogenous SA measurement by spectrophotometer: Measuring the absorbance of the prepared samples (violet-colored complex) was done by UV-Visible spectrophotometer

(Jenway-Model 6305) at a wavelength of 540 nm against blank sample (without salicylic acid). Using Microsoft Excel software, plotting a graph with the absorbance on Y-axis and concentration on X-axis, results in an equation formatted as follows: y= 0.0298x + 0.3466, where solving for x determines the SA concentration of the sample.

Disease incidence and severity: Disease incidence (I = Σ x/N) was the proportion of diseased plants, which consisted of the number of diseased plants (x) divided by the total number evaluated (N). Disease severity (S) was estimated by the equation S = Σ (xi × ni)/N, in which xi represented disease grade (0 = free of infection, 1= trace - 25% leaf area spotted, 2= 26-50%, 3= 51-75%, 4= 76-100% leaf areas killed; ni, represented the number of diseased plants on the grades of disease scale and N was the total number of diseased plants evaluated (Cardoso et al., 1998)**.**

Statistical analysis: Data obtained were subjected to ANOVA and statistically analyzed (Gomez & Gomez, 1984) SA absorbance values were compared by Tukey HSD test, while disease incidence and severity were compared by Least Significant Difference (LSD) test at a confidence level of 95%. The package used for analysis was NCSS version 2021.

RESULTS

To detect the initiation of signaling pathway and plant response, a fast, sensitive method is required for determination of SA in the plant at different times. For the determination of endogenous salicylic acid, the property of its violet color production when bound to ferric chloride facilitates its detection by spectrophotometer.

In the present work, wheat plants infected with *Alternaria* leaf blight pre-treated with salicylic acid spray or water as control. Leaf samples were removed after 10, 20, and 30 days of *Alternaria* inoculation to test their endogenous content of salicylic acid by

measuring the absorbance of UV light at 540 nm by spectrophotometer. Table (1) showed the increase of endogenous SA absorbance values by spectrophotometer over the days and more in the case of treatment with salicylic acid than distilled water (control). SA application compared to distilled water proved its success in recording high levels in endogenous SA after 10, 20, and 30 days of inoculation, (37.45, 45.31, 52.64) and were differs significantly. Therefore a spray of wheat leaves with H_2O (control) exhibit lower values (16.34, 16.62, 18.26) in all day's periods, respectively without significant differences between time intervals.

Table: (1). Colorimetric absorbance (540 nm) of endogenous SA of wheat leaves treated with SA or H_2O (control) after three time intervals (10, 20, and 30 days) of inoculation.

Treatments	Days after treatment		
	10	20	30
SA	37.45[*a]	45.31[* b]	52.64[*c]
H_2O (control)	16.34[**d]	16.62[**d]	18.26[**d]

According to Tukey HSD test (α=0.05): (*), (**) indicate significance in the same column, while rows with the same letters don't differ significantly.

As shown in Table 2, disease incidence values recorded at three periods; 10, 20, and 30 days of inoculation for wheat treated with exogenous salicylic acid indicated that *Alternaria* leaf blight disease was significantly decreased, it showed a low value of 4.19 after 10 days of inoculation, this value increased significantly after 20 days of inoculation (7.76), It has continued to increase significantly until the 30th day by 10.62. On the other hand, the use of water instead of salicylic acid showed high values of disease incidence with highest significant differences in all time periods during the experiment (9.05 after 10 days, 21.84 after 20 days and 54.15 after 30 days). Disease incidence reduction was 54% after 10 days, followed by 64%, after 20 days and 80% after 30 days.

Table: (2). Leaf blight disease incidence at three time intervals (10, 20 , and 30 days) of inoculation in treated

plants with exogenous SA compared to plants treated with H_2O (control).

Treatments	Disease Incidence			LSD (α=0.05)
	10	20	30	
SA	4.19[a]	7.76[c]	10.62[e]	2.81
H_2O (control)	9.05[b]	21.84[d]	54.15[f]	9.99
LSD (α=0.05)	1.5	7.37	10.27	

 Rows and columns with the same letters don't differ significantly, according to LSD test (α=0.05).

Disease severity values listed in Table (3) indicated the decrease trend in disease severity for the plants were treated with a concentration of 1 Mm of salicylic acid compared to untreated plants with statistically significant differences. It is evident from Table (3) that the disease severity after 10 days recorded a low value of 2.43 and increased after 20 days of inoculation to 5.86 and then to 12.73 on day 30th of *Alternaria* inoculation with a significant difference between them. On the other hand, untreated plants (control) exhibited high values of disease severity in all periods, where it recorded 14.34, 21.13, and 46.04 in the day periods, respectively, with significant differences between them, except 10th and 20th day. In comparison between the time intervals, it is clear the severity reduced by 83% after 10 days of inoculation followed decrease to 72% for both the 20th and 30th days of inoculation compared to control.

Table: (3). Leaf blight disease severity at three time intervals (10, 20 , and 30 days) of inoculation, in treated plants with exogenous SA compared to plants treated with H_2O (control).

Treatments	Disease Severity			LSD (α=0.05)
	10	20	30	
SA	2.43[a]	5.86[b]	12.73[c]	3.06
H_2O (control)	14.34[d]	21.13[d]	46.04[e]	10.42
LSD (α=0.05)	5.17	9.3	8.12	

 Rows or columns with the same letters don't differs significantly according to LSD test (α=0.05).

DISCUSSION

In plants, the positive correlation between endogenous levels of SA and resistance responses against biotrophic and hemibiotrophic pathogens is well established (Glazebrook, 2005). In this study, the applied of exogenous SA was suitable at concentration (1mM) as a plant activator to induce internal systemic resistance in wheat plants against leaf blight disease by enhancing significant levels of endogenous SA, which plays a key role in the emergence of systemic acquired resistance. (Vallad & Goodman, 2004) explained that exogenous application of SA on plants due to induction of endogenous SA accumulation causes SAR genes activation which leads to resistance against different kinds of pathogens. Exogenous application of SA or one of its active analogs is acceptable to induce plant defense against biotrophic and semibiotrophic pathogens (Koo et al., 2020; Lu, 2009; Vallad & Goodman, 2004) endorsed the use of SA at a concentration of 2 mM, stating that it was not only induces enhanced disease resistance but also has adverse effects on plant growth and productivity, which caused by imbalance between cost and benefit of limited energy that plant can use.

In this study a simple technique to detect endogenous SA in plant extraction by using the colorimetric method with a spectrophotometer was used, although there are other advanced techniques used in SA estimation such as HPLC, GC-MS; this was similar to (Warrier et al., 2013) who used the spectrophotometric method in comparison with the well-known sophisticated methods like HPLC, GC, MS; these extraction procedures are very cumbersome and time-consuming, the spectrophotometric method is simple, fast, reliable and accurate. Accumulation of endogenous SA level, throughout the present work, was associated with significant activation of systemic resistance against *A. triticina* in wheat plants grown under experimental conditions. SA was known to be an important signal molecule and its level may increase endogenously prior to the activation of SAR in each of the host-pathogen interactions (Malamy et al., 1990;

Métraux et al., 1990) Other authors concluded that endogenous levels of methyl salicylic acid (MeSA) increase in plants resisting pathogen infection (Tripathi et al., 2010; van den Burg & Takken, 2009). SA has been found to activate through a redox mechanism (Hadi & Balali, 2010). According to (Sticher et al., 1997), the SA signaling pathway can be triggered by exogenous SA, which improves disease resistance, because this pathway is related to systemic acquired resistance (SAR), which can emerge when endogenous SA accumulation and is triggered after plant pathogen infection.

In this experiment, after application of SA in wheat infected with *A. triticina* showed that an increase in the endogenous SA levels, the results agreement with (Gholamnezhad et al., 2016), who recorded increase in the endogenous SA levels which led to oxidation of phenolic compounds, that may limit the fungal growth. (Sahu & Sabat, 2011), after applying the SA in wheat plants, they found increased roots, superoxide dismutase (SOD) and malondialdehyde (MDA) activity, these enzymes protect the cell from oxidative stress

CONCLUSION

Our study proved the reduction wheat leaf blight incidence and severity as a result of treatment with 1mM of SA which leading to accumulation of significant levels of endogenous SA in which, it is the key role in initiation of systemic acquired resistance. It recommend to further investigations to determine the induced compounds responsible of resistance and other induced structures which, prevents the disease progression in the plant, and due to the lack of appropriate facilities, we were unable to make those estimates in our study.

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ETHICS

Authors should address any ethical issues that may arise after the publication of this manuscript.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: The first author did the practical experiments and wrote the results, while the second author did the chemical analyzes and calculations. Both authors contributed to the final version of the manuscript.

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Changes in Hematological Parameters of Common Carp (*Cyprinus carpio*) Fingerlings Fed on Pomegranate (*Punica granatum*) Peel Supplement

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Abstract: The present study aimed to determine the effect on different blood parameters (WBCs, RBC, HGB, HCT, MCV, MCH, and MCHC) in fingerlings of common carp (*Cyprinus carpio*) weighing 13.5 ± 1 g with pomegranate peels (*Punica granatum*) in their different forms, raw (PPR), alcoholic (PPA) and water (PPW), which were added to their feed as 0.5% and 1% respectively, in addition to the control treatment (21 replicates). A commercial diet was used containing 35% crude protein, 6% lipid, 12% Ash and 50 TVN for ten weeks. At the end of the experiment, blood was drawn from the heart of the fish, and analyses were performed. The results showed that RBCs, HGB and HCT improved significantly ($P< 0.05$), while there were no significant differences ($P> 0.05$) in WBCs, MCH, and MCHC. In short, we recommend adding pomegranate peels or extracts (alcoholic or aqueous) to common carp food at a rate not exceeding 1%.

التغيــرات فــي مصــل دم إصـبعيات أسـماك الكـارب الشــائع (*Cyprinus carpio*) المغـذاة علـى قشـور الرمـان (*Punica granatum*)

الكلمـــات المفتاحيـــة :
قشور الرمان؛ خلايا الدم البــيض؛ خلايــا الــدم الحمر؛ الكارب الشائع.

المستخلص : تهدف الدراسة الحالية إلى تحديد تأثر معاملات الدم المختلفة (WBC, RBC ،HGB، HCT، MCV، MCH و MCHC) في إصبعيات الكارب الشائع (*Cyprinus carpio*) التي تزن 13.5 ± 1 غم بقشور الرمان (*Punica granatum*). حيث تمت إضافته مسحوقا خاما (PPR) ومستخلصا كحوليا (PPA) ومستخلصا مائيا (PPW) بنسب 0.5 %، و 1 % على التوالي بالإضافة إلى معاملة السيطرة (21 مكرر). استخدمت عليقة تجارية تحتوي على 35٪ بروتين خام، و6٪ دهون، و12٪ رماد، و50 TVN لمدة 70 يومًا. في نهاية التجربة، تم سحب الدم من القلب، وإجراء التحليلات. أظهرت النتائج أن RBCs، HGB، وHCT تحسنت بشكل ملحوظ (P<0.05)، بينما لم تكن هناك فروق معنوية (P>0.05) في WBCs، HCT، MCH، وMCHC. باختصار، نوصي بإضافة قشور الرمان، أو أحد مستخلصاته إلى عليقة الكارب الشائع بمعدل لا يتجاوز 1٪.

INTRODUCTION

In the intensive aquaculture industry, several environmental stressors influence the fish species. Fish have stress reactions when exposed to harmful stimuli such as temperature fluctuations, pH variations, decreases in oxygen levels, increases in ammonia levels, handling, transport, and osmotic changes in water (Pickering, 1993; Everly and Lating, 2013). The fish individual's integrated stress response, which consists of behavioral, neurological, hormonal, and physiological components, can alter the fish's health status and diminish its resistance to diseases and stress. It

also takes some time for the fish to get back to normal. (Lebelo et al., 2001; Suljević et al. 2016). Additionally, antibiotics are frequently used to treat fish infections, despite having adverse side effects. As a result, scientists have looked for cheaper, safer, and more efficient natural alternatives, such as herbs, vegetables, and other edible plants, to employ as growth stimulants or immunostimulants (Badrey et al., 2019). Pomegranate (*Punica granatum*) peel products have higher antioxidant levels than pomegranate juice, making them attractive candidates as a nutritional supplement for animal feed (Badawi and Gomaa 2016). However, Türkyılmaz et al., (2013) reported pomegranate peel is also a good source of flavonoids, phenolic acids, and tannins (ellagitannins as punicalin, punicalagin, gallic acid, and ellagic acid). Therefore, pomegranate peel's beneficial components make it a potential candidate for the discovery of new natural agents with a variety of biological functions, antibacterial activity, and potential health benefits (Kaderides et al., 2015). Pomegranate peel extract has been extensively studied, in particular, for its potent anti-microbial effect, high anti-oxidant activity, cytotoxic effect, hypoglycemic effect (Rajput et al., 2011) hypolipidemic effect (Belal et al., 2009), hepatoprotective effect, and anti-inflammatory activity (Ibrahim, 2010; Jurenka, 2008).

The hematological variables can be used as an indicator for assessing the health conditions of aquatic organisms. However, this can be very difficult due to many endogenous and exogenous factors. According to Fernandes and Mazon (2003), the blood traits of fish are closely related to environmental and biological factors. Physiological changes can be a result of stress, and several blood characteristics are used to determine these conditions. Adams et al., (1996) stressed the importance of using hematology as a tool to track changes in fish diet, water quality, and disease while also monitoring their biological state. Since fish are closely entwined with their environment, they are subject to physical and chemical changes that may be reflected in the components of blood cells. Other factors, including behavior, environment, and climate, can also affect blood values (Fazio, 2019). Therefore, the aim of the present study was to investigate how supplementing pomegranate peel to the diet of common carp (*Cyprinus carpio*) influenced hematological parameters.

MATERIALS AND METHODS

Preparation of pomegranate peels extract
Water extract (PPW): The aqueous extract was created by mixing 25 g of pomegranate peel powder with 250 ml of distilled water (1:10), shaking the mixture for 30 minutes at a speed of 150 cycles per minute, and letting it soak for 24 hours in the refrigerator, as described by Handa et al. (2008). A concentrated extract was then obtained at the bottom of the drying vessel after the mixture had been filtered through several layers of gauze to remove the insoluble plant matter, and again using filter papers (Whatmann No. 2). The filter was then removed and dried in an electric oven at 40 C° until it was no longer visible. The extract was then put in clean, opaque glass bottles and kept in the refrigerator at 4 C° until use. The process was then repeated using the same steps and conditions until a sufficient amount of extract was obtained.

Alcoholic extract (PPA): The method of Gülçin et al., (2003) was used in the preparation, as 25 gm of pomegranate peel powder was mixed with 250 ml of ethanol alcohol with a concentration of 96%. The mixture was stirred for 24 hours on a magnetic stirrer and then filtered through gauze two successive times, then using filter paper (Whatmann No.1). The filtrate was then concentrated using the rotary evaporator and then dried in the electric oven at a temperature of 40 C° and placed in sealed opaque bottles and kept in the refrigerator until use. The process was repeated by following the same steps and conditions until a sufficient amount was reached from the extract.

Experimental fish: The fingerlings of common carp used in the experiments were obtained from the aquaculture station of the College of Agriculture. Fish that were highly stressed or oversized were excluded. The fish were placed in a bowl of water and individually weighed to the nearest 13.5±1 g on an electronic scale. The fish were divided into seven treatments, each treatment had three replicates (five fish). The fish were allowed to acclimate to the laboratory conditions for two weeks before the start of the experiment. 60 × 40 × 50 cm aquaria (experimental units) were used where fish were distributed.

Fish diets and feeding regime: An Iranian commercial diet of known chemical composition was used, as shown in Table (1). Seven experimental diets were supplemented by pomegranate peel powder (PP) at (0.5% and 1%) and its aqueous and alcohol extracts (0.5% and 1%), as well as the control diet (C). The fish were fed diets two times daily (9 am and 4 pm) at a rate of 2 % of body weight for ten weeks. Water quality parameters measured during the trial period (pH = 8.78, EC = 2.71 ds\cm, DO= 9.43 ppm, Temp = 24.34 C°, Sal. = 1.30 psu).

Table (1). The proximate chemical composition of the commercial diet used in the experiment

Nutrition	Amount
Moisture (Max)	10
Crude protein %	35
Ash (Max)	12
TVN (Max) mg/100g	50
Metabolizable Energy (Kcal/kg)	3700
Crude Fiber (Max)%	5.5
Crude Fat %	6
TVN (Max) mg/100g	50
Lysine %	1.8
Methionine %	0.48
Threonine %	1.15

Composition: Wheat Flour, Barley, Corn Gluten, Vegetable Meal, Fish Meal, Yeast, Fish Oil, Vegetable Oil, Choline Chloride, Lysine, Methionine, Threonine, Vitamin Premix, Special Mineral Premix, Anti-Oxidant, Inositol.

Hematological analysis: In test tubes containing an anticoagulant, blood samples from the heart were collected using a 2-ml glass sy-ringe. The Mindrary- BC-30S hematology analyzer was used to measure the hemoglobin concentration (HGB), hemocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cells (RBC) and white blood cells (WBC).

Statistical analysis: Data were presented as mean ±SD. The results were subjected to a one-way analysis of variance (ANOVA) to test the effect of treatment inclusion on fish performance. Data were analyzed using IBM SPSS (2013) program, Version 22. Differences between means were compared using LSD's multiple range tests at P< 0.05 level.

RESULTS AND DISCUSSION

Figure (1) of the results shows the values for RBCs, WBCs, MCV, and HGB. The inclusion of PP and PPE has been shown to improve blood hematological parameters. Except for PP1, the increase in RBC coefficients was not significant (P>0.05) when compared with the control (C) treatments. In terms of WBCs, no significant differences (P>0.05) were found between any of the treatments. There were no significant differences (P>0.05) in MCV. Both the PPA1 % and the water extract treatments enhanced HGB.

In aquaculture, hematology is gaining importance because of the ease and reliability of monitoring the health status of cultured species (Hrubec et al., 2000). WBCs are circulatory cells that function as a cellular defense, aid in acquired and inherent immunological responses, and are correlated with BMI. The thymus, spleen, and kidneys are additional organs where WBCs are made (Esmaeili, 2021). The findings revealed that the WBCs values in the blood of carp fish did not differ significantly (P>0.05). The active components in the pomegranate peel may have decreased free radicals compared to the control group, which may have decreased the vulnerability to stress brought on by the buildup of free

radicals. Fish subjected to stress will have decreased blood WBC counts, especially lymphocytes (Esmaeili, 2021). Additionally, no bacterial infections were present in the fish used in the experiment. According to Hrubec et al., (2000) exposure to low-quality water with a high bacterial load affects the WBCs count and tends to lower the number of RBCs. The present findings are consistent with the study of Shafiei et al., (2016) who used PPE in the diets of common carp fingerlings. This result agrees with the findings of Badrey et al., (2019), which indicated that the increase in WBC in the blood of monosex ti-

lapia (*Oreochromis niloticus*) fed on PPR is a result of a possible immune modification in its body. The results of Sönmez et al., (2022) on rainbow trout (*Oncorhynchus mykiss*), Avazeh et al., (2021) indicated an increase in WBC with increased levels of PPR in rainbow trout (*O. mykiss*) diets, which indicated the immunomodulatory effect of PPR, while the results of Harikrishnan et al., (2012) found that the olive flounder (*Paralichythys olivaceus*) had increased resistance against *Philasterides dicentrarchi* due to the increase in WBCs after feeding with pomegranate peels.
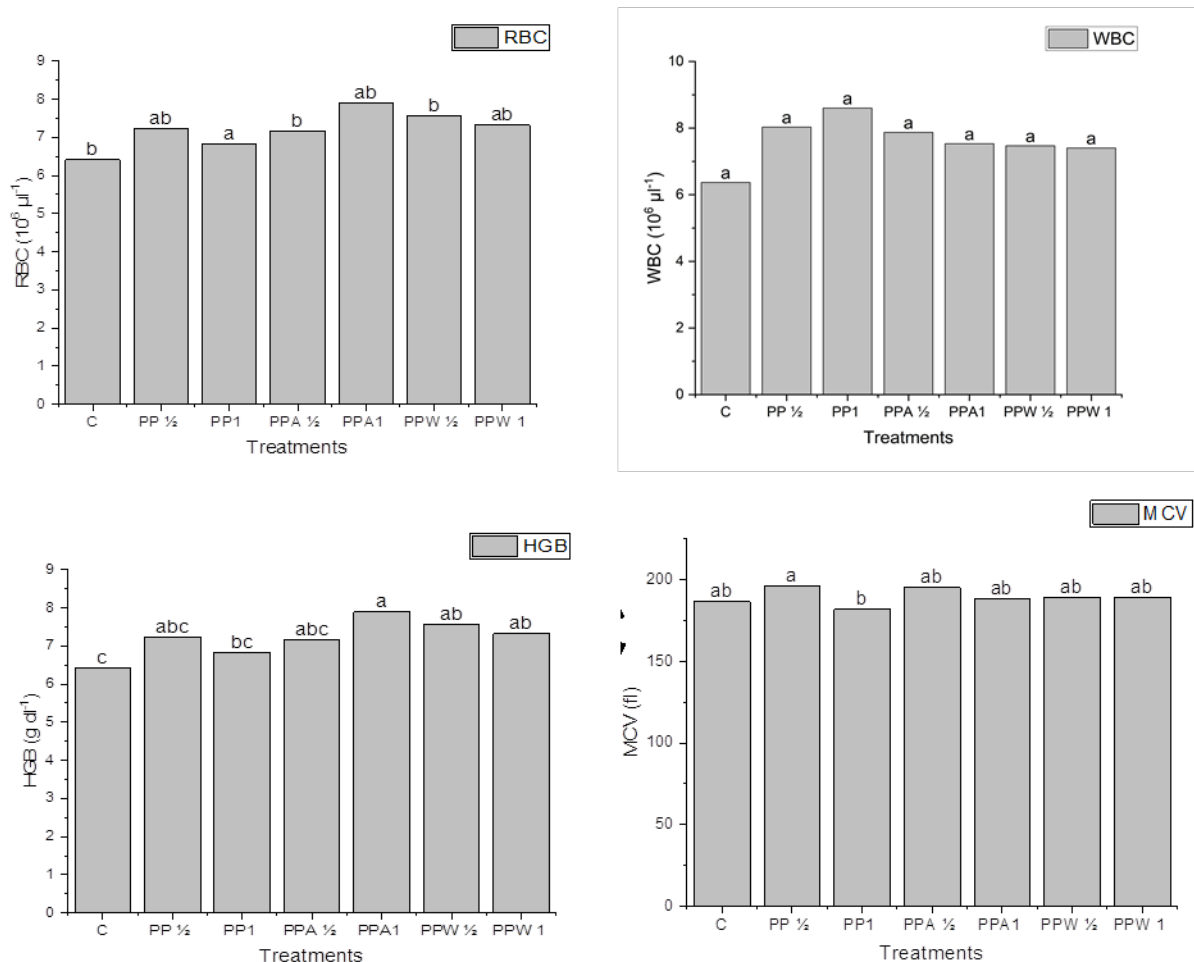
Figure: (1). Changes in the white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB) and mean corpuscular volume (MCV) in treatments fed supplementation with (PPR) and (PPE) pomegranate diets. Data are represented as mean±SD. (n = 3, in replicate). The **ab** Mean in the same columns with different superscripts are significantly different at (P<0.05).

RBCs, HGB, and HCT are measures of oxygen-carrying capacity and general health. PVC, RBCs, and HGB levels in the blood are absolutely necessary for MCV, MCH, and MCHC to function (Tavares-Dias 2006; Singh et al., 2020). The absorption of iron, that the body needs, due to the compounds in pomegranate peels, or the effect of antioxidants in the peels that can reduce the dissolution of blood cells due to hydrogen peroxide H_2O_2 caused by oxidative stress in RBCs, could be the reasons why HGB was improved by the aqueous extract treatments in addition to PPA at 1% (Ma et al., 2000). The polyphenolic compounds found in plant components have the capacity to form complexes with metal ions like iron and obstruct physiological processes involving iron and other minerals (Satyakeerthy, 1999). When several polyphenolic compounds interact with

the cell membrane's surface, they can act as a barrier against soluble free radicals (Shafiei et al., 2016).

According to Gallaugher et al., (1992), MCV and MCHC are indicators of RBCs size. The present results showed no significant (P>0.05) differences in the values between the treatments. Therefore, it appears that the concentration of pomegranate peels had an impact on that; when it was increased in the ration, it decreased MCV and MCHC. Pomegranate peels contain tannic acid, which lowers the bioavailability of iron and results in a drop in RBCs. According to Lee et al., (2010), consuming 125, 250, 500, and 1000 TA/kg of tannic acid lowers iron levels in the blood. As a result, RBCs, HGB, and HCT counts will fall. Tannin levels also lower RBCs, HGB, and PCV quantities (Delimont et al., 2017).
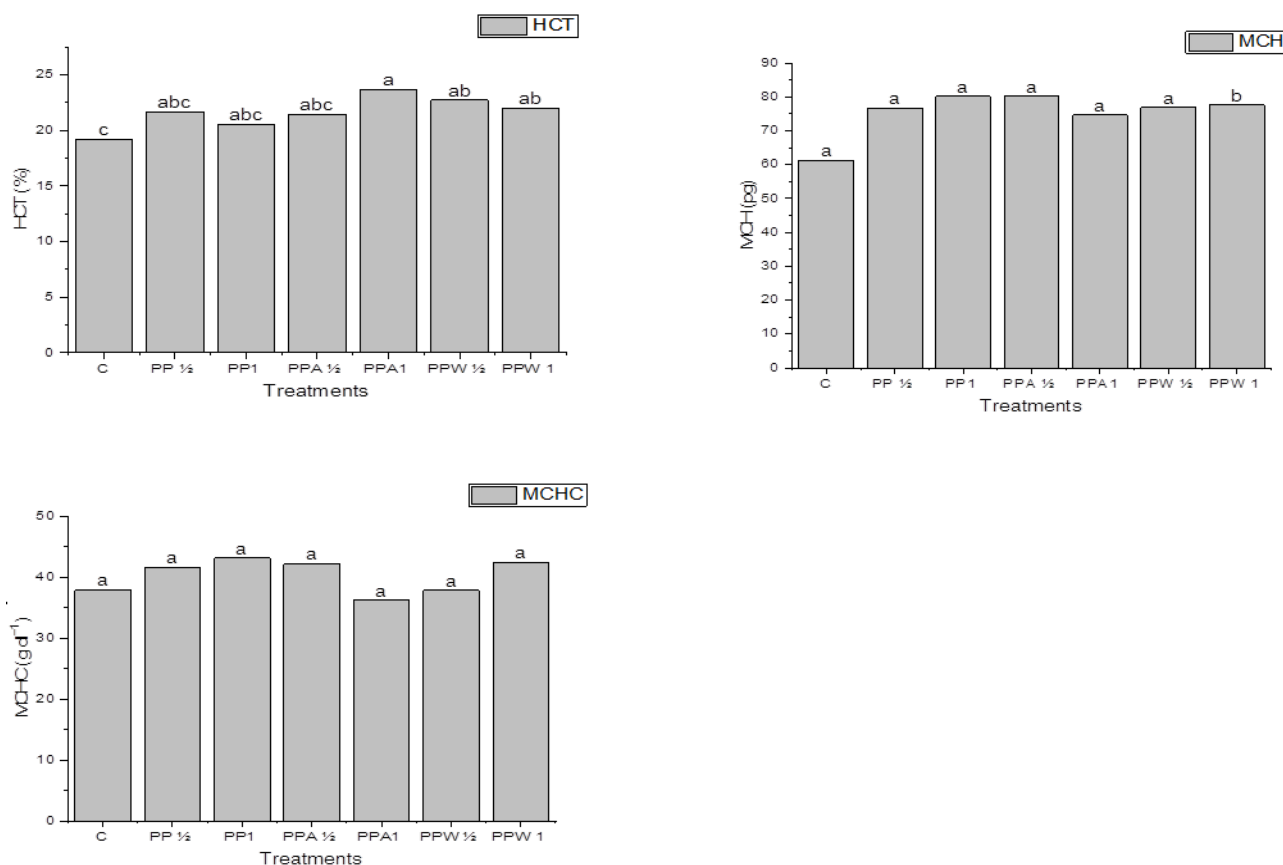

Figure: (2). Changes in the hematocrit (HCT), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in treatments fed supplementation with (PPR) and (PPE) pomegranate diets. Data are represented as mean±SD. (n = 3, in replicate). See Figure. (1) for statistical information.

CONCLUSION

The present study showed that adding pomegranate peels, whether raw or extracts (alcoholic or aqueous), improved hematological parameters of common carp without exceeding 1%. In addition, more studies should be conducted to verify the ideal ratios in fish diets to improve immunity and health of fish cultured using plant additives rather than resorting to antibiotics.

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ETHICS

The authors declare that they have no conflicts of interest associated with this manuscript.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

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Soil Science Education: Adaptation of Soil Judging (Evaluation) to Libya

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Abstract: Soil judging is a field area of soil science, which is a diverse discipline for soil science education that combines geology, physics, chemistry, and biology to improve the understanding and protection of natural resources. Adaptation of Soil Judging to Libya involves tailoring Soil Judging materials to the country's local context. The objectives of this study were to adapt Soil Judging to Libya and evaluate it in various locations in Libya. Different soil judging handbooks from the United States (US) were used to develop teaching materials for Libya (including tables of soil physical and chemical properties and scorecards). The soil judging scorecard was enhanced by adding more specific information relevant to Libya (e.g., soil salinity, calcium carbonate, etc.). Libyan users were asked to complete a survey on the usefulness of Soil Judging in Libya. Eighty-two percent of those surveyed were unaware of Soil Judging prior to this study. After completing Soil Judging trials in various locations in Libya, 95% of those surveyed indicated that Soil Judging is helpful in natural science education in Libya. Future improvements to Soil Judging should include better equipment and explanation.

تعليم علوم التربة: تكييف تقييم التربة (SOIL JUDGING) لليبيا

المستخلص: يعتبر تكييف تقييم التربة (Soil Judging) المكون الميداني لعلوم التربة وهو تخصص متنوع لدراسة التربة التي تجمع بين الجيولوجيا، الفيزياء، الكيمياء والبيولوجيا لزيادة فهم الموارد الطبيعية وحمايتها. إن عملية تكييف تقييم التربة لليبيا تحتاج إلى تعديل بطاقات قياس الأداء (Scorecards) المستخدمة في هذه العملية بما يتماشى مع البيئة المحلية في البلاد. هدفت هذه الدراسة إلى تكييف عملية تقييم التربة لليبيا وتقييمها في مواقع مختلفة من البلاد. تم استخدام كتيبات مختلفة لتقييم التربة من الولايات المتحدة الأمريكية لتطوير وتجهيز المواد التعليمية المستخدمة في تقييم التربة (بما في ذلك جداول الخصائص الكيميائية، والفيزيائية للتربة، وبطاقات قياس الأداء). تم تحسين بطاقات قياس الأداء المستخدمة في عملية تقييم التربة (Soil Judging) بإضافة المزيد من المعلومات المحددة ذات الصلة بليبيا (على سبيل المثال، ملوحة التربة، كربونات الكالسيوم، إلخ). طُلب من المستخدمين المحليين في ليبيا استكمال دراسة استقصائية حول فائدة تقييم التربة في ليبيا. وأظهرت النتائج أن 82٪ ممن شملهم الاستطلاع لم يكونوا على دراية بعملية تقييم التربة قبل هذه الدراسة. أضف إلى ذلك أنه بعد الانتهاء من تطبيق عملية تقييم التربة في مواقع مختلفة في ليبيا، أشار 95٪ ممن شملهم الاستطلاع إلى أن عملية تقييم التربة (Soil Judging) مفيدة في تدريس العلوم الطبيعية بشكل عام، وعلم التربة بشكل خاص في ليبيا، ويجب أن تتضمن التحسينات المستقبلية لتحكيم التربة معدات، وشرحًا أفضل.

INTRODUCTION

Soil classification is an important component in the exchange and advancement of soil knowledge worldwide. Field descriptions and laboratory analysis results are the foundation of soil classification. Soil judg-

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ing can be defined as a field area of soil science, which is a diverse discipline for soil science education that combines geology, physics, chemistry, and biology to improve the understanding and protection of natural resources. Soil judging (Evaluation) helps us better understand and protect the natural resource that sustains us all. Soil judging is a national collegiate soil judging contest in the USA held at different host institutions each year since 1961 (Post et al., 1974).

In this competition, the soil judging teams compete in contests that test their knowledge of basic soil properties, such as texture, color, and structure, and the student's ability to make interpretations based on these properties and local site characteristics. Soil Judging is used in several countries of the world (e.g., the U.S. and Germany) to train soil scientists on how to describe, classify, and interpret soil for different uses in the field. The first International Soil Judging Contest took place in June 2014 at the 20th World Congress of Soil Science in Korea, with a limited number of countries participating in it (USA, Japan, China, Korea, South Africa, Australia, Taiwan, Mexico, Hungary, and the United Kingdom). Currently, Libya does not have Soil Judging, and adaptation of soil judging to Libya can improve soil science knowledge exchange and can potentially alleviate land use problems in Libya by educating students and planners about important soil properties related to land use, such as: soil infiltration rate, hydraulic conductivity, available water, soil wetness class, and soil interpretations related to suitability for dwellings with basements, septic tank absorption field, and local roads and streets. Numerous studies documented various benefits of soil judging and field trips to enhance soil science learning in the US (Cavinder et al., 2011; Cooper & Dolan, 2003; Galbraith, 2012), and it would be beneficial to use this experience in other parts of the world. Adaptation of educational materials to other countries is a necessary process, which can be divided into the following steps: 1) identifi-

cation of a reason for adaptation of environmental materials; 2) identification of people that need to be involved; 3) identification of critical environmental issues; 4) identification of solutions to environmental problems; 5) identification, screening, and selecting environmental education materials; 6) copyright issues; 7) adapting and testing materials; 8) implementing an environmental education program; 9) evaluating a program and the effectiveness of adapted materials; 10) following principles of successful adaptation (Corps, 1999). Examples of adaptations of educational materials can range from lessons to programs (Corps, 1999).

Soils in Libya are classified according to the U.S. Soil Taxonomy (Zurqani et al., 2012). Libya has six soil orders according to the U.S. Soil Taxonomy (Entisols, Aridisols, Alfisols, Inceptisols, Vertisols, and Mollisols) and the most common soil orders are Entisols and Aridisols. Most Libyan soils have a sandy or loamy sand texture with rapid soil infiltration. (Abdelnaser et al., 2011) reported that rapid expansion of industry, urbanization, and increasing population led to dramatic increases in the amount of municipal solid waste generated in Libya. Libyan soils texture are sands and loamy sands; they have very low available water. Water stress is a common factor limiting crop yield, especially in arid and semi-arid areas where the annual average precipitation does not exceed 300 mm (Zurqani et al., 2019). Septic tanks are used in many parts of Libya, but there is a lack of appropriate wastewater management including collection and treatment facilities in the rural area, which could cause environmental pollutions.

Soil salinity problems in Libya very often result from extensive agricultural activities, lack of precipitation and overdraw of fresh groundwater to the extent of causing seawater intrusion. In addition, low amounts of rainfall and high temperatures are also contributed to soil salinity problems (Zurqani et al., 2018). Sodicity also is common in

semi‑arid areas, particularly in sites where incoming water containing dissolved salt is lost by evaporation. The objectives of this study were to adapt Soil Judging for Libya, conduct Soil Judging in various locations in Libya, and evaluate the effectiveness of adapted materials using a survey.

MATERIALS AND METHODS

Study Area: Libya is situated in the northern portion of the African continent and covers 1,759,540 million km^2 (Zurqani, 2021). Desert covers more than 95% of the country while cultivated areas cover slightly over 2% (Zurqani et al., 2019). The population is about 5,673,031 (13% is rural) (Bureau of Statistics and Census Libya, 2012). There are four administrative territories in Libya (Fig. 1). Libya has an arid and semi-arid area climate influenced by the Mediterranean climate (Xeric), characterized by rainfall in the winter and almost no rainfall in the summer, which is the major heat and drought period of the year (Zurqani, 2021). However, the southern part of Libya is under the (Torric) moisture regime (Ben-Mahmoud, 1995).
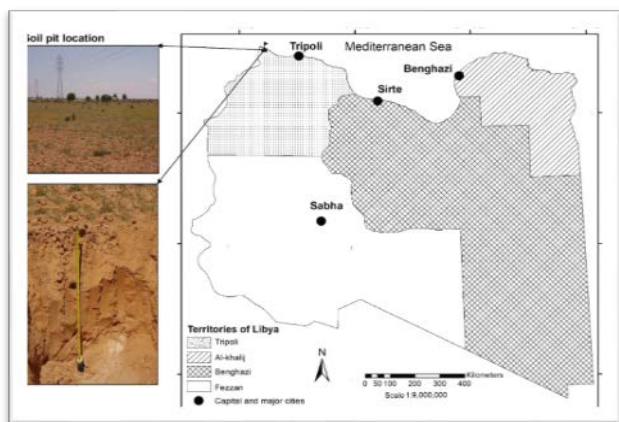
Figure (1). Map of Libya with soil pit location.

The average monthly temperatures range from 13.2 C° to 27.9 C° with an annual level of 20.7 C°, and the soil temperature regime in the study area is thermic (Ben-Mahmoud, 1995). The average annual rainfall varies from region to region according to geographic position and topography. Rainfall occurs during the winter months (October to March) (Zurqani et al.,

2012). Land degradation and desertification are the main soil threats facing agricultural development.

Soil Judging Equipment and Materials: Most Soil Judging equipment can be obtained in Libya or ordered from suppliers (Fig. 2). This set of equipment that must be provided for each student involved in a soil judging completion includes: a scorecard, official rules, an Abney level or clinometer, garden spade, bucket, clipboard, soil collection trays, water bottle, measuring tape, a calculator, a pencil, and a Mussel color chart. Soil samples to provide soil physical and chemical data for the students (Fig.3). These soil properties can be analyzed in any one of the several soil nutrient analysis laboratories in Libya: Libyan Universities Institutes, and Libyan Agriculture Research Centers.

Figure (2). Soil Judging equipment.

PIT No. 3

No. of horizons ___3

Depth to be described ___70 cm

Nail in 3rd horizon @ ___60 cm

HORIZON	OC (%)	BS (meq/L)	pH	CaCO$_3$ (%)	SAR (%)	ECe (dS/m)
1	0.19	29.21	7.1	38.51	22.04	4.00
2	0.01	3.37	7.5	36.75	15.85	0.25
3	-	-	-	-	-	-

Flooding: None   Ponding: None

Figure: (3). Soil physical and chemical properties for the soil pit No. 3 in Zuwarah, Libya (Zurqani, 2010).

COURSES BACKGROUND

A soil judging course can be incorporated in various soil science programs currently taught in Libya, for example: University of Tripoli, Omar Al-Mukhtar University, Sebha University, Al Zawia University, Sirte University, University of Elmergib, University of Al-Jabal Al-Gharbi, Higher Institute of agricultural techniques (Al-Gheiran, Tripoli). All of these institutions can use soil judging to improve the soil courses such as Fundamentals of Soil Science course or in a more specific course such as Soil Survey Genesis and Classification course. Soil judging education can directly benefit the agriculture, housing and town planning, transportation, and health services. Computer laboratories and internet services in most of Libyan universities can be used in creating Modular Object-Oriented Dynamic Learning Environment (Moodle). This environment could be used for e-learning learning (e.g. storing course materials and assessing student's learning via electronic quizzes and tests).

RESULTSAND DISCUSSION

Libya - Specific Modifications to Soil Judging Scorecard: The main soil orders in Libya are Entisols, Aridisols, Mollisols, Alfisols, Vertisols, and Inceptisols (Agriculture–, 2003; Ben-Mahmoud, 1995; Export, 1980; Zurqani et al., 2019). In general, apart from the JabalAkhdar and some of the Tripoli Mountains (JabalNafusah), the most commonly soil orders are Entisols and Aridisols (Zurqani et al., 2018). Dry climatic conditions and soil parent materials in Libya result in high accumulation of calcium carbonate, and the presence of gypsum in some areas (Zurqani et al., 2018). The precipitation and accumulation of calcium carbonate may result in the development of calcic/petrocalcic horizons that vary in the extent of their development depending on the circumstances and composition. According to Ben Mahmoud (1995) these soils generally cover large areas in the northern region of the country. In order to adapt the soil judging score-

card to Libya, the following additions/modification were made to the already existing soil judging scorecard: adding a column for testing of carbonates, plant sensitivity to salt-affected soils (Table 1), and wind erosion potential classes (Table 2).

Table (1). Plant Sensitivity to salt affected soils (Adapted from (Brady et al., 2008))

Factors affecting use	Degree of limitation			
	Slight (1) (Normal Soils)	Moderate (2) (Saline Soils)	Severe (3) (Sodic Soils)	Extreme (4) (Saline-Sodic Soils)
ECe (dS/m)	< 4	≥ 4	< 4	≥ 4
pH	< 8.5	< 8.5	≥ 8.5	< 8.5
SAR* (%)	< 13	< 13	≥ 13	≥ 13

* If you are using Exchangeable Sodium Percentage (ESP) the degree of should be 15 %

Table (2). Wind erosion potential classes. Adopted from (Blanco-Canqui & Lal, 2008)[1], (Ludwig et al., 1995)[2].

Factors influence wind Erosion relative to the surface horizon texture (Barriers[1], surface roughness[2])	Wind Erosion Potential Classes Surface horizon texture class[3]				
	L, (SiL > 20 % clay), CL, Si, SiCL,	L, (SiL< 20 % clay), SCL, SC	C, SiC, CL, (SiCL> 35 % clay)	L, SL, SiC, CL,	S, LS
- Vegetative barriers with feedlot wind-break - Very Rough Soil surface	Very low	Very low	Very low	Low	Low
- Vegetative barriers (perennial plants or annual plants combination) - Rough Soil surface	Low	Low	Low	Medium	Medium
- Strip Cropping - Medium Soil surface	Medium	Medium	Medium	High	High
- Lack of crop residue - Smooth Soil surface	High	High	High	Very high	Very high
- Bare soil - Very Smooth Soil surface	Very high	Very high	Very high	Very high	Very high

Notes: This table did not take in the consideration of the slope and the water quantity in the\ surface horizon.
Soil Texture Abbreviations: Sand = S, Sandy Loam = SL, Sandy Clay Loam = SCL, Sandy Clay = SC, Silt = Si, Silt Loam = SiL, Silty Clay Loam = SiCL, Silty Clay = SiC,, Clay = C, Clay Loam = CL, Loam = L, Sandy Clay = SC, and Loamy Sand = LS.

No. of Horizons ___3___
Depth to be described ___70___ cm
Nail in 3rd horizon @ ___60___ cm

Contestant _____

A. Morphology

Horizon						Texture			Color			Structure		Consist.	Redox. Features			CaCO3	Score
					Rock										Redox	Redox	Red.	HCl 1N (N,	
			Lower	Bound.	fragmnt	USDA	Clay								conc.	depltn.	matrix	Vsli, Sli, St,	
Pre.	Master	Sub	No.	depth	distnct.	modif.	class	content	Hue	Val.	Chr.	Grade	Shape	Moist	y/n	y/n	y/n	Vio)	
1	3	2	1	3	1	1	3	1	1	1	1	2	2	1	1	1	1	3	30
-	A	p	-	20	C	-	LS	2.2	7.5YR	5	4	SLS	MA	L	N	N	N	Vio	
-	C	-	1	50	C	-	LS/S	6	7.5YR	5	4	SLS	MA	L	N	N	N	Vio	
-	C	-	2	70+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

B. Soil Profile and Interpretations

Infiltration Rate (5)
___ Rapid
_X_ Medium
___ Slow

Available Water (5)
_X_ Very Low   ≤ 7.5 cm
___ Low   > 7.5 and ≤ 15.0 cm
___ Moderate   > 15.0 and ≤ 22.5 cm
___ High   > 22.5 cm

Soil Interpretations (2 each)
_2_ Dwellings with Basements
_3_ Septic Tank Absorption Field
_2_ Local Roads and Streets
_4_ Plant Sensitivity to salt affected soils
(1 = slight, 2 = moderate, 3 = severe, 4 = Extreme)

Part A _____
Part B _____
Part C _____
Part D _____
Total _____

Hydraulic Conductivity (5)
___ High
___ Moderate
_X_ Low

Soil Wetness Class (5)
_X_ > 150 cm
___ 101-150 cm
___ 51-100 cm
___ 25-50 cm
___ < 25 cm

C. Site Characteristics

Position of Site (5)
_____ Depression
_____ Drainage Way
_____ Flood Plain
_____ Footslope
_____ Stream Terrace
__X__ Upland

Parent Material (5)
_____ Alluvium
_____ Colluvium
_____ Residuum
__X__ Loess

Soil Slope (5)
__X__ Nearly Level (0 to 2%)
_____ Gently Sloping (>2 to 6%)
_____ Sloping (>6 to 12%)
_____ Moderately Sloping (>12 to 20%)
_____ Strongly Sloping (>20 to 30%)
_____ Steep (>30%)

Surface Water Runoff (5)
_____ Ponded
_____ Very Slow
__X__ Slow
_____ Medium
_____ Rapid
_____ Very Rapid

Wind Erosion Potential (5)
_____ Very Low
_____ Low
_____ Medium
_____ High
__X__ Very High

Water Erosion Potential (5)
__X__ Very Low
_____ Low
_____ Medium
_____ High
_____ Very High

D. Soil Classification

Epipedons (5)
_____ Mollic
__X__ Ochric
_____ Umbric
_____ Anthropic

Subsurface Horizons
and Characteristics (5 each)
_____ Albic
_____ Argillic
_____ Cambic
_____ Calcic
__X__ Petrocalcic
_____ Gypsic
_____ Petrogypsic
_____ Natric
_____ Salic
_____ Duripan
_____ Fragipan
_____ Lithologic Discontinuity
_____ Lithic Contact
_____ Paralithic Contact
_____ None

Order (5)
_____ Alfisols
_____ Entisols
_____ Inceptisols
_____ Mollisols
_____ Vertisols
__X__ Aridisols

Part C Score _____

Part D Score _____

Figure (4). A) Example of the front side of completed scorecard for the soil pit No. 3 in Zuwarah, Libya (scorecard adapted from (Karathanasis et al., 2013)). **B)**. Example of the back side of completed scorecard for the soil pit No. 3 in Zuwarah, Libya (scorecard adapted from (Karathanasis et al., 2013)).

The scorecard that used for grading in soil judging competitions must be adapted to local soils and classification. A newly developed soil judging scorecard (Fig. 4a and 4b) is adapted for Libyan soils classified using USDA/SCS Soil Taxonomy (Arabic version in Appendix A). In order to demonstrate how to use soil judging scorecard, soil pit No. 3 (Zurqani, 2010) was used to fill out the "practice" soil scorecard (Fig. 4a and 4b).

In addition to the soil judging scorecard, other supplemental materials were used: 1) Soil physical and chemical properties, 2) optional topographic map of the area, 3) textural triangle (not shown, but it is the same used in Libya and USA), 4) abbreviations of distinctness of soil boundary, texture, modifiers of rock fragment quantity and size, structure grade, structure shape, consistence, redoximorphic features (Appendix B), 5) tables of surface and soil erosion potential classes, and 6) tables of soil use interpretations for dwellings with basement, septic absorption fields, and local roads and streets. Soil pit No. 3, which was one of soil profiles conducted by Zurqani (2010) in the northwest of Libya near the coastal strip. The soil pit has been classified as NatricPetrocalcids in the USDA/SCS Soil Taxonomy (1999). In part B, the infiltration rate was determined to be medium based on soil texture (LS/S) and soil organic carbon content (0.19%) in the Ap horizon (Karathanasis et al., 2013). Hydraulic conductivity was determined to be low based on subsurface horizon characteristics (Karathanasis et al., 2013). Available water was calculated based on depth of 70 cm x 0.05 = 3.5 cm (multiplier for LS and LS/S in all of the horizons) (Karathanasis et al., 2013). The soil wetness class is > 150 cm (not wet at depths of less than 151 cm) because of lack of redoximorphic features through the soil pit (Karathanasis et al., 2013). Soil interpretation for dwellings with basements was identified as "2 = moderate" based on using the following criteria: flooding or ponding (none), slope (< 6 %), depth to seasonally high water table (> 100 cm),

and depth to duripan layer (kqm))50 - 100 cm(, and depth to hard rock, R (cm) > 150 cm. Soil interpretation for septic tank absorption fields was identified as "3 = severe", based on using the following criteria: flooding or ponding (none), slope (< 6 %), depth to seasonally high water table (> 150 cm), the limiting hydraulic conductivity "low", and depth to duripan layer (kqm) 50 - 100 cm, and depth to hard rock, R (cm) > 150 cm. Soil interpretation for local roads and streets was identified as "2 = moderate" based on using the following criteria: flooding or ponding (none), slope (< 6 %), depth to seasonally high water table (> 100 cm), and depth to duripan layer (kqm) 50 - 100 cm, and depth to hard rock, R (cm) > 150 cm. Soil interpretation for plant sensitivity to salt affected soils was "4 = extreme" based on the surface horizon, and using the following criteria: pH = 7.1, SAR (%) = 22.04, and the ECe (dS/m) = 4. In Part C, surface runoff class was "slow" based on > 1 – 2 % slope and "medium" infiltration determined in the Part B of the scorecard. In Part C, erosion potential was "very low" based "slow" surface runoff and LS/S surface horizon texture determined in the Part A of the scorecard.

In general, the U.S. scorecard can be used in Libya and other countries which have the same climatic conditions with necessary modifications depending on the region and soil interpretations to be used. The scorecard can be further adapted to simultaneously train the user to describe and classify soil in multiple soil classifications. Soil Judging was conducted by professors in soil science departments in Libyan universities by various participants: 54% were students, 23% were researchers, 10% were educators, and 10% were workers. Fifty percent of participants had a high school degree, 35% had a bachelor's degree, 11% had a master's degree, and four percent had a doctorate.

Initially, participants were asked about their knowledge of soil science: 73% responded that they had a fundamental soil science

course before and 40% indicated that they had conducted field work related to soil science. Eighty-two percent of participants stated that they had no prior knowledge of Soil Judging, and 95% stated that Soil Judging is useful to natural science education in Libya (Table 3)

Adapted materials (e.g., scorecard) and explanatory materials were evaluated between "good" and "excellent" (Table 4). The evaluation of the soil judging equipment was be-

tween "poor" and "good" (Table 4) Specific feedback (Appendix C) from the participants is valuable to provide more specific guidelines on positive and negative aspects of the project (Table 5).

Responses included the desire for additional seminars to increase the awareness and potential impact of Soil Judging in Libya as well as including additional field locations. Access to equipment including soil pH and EC tests was listed as a need.

APPLICATION OF SOIL JUDGING INLIBYA

Figure (5). Participants are examining the soil pit during Soil Judging practice in Libya; (a) University of Tripoli, and (b) University of Zawia

Table (3). Responses from Libyan users to questions about the Soil Judging project (total number of participants = 53).

Survey questions	Yes (%)	No (%)	N/A†
1. Have you ever had a soil science course? (Yes / No)	73	27	-
2. Have you ever had field work related to soil science? (Yes / No)	40	60	1
3. Did you know about Soil Judging before this power point presentation? (Yes / No)	18	82	3
4. Is Soil Judging useful to natural science education in Libya? (Yes / No)	95	5	1

† N/A = not answered.

Table (4). Responses about the quality of the Soil judging (total number of participants = 53).

Survey question	Mean ±, SD†	N/A†
1. Did you find the Soil Judging power point presentation informative? (1 = not at all, 3 = somewhat, 5= very informative)	4.5 ± 0.8	-
2. How did you find quality of explanation? (1 = poor, 3 = good, 5 = excellent)	3.7 ± 1.0	-
3. Did you find the field demonstration for Soil Judging informative? (1 = not at all, 3 = somewhat, 5 = very informative)	4.6 ± 0.9	1
4. How did you find quality of field demonstration and explanation? (1 = poor, 3 = good, 5 = excellent)	3.8 ± 1.0	1
5. Did you find the Soil Judging field work informative? (1 = not at all, 3 = somewhat, 5 = very informative)	4.5 ± 0.9	-
6. How did you find quality of Soil Judging equipment? (1 = poor, 3 = good, 5 = excellent)	2.0 ± 1.0	1
7. How did you find quality of the Soil Judging scorecard? (1 = poor, 3 = good, 5 = excellent)	4.4 ± 1.0	2

† SD = standard deviation; N/A = not answered.

Table (5). Specific recommendations to improve Soil Judging in Libya.

Do you have any suggestion to improve the adaptation of Soil Judging power point presentation (or suggestion for other "field demonstration and explanation" and "field work related to Soil Judging")
You should organize conferences and seminars about soil judging that will raise awareness about how it is importance and how it is work for soil evaluation as big part of applied science.
I hope if you organize field trips and visits to different fields to practices on with different types of soil and places.
I wish next visit we have the necessary support for equipment and transportation.
You should seek to teach soil judging approach as field practices will help students recognize the importance of soil and study its various properties evaluated.
It was very informative that will help me on both sides an academic and field work in my M.S. research.
We need to know more details about Soil Judging.
We need more field work.
Provide all the equipment that we needed in soil judging test.
Provide the hand measurement for the soil pH test and Soil EC test will help us a lot.
This is my first time I visit the field to study soil properties, and I like it a lot.

CONCLUSION

The introduction of soil judging in Libya could have numerous benefits for the country. One of the main advantages is the potential for low-cost, non-traditional education in the techniques of land management and use. This could be especially useful for students and government workers, as it would provide them with hands-on experience and practical knowledge in this important field. Besides, Libya already has the necessary infrastructure in place to support theimplementation of soiljudging competitions in schools (including middle and high schools, colleges, and universities), as well as in various government sectors such as agriculture, health, road construction, and building and town planning.

This makes it an ideal location to introduce and promote the benefits of soil judging. Furthermore, soil nutrient analysis data can be easily obtained from any of the soil nutrient analysis laboratories in Libya, making it possible to conduct accurate and comprehensive soil assessments. Overall, the introduction of soil judging to Libya has the potential to greatly improve land management practices and increase the country's overall sustainability.

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Appendix A: The front (a) and back (b) sides of developed Libyan scorecards.

ج: خصائص الموقع

(5) مكان الموقع
منخفضات: ....
نظام الصرف: ....
الفياضانات: ....
قم المنحدر: .... علامة الانقباض السطحية في الاسفل
مصاطب المياه: .... علامة الطمي في الاسفل
مرتفعات: ....

(5) مواد الأصل
طمي: .... تكونت بفعل جريان المياه
الانقاض السطحية: .... تكونت بفعل الجاذبية، تتواجد اسفل قدم المنحدر
محلية أو متبقية: .... تكونت في نفس المكان

(5) ميل التربة
قريب المستوى (0 الى 2%): ....
قليل الانحدار (<2 إلى 6%): ....
منحدر (<6 إلى 12%): ....
متوسط الانحدار (<12 إلى 20%): ....
شديد الانحدار (<20 إلى 30%): ....
شديد الانحدار (<30%): ....

(5) الجريان السطحي (الشروط في الجدول، تعمل كيفية استخدامها)
نادر الحدوث: ....
قليل جدا: ....
قليل: ....
متوسط: ....
سريع: ....
سريع جدا: ....

(5) حدوث تعرية (الشروط في الجدول، تعمل كيفية استخدامها)
قليلة جدا: ....
قليلة: ....
متوسطة: ....
عالية: ....
عالية جدا: ....

نتيجة الجزء ج: ....

د. تصنيف التربة

(5) الآفاق السطحية
الأفق العضوي المعدني: .... (الأفق أ 10 سم، ثم ص او ج ص)
الأفق الأوكري: ....
الأفق المعدني: ....

الأفاق تحت السطحية وخصائصها (لكل 5)
أفق الغسيل: .... فاتح اللون > 1 سم
الأفق الطيني: .... (وجود علامات نزوح الطين لاسفل بالرشح)
أفق التغيير: ....
الطبقة الصماء القابلة للكسر: ....
طبقة متقطعة التطور: .... (التغير في حجم الحبيبات)
طبقة حجرية متصلة: .... (تفصل التربة بالأفق ص)
طبقة شبه حجرية متصلة: .... (تفصل التربة بالأفق ج ص)
لاشيء: ....

الرتبة (6)
كرب مناطق الغابات: .... (oc, ar, um A, Bt, C)، نسبة التشبع بالقواعد > 35 % ولكن < 50 %
الكرب حديثة التكوين: .... (الأفق الأوكري، أ، ج)
الكرب البسيطة التطور: .... (och or um, A, Bw, C)
كرب مناطق الحشائش والأعشاب الطبيعية: .... (m, ar, A, Bt, C) (B.S. >50%, OC >0.6%)
الكرب الجافة: .... (arg, oc, A, E, Bt, C)، نسبة التشبع بالقواعد > 35 %
الكرب الطينية المتشققة السوداء: .... (a/g, oc, A, E, Bt, C)

نتيجة الجزء د: ....

(a). The front side of developed Libyan scorecards.

بطاقات تقييم التربة (Soil Judging)

(b). The back side of developed Libyan scorecards.

Appendix B

Abbreviations (Adapted from Handbook for Collegiate Soils Contest, 2011).

Distinctness of Boundary			
Abrupt = A	Gradual = G		
Clear = C	Diffuse = D		
Texture			
Sand = S	Silt = Si	Clay = C	Loam = L
Sandy Loam = SL	Silt Loam = SiL	Clay Loam = CL	Sandy Clay = SC
Sandy Clay Loam = SCL	Silty Clay Loam = SiCL		Loamy Sand = LS
Sandy Clay = SC	Silty Clay = SiC		
Modifiers of Rock Fragment Quantity and Size			
Gravelly = GR	Cobbly = CB	Stony = ST	
Very Gravelly = VGR	Very Cobbly = VCB	Very Stony = VST	
Extr. Gravelly = XGR	Extr. Cobbly = XCB	Extr. Stony = XST	
Structure Grade			
Structureless = SLS	Weak = WK	Moderate = MO	Strong = ST
Structure Shape			
Granular = GR	Prismatic = PR	Angular Blocky = ABK	
Platy = PL	Single Grain = SG	Subangular Blocky = SBK	
Massive = MA			
Consistence			
Loose = L	Friable = FR	Very Friable = VFR	
Firm = Fi	Very Firm = VFi	Extremely Frim = EFi	
Redoximorphic Features			
Enter "Yes" (Y) if present, and "No" if none are present.			
presence or absence of carbonates (e.g., CaCO3)			
Effervescence class		Criteria	
Non effervescent (N)		No bubbles detected	
Very slightly effervescent (VSli)		Few bubbles seen	
Slightly effervescent (Sli)		Bubbles readily seen	
Strongly effervescent (St)		Bubbles from low foam	
Violently effervescent (Vio)		Thick foam from quickly	

Appendix C

Soil Judging survey form.

<div align="center">Soil Judging Survey</div>

Thank you for participating in this Survey of "Potential Adaptation of Soil Judging in Libya."
Your responses will be very useful in further improvement and development of this project.
Please, fill in the bubble with your answers ●or circle the appropriate answer.
Part A. General Information.
I am a: ○ student; ○ farmer; ○ worker; ○ engineer; ○ educator; ○ researcher; ○ other
My highest education is: ○ school; ○ university; ○ M.S; ○ Ph.D.; ○ other: _____
My academic major or specialty is: _____
1. Have you ever had a soil science course? (Yes / No)
2. Have you ever had field work related to soil science? (Yes / No)
Part B. Questions about Soil Judging power point presentation.
3. Did you find the Soil Judging power point presentation informative?
    1 = not at all   3 = somewhat   5 = very informative
4. How did you find quality of explanation?
    1 = poor   3 = good   5 = excellent
5. Did you know about Soil Judging before this power point presentation? (Yes / No)
6. Is Soil Judging useful to natural science education in Libya? (Yes / No)?
7. Please, provide specific comments about further improvements to this power point presentation:
Part C. Questions about field demonstration:
8. Did you find the field demonstration for Soil Judging informative?
    1 = not at all   3 = somewhat   5 = very informative
9. How did you find quality of field demonstration and explanation?
    1 = poor   3 = good   5 = excellent
10. Please, provide specific comments about further improvements to this field demonstration and explanation:
Part D. Questions about field work.
11. Did you find the Soil Judging field work informative?
    1 = not at all   3 = somewhat   5 = very informative
12. How did you find quality of Soil Judging equipment?
    1 = poor   3 = good   5 = excellent
13. How did you find quality of the Soil Judging scorecard?
    1 = poor   3 = good   5 = excellent
14. Please, provide specific comments about further improvements to field work related to Soil Judging:

Bee Honey as a Bioindicator of Environmental Pollution with Some Heavy Metals in Zawia and Janzour Regions, Libya

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Abstract: This study aims to estimate the concentration of some heavy metals in bee honey and its various products and the possibility of inferring bees as a bioindicator in determining pollution by these metals. Eight hives were distributed; seven were close to different sources of pollution and one hive was in an agricultural site far control sample. Twenty honey samples were collected from the mentioned sites during three seasons and some samples of pollen, wax, and gum were also collected from one of the sites of pollution sources and the control site. Obtained results indicated that most of the honey samples were above the permissible limit for lead and cadmium compared to the European Union (EU) standard (1.0) mg/kg and the Codex standard. It was found that all samples contained relatively high concentrations of lead and cadmium in all sites. As for copper and zinc, all samples contained concentrations less than the permissible limit according to the Codex standard, which is (5.0) mg/kg. As for the concentration of these elements in pollen, wax, and gum samples, the highest concentration was in bee gum samples. It became clear through the results of this study those bees, and through their various products: honey, pollen, beeswax, and gum, can be consider a bioindicator of the environment to determine the extent of pollution by some heavy metals in the sites surrounding the beehives.

عسل النحل بوصفه مؤشراً حيوياً للتلوث البيئي ببعض العناصر الثقيلة في منطقة الزاوية وجنزور بليبيا

المستخلص: تهدف هذه الدراسة الى تقدير تركيز بعض العناصر المعدنية الثقيلة بعسل النحل ومنتجاته المختلفة وإمكانية الاستدلال بالنحل كمؤشر حيوي في تحديد التلوث بهذه العناصر. وزعت عدد ثمانية خلايا نحل، سبعة منها قريبة من مصادر تلوث مختلفة وخلية في منطقة زراعية كعينة شاهد. تم تجميع عدد عشرون عينة عسل من المواقع المذكورة وذلك خلال ثلاث فصول من السنة، وكذلك تم تجميع بعض العينات من حبوب اللقاح، الشمع والصمغ من أحد مواقع مصادر التلوث وموقع المنطقة الزراعية. أشارت النتائج المتحصل عليها أن أغلب عينات العسل كانت فوق الحد المسموح به للرصاص والكادميوم مقارنة بمعيار الاتحاد الأوربي (EU) (1.0) مليغرام/كيلوغرام (مغ/كغ) ومعيار هيئة دستور الأغذية (Codex). فقد وجد بان جميع العينات احتوت على تركيزات عالية نسبياً من الرصاص والكادميوم في جميع المناطق. أما عنصري النحاس والزنك فاحتوت جميع العينات على تركيزات أقل من الحد المسموح به حسب معيار هيئة دستور الأغذية وهو (5.0) مغ/كغ. أما فيما يتعلق بتركيز هذه العناصر بعينات حبوب اللقاح، الشمع والصمغ فكان أعلى تركيز لها في عينات صمغ النحل. اتضح من خلال نتائج هذه الدراسة بأن النحل ومن خلال منتجاته المختلفة: العسل، حبوب اللقاح، شمع النحل والصمغ يمكن أن يعتبر مؤشر حيوي للبيئة لتحديد مدى التلوث ببعض العناصر الثقيلة في المناطق المحيطة بخلايا النحل.

الكلمات المفتاحية :
العناصـــر المعدنيـــة الثقيلة،
عسل النحل،
حبوب اللقاح؛،
شمع النحل،
صمغ النحل.

المقدمة

أصبح التلوث البيئي ظاهرة عالمية واكبت التقدم العلمي حيث شملت الدول النامية والمتقدمة على السواء مع اختلاف نوعية التلوث. فالدول المتقدمة تعاني من أثار الصناعات التكنولوجية

المتقدمة والتي من أهمها التلوث الذري والكيميائي. أما بالنسبة للدول النامية فإنها تعاني من التلوث نتيجة لسوء إدارة الأنظمة البيئية وإهمال عنصر البيئة عند وضع خطط التنمية، ونظرا للكثافة السكانية العالية والتطور الصناعي والاقتصادي وحركة

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المواصــلات المتزايــدة، والاسـتخدام غيـر المـنظم للمبيدات الزراعيـة المختلفـة والأسـمدة الكيميائيـة المسـتخدمة فـي قطـاع الزراعيـة، بالإضافـة الـى مخلفـات الصـرف الصـحي لهـا دوراً كبيـراً فـي تفاقـم هـذه المشـكلة (حسـين، 2007). كـل هـذه المخاطـر تهـدد مكونـات النظـام البيئـي الطبيعـي ذلـك بتغيـر فـي خـواص احـدى مكونـات هـذا النظـام أو جميعهـا الأمـر الـذي يجعلهـا غيـر صـالحة للاسـتخدام فـي الأغـراض التـي كانـت تسـتعمل فيهـا. إن التلـوث البيئـي هـو كل تغيـر كمـي أو كيفـي فـي مكونـات البيئـة الحيـة أو غيـر الحيـة، ولا تقـدر الأنظمـة البيئيـة علـى اسـتيعابه دون أن يختـل توازنهـا، ولقـد طغـى تأثيـر التلـوث علـى كـل مجـالات الحيـاة البشـرية والماديـة والصـحية والنفسـية والاجتماعيـة (أرنـاؤوط، 2000). ومـن أهـم المشـاكل التـي تواجـه الإنسـان اليـوم هـي مشـكلة تلـوث الغـذاء بالعناصـر المعدنيـة الثقيلـة ممـا يؤثـر علـى صحـة وسـلامة المسـتهلك. لذلـك يجـب أن نهتـم بالوقايـة كوسـيلة للإقـلال مـن التلـوث إن لـم يكـن القضـاء عليـه نهائيـاً، ومـن هـذه الأغذيـة سـنتناول دراسـة تلـوث عسـل النحـل المنتـج بالقـرب مـن مصـادر التلـوث المختلفـة وكذلـك علاقـة النحـل بالتعـرف علـى مصـادر التلـوث بالبيئـة المحيطـة. إن أهـم عامـل خطـر للكائنـات الحيـة مثـل نحـل العسـل الـذي يعيـش فـي بيئـة ملوثـة بسـموم ومعـادن ثقيلـة ومبيـدات حشـرية وغيرهـا، هـو إنتـاج منتجـات ملوثـة وغيـر صـحية ويمكـن قيـاس مسـتوى التراكـم مـن خـلال تقديـر كميـة الملوثـات المختلفـة فـي هـذه المنتجـات والـذي يعكـس تلـوث البيئـة المحيطـة (Goretti et al., 2020).

لمـا للعسـل مـن قيمـة غذائيـة كبيـرة، وكذلـك قيمـة صحيـة، حيـث يرجـع ذلـك إلـى التركيـب الكيميائـي للعسـل، فقـد ورد ذكـر العسـل بلفظـه فـي القـرآن الكريـم مـرة واحـدة؛ فـي سـورة محمد صل الله عليه وسـلم، وذلـك فـي سـياق بعـض مـا يتنعـم بـه أهـل الجنـة فيهـا، قـال تعالـى: (فِيهَا أَنْهَارٌ مِنْ مَاءٍ غَيْرِ آسِنٍ وَأَنْهَارٌ مِنْ لَبَنٍ لَمْ يَتَغَيَّرْ طَعْمُهُ وَأَنْهَارٌ مِنْ خَمْرٍ لَذَّةٍ لِلشَّارِبِينَ وَأَنْهَارٌ مِنْ عَسَلٍ مُصَفًّى) (محمد:15). كمـا جـاء ذكـره بغيـر لفـظ العسـل فـي سـورة النحـل، وأن فيـه شـفاء للنـاس، قـال تعالـى: (يَخْرُجُ مِنْ بُطُونِهَا شَرَابٌ مُخْتَلِفٌ أَلْوَانُهُ فِيهِ شِفَاءٌ لِلنَّاسِ). (النحـل:69). قـد أصبـح

التلـوث منتشـراً بشـكل كبيـر فـي معظـم المناطـق التـي يرعاهـا النحـل وخاصـة القريبـة مـن مصـادر التلـوث بشـكل عـام والتـي مـن مخلفاتهـا العناصـر الثقيلـة. يعـد عسـل النحـل وحبـوب اللقـاح مؤشـر حيـوي لمراقبـة تلـوث البيئـة المحيطـة والقريبـة مـن خلايـا النحـل كالمناطـق الصناعيـة، الزراعيـة والحضريـة، حيـث يـزداد مسـتوى بعـض العناصـر المعدنيـة الثقيلـة فـي العسـل وحبـوب اللقـاح وفـق تلـوث هـذه المناطـق الصناعيـة والزراعيـة وغيرهـا (Aldgini et al., 2019). يتأثـر العسـل ومنتجاتـه المختلفـة مثـل حبـوب اللقـاح وغيرهـا بالملوثـات المعدنيـة كنتيجـة للمناطـق الجغرافيـة والبيئـة المحيطـة (,.Gonzalez-Martin et al 2018). أوضحـت الدراسـات أن النحـل يقـوم بزيـادة التلقيـح الزراعـي بنسـبة حوالـي 70% كمـاً ونوعـاً. إضافـة إلـى مردـوده الاقتصـادي والبيئـي، فيعتبـر نحـل العسـل مؤشـر بيئـي يجـب حمايتـه، حيـث تعتمـد كثيـر مـن وسـائل رصـد البيئـة علـى وجـود أو غيـاب نحـل العسـل، وعلـى هـذا الأسـاس يعتبـر نحـل العسـل مؤشـر قـوي وهـام لرصـد التنـوع الحيـوي، خاصـة فـي البيئـات التـي بهـا مصـادر تلـوث مختلفـة (هيئـة أبوظبـي للزراعـة والسـلامة الغذائيـة، 2019).

تكمـن فائـدة نحـل العسـل كعامـل بيئـي فـي التراكـم لبعـض الملوثـات فـي رحيـق الأزهـار وحبـوب اللقـاح والنباتـات الملوثـة بمـواد كيميائيـة زراعيـة مختلفـة، والتـي لا يسـتطيع النحـل التمييـز بينهـا وبيـن المصـادر غيـر الملوثـة علـى الرغـم مـن أن نحـل العسـل متخصـص فـي التحسـس الجينـي للعديـد مـن حبـوب اللقـاح ورحيـق الأزهـار لأنـواع مختلفـة مـن النباتـات، وخيـارات النحـل فـي البحـث عـن الغـذاء عـادة مـا تكـون مـن خـلال جمعـة مـن المناطـق المحيطـة بالخلايـا. لذلـك يعتبـر نحـل العسـل مؤشـر حيـوي ممتـاز لتلـوث البيئـة نظـرًا لقدرتـه علـى توفيـر معلومـات عاليـة الدقـة حـول وجـود الكيماويـات الزراعيـة والملوثـات الأخـرى فـي المناطـق المحيطـة (Quigley et al., 2019).

إن انتشـار مصـادر التلـوث مـن عـوادم حركـة الموصـلات وصناعـة التعديـن ومحطـات إنتـاج الطاقـة الكهربائيـة، ومصافـي النفـط وكذلـك اسـتخدام المبيـدات والأسـمدة الزراعيـة المختلفـة

والتي تحتوي على بعض العناصر الثقيلة قد يؤدي إلى تلوث العسل ومنتجاته. لذلك تهدف هذه الدراسة إلى تقدير تركيز بعض العناصر المعدنية الثقيلة بعسل النحل ومنتجاته ومدى مطابقتها للمواصفات القياسية ومقارنتها مع نتائج دراسات دولية أخرى وتحديد العلاقة بين تركيز هذه العناصر ومصادر التلوث المختلفة، إضافة إلى إمكانية الاستدلال بعسل النحل كمؤشر حيوي في تحديد التلوث بالعناصر المعدنية الثقيلة.

المواد وطرق البحث

جمع العينات: تم توزيع عدد ثمانية خلايا نحل أو طرائد، سبعة منها قريبة من مصادر تلوث مختلفة وخلية في منطقة زراعية بعيدة عن مصادر التلوث لغرض المقارنة كعينة شاهد، حيث شملت المواقع مصفاة تكرير النفط بالزاوية، محطة توليد كهرباء الزاوية، مصنع لصهر معادن الخردة بجنوب الزاوية، محطة توليد كهرباء جنزور على بعد 250م، محطة توليد كهرباء جنزور على بعد 1000م، طريق عام بمنطقة جودائم، محجر ومكب نفايات مختلفة بجودائم، منطقة زراعية بالحشان الزاوية كعينة مراقبة أو شاهد، حيث تعتبر من المناطق ذات سقوط امطار جيدة ومناخ معتدل ومراعي غنية بالنباتات والأشجار المناسبة لتربية النحل، وإنتاج العسل في مواسم مختلفة. أجريت الدراسة في الفترة الممتدة من شهر فبراير إلى شهر أكتوبر. تمت مراقبة الطوائف ومتابعتها من خلال عدة زيارات ميدانية، جمعت العينات الأولي من جميع المواقع في شهر ابريل والعينات الثانية في شهر يونيو أما العينات الثالثة فقد جمعت في شهر أكتوبر. تمت عملية فرز العسل من أقراص الشمع بطريقة الطرد المركزي، حفظت العينات في قنينات من الزجاج محكمة القفل، في درجة حرارة الغرفة إلى حين إجراء التجارب المعملية وفي نفس الوقت تم تجميع عينتين من كل من حبوب اللقاح، الشمع وصمغ النحل البربولس من موقع محطة توليد كهرباء جنزور (250م) والمنطقة الزراعية بالحشان الزاوية بهدف مقارنة تركيز العناصر الثقيلة بينها وبين عينات عسل النحل.

هضم وتجهيز العينات: تمت عملية هضم العينات بالطريقة الرطبة باستخدام جهاز الهضم بالموجات الدقيقة (Milestone Microwave)، سخنت العينات على حمام مائي لغرض تسهيل عملية الخلط والمزج، ثم وزن حوالي واحد غرام من العينة في وعاء بلاستيكي مخصص لعملية الهضم وأضيف 6 مل من حمض النيتريك المركز (65%) و2 مل من فوق أكسيد الهيدروجين بتركيز (30%) شركة (Riedel–DeHaena Germany)، ثم غلق الوعاء البلاستيكي ووضعت العينات في جهاز الهضم بالموجات الدقيقة وتم تشغيل الجهاز بواسطة برنامج مخصص لغرض التحكم في درجة الحرارة، الضغط وزمن الهضم، وبعد ذلك تم تخفيف العينة بالماء المقطر مرتين إلى العلامة 10مل وتم تحضير ثلاثة مكررات لكل عينة (الزقطاط، 1992 وإبراهيم وعاطف 2003 و Tuzen & Soylak, 2005).

تقدير العناصر المعدنية: تم تحضير سلسلة من المحاليل القياسية بتركيزات مختلفة من عنصر الرصاص، الكادميوم، الزنك والنحاس موردة من شركة (Fluka Chemika Germany)، وذلك باستخدام (Metal Atomic Spectroscopy standard)1.0غ/لتر ولعدد ثلاث مكررات. كما استخدم جهاز الامتصاص الذري نوع (Atomic Absorption Spectrophotometer Byunicam sp 9 flame) لتقدير العناصر المعدنية المختلفة.

أخذت القراءات والنتائج لجميع العينات لثلاث مكررات، وتم التعبير عن النتائج المتحصل عليها بالمتوسطات مع الانحراف المعياري (SD ±). استخدم التصميم العشوائي الكامل (CRD) لدراسة تأثير المناطق المختلفة على كمية العناصر المعدنية وقورنت الفروق المعنوية بين المتوسطات بواسطة اختبار دانكن (Duncan) متعدد الحدود عند مستوى معنوية 5%، واستعمل البرنامج (SPSS, 2018) في عمليات التحليل الإحصائي وتم عرض النتائج بواسطة (Microsoft Excel ver. 10).

النتائج والمناقشة

تركيز الرصاص في عينات العسل: تبين من خلال النتائج المتحصل عليها أن جميع العينات احتوت على كميات متفاوتة من عنصر الرصاص، حيث إن جميع العينات كانت فوق الحد المسموح به حسب معيار الاتحاد الأوربي الذي يشير إلى أن لا تتجاوز كمية الرصاص في العسل عن (1.0) مغ/كغ (EU, 2000a). بلغ متوسط تركيز الرصاص في عينات العسل 0.082±1.209 مغ/كغ بمدي تركيز تراوح بين 1.286-1.139 مغ/كغ، مقارنة مع متوسط تركيز عنصر الرصاص في المنطقة الزراعية حيث كان 0.081±1.016 مغ/كغ كما يشير الجدول رقم (1)، وكان أعلى تركيز في العينات القريبة من الطريق العام بمنطقة جودائم 0.084±1.286 مغ/كغ وقد يعزي ذلك إلى عدد من مصادر التلوث في هذا الموقع من طريق عام ومكب نفايات ومحاجر. كما كان اقل تركيز (0.052±1.139) مغ/كغ بالقرب من محطة توليد الكهرباء جنزور 1000م حيث لوحظ وجود فروق معنوية عند مستوى معنوية 5% ما بين المواقع السبعة المختلفة وكذلك موقع عينات المراقبة. تبين أيضا من نتائج التحليل الإحصائي إن العينات التي كانت قريبة من مصادر التلوث مجتمعة احتوت على أعلي تركيز من العينات التي كانت في المنطقة الزراعية وهذا مؤشر يؤكد على أن هذه المصادر لها تأثير مباشر على مستوى التلوث البيئي بهذه المناطق.

كما أن نتائج هذه الدراسة كانت مرتفعة مقارنة ببعض الدراسات مثل الدراسة التي أجراها كل من (Fredes & Montenegro, 2006) حول تركيز عنصر الرصاص في العسل المنتج في التشيلي والذي تراوح ما بين 0.11-0.01 مغ/كغ، وأيضا الدراسة التي أجراها كل من (,.Osman et al 2007) في منطقة القصيم بالمملكة العربية السعودية والتي تراوح تركيز الرصاص في عينات العسل 0.380-0.373 مغ/كغ، وكذلك الدراسة التي أجراها كل من (Bogdanov et al., 2007) في عينات من العسل السويسري فكان تركيز

الرصاص 0.329-0.003 مغ/كغ. هناك دراسات تجاوز فيها تركيز الرصاص النتائج المتحصل عليها بهذه الدراسة مثل الدراسة التي أجراها (الهدار، 2006) علي 20 عينة عسل من مناطق مختلفة من ليبيا فكان تركيز الرصاص في بعض العينات وصل إلى 50.0 مغ/كغ وكذلك الدراسة التي أجراها كل من (Rashed & Soltan, 2004) في عينات من العسل المصري فكان تركيز الرصاص في العسل في مدى ما بين 9.3-4.2 مغ/كغ. بذلك يمكن أن يتم استخدام نتائج هذه الدراسة كمؤشر بيولوجي لتلوث البيئة بالمعادن الثقيلة مثلما أوضح أيضاً (Matin et al., 2016) من خلال نتائج دراستهم لعسل النحل في بعض المناطق الصناعية بتركيا.

مستوى الرصاص المرتفع في جميع العينات يحتمل أن يكون ناتج بفعل مصادر التلوث القريبة من خلايا النحل، والتي من شأنها أن تزيد من تركيز الرصاص في العسل، كما تبين بأن جميع النتائج المتحصل عليها من الدراسة متوافقة مع الدراسة التي أجراها كل من (Bratu & Georgescu, 2005) برومانيا حيث كانت نسبة تركيز الرصاص في العينات (1.40-0.550) مغ/كغ، كذلك تطابقت نتائج هذه الدراسة مع نتائج الدراسة التي اجريت في الأردن (,.Atrouse et al 2004) في حوالي 75% من العينات والتي تراوح تركيز الرصاص بعينات العسل في مدى ما بين 1.234-0.853 مغ/كغ وكذلك تطابقت بنفس النسبة حوالي 75% مع نتائج الدراسة التي أجراها (Čelechovská & Vorlová, 2001) في العسل المنتج في التشيك، والتي كان تركيز الرصاص بها ما بين 1.3-0.02 مغ/كغ. كانت نتائج تركيز الرصاص في عينات العسل المتحصل عليها من هذه الدراسة أعلي من نتائج الدراسة التي أجراها كل من (,Demirezen & Aksoy 2005) في منطقة القيصري بتركيا والتي تراوح تركيز الرصاص في عينات العسل 0.85-0.10 مغ/كغ، وكذلك كانت نتائج هذه الدراسة اعلى من الدراسة التي أجراها (Tuzen & Soylak, 2005) في منطقة الأناضول بتركيا والتي تراوح تركيز الرصاص في مجموعة من عينات العسل 0.321-0.0176 مغ/كغ، كما أن نتائج هذه الدراسة كانت

مرتفعة مقارنة ببعض الدراسات مثل الدراسة التي أجراها كل من (Fredes & Montenegro, 2006) حول تركيز عنصر الرصاص في العسل المنتج في التشيلي والذي تراوح ما بين 0.01-0.11 مغ/كغ، وأيضا الدراسة التي أجراها كل من (Osman et al., 2007) في منطقة القصيم بالمملكة

العربية السعودية والتي تراوح تركيز الرصاص في عينات العسل 0.380-0.373 مغ/كغ، وكذلك الدراسة التي أجراها كل من (Bogdanov et al., 2007) في عينات من العسل السويسري فكان تركيز الرصاص 0.003-0.329 مغ/كغ.

جدول (1) تركيز العناصر المعدنية (الرصاص، الكادميوم، النحاس والزنك) مغ/كغ في عينات العسل بالمناطق المختلفة

	كمية العناصر المعدنية مغ/كغ			مصدر التلوث (المنطقة)
الزنك	النحاس	الكادميوم	الرصاص	
0.014±0.068[d]	0.064±0.454[c]	0.062±0.293[b]	0.122±1.188[d]	مصفاة تكرير نفط الزاوية
0.012±0.028[c]	0.056±0.496[d]	0.058±0.247[c]	0.087±1.216[d]	محطة توليد الكهرباء بالزاوية
0.011±0.029[c]	0.045±0.505[b]	0.086±0.209[c]	0.079±1.271[c]	مصنع لصهر المعادن والخردة جنوب الزاوية
0.012±0.039[b]	0.044±0.469[d]	0.056±0.136[d]	0.064±1.218[d]	محطة توليد الكهرباء جنزور250م
0.013±0.043[b]	0.084±0.521[b]	0.033±0.117[d]	0.052±1.139[b]	محطة توليد الكهرباء جنزور1000م
0.009±0.029[c]	0.053±0.454[c]	0.061±0.241[c]	0.084±1.286[de]	طريق عام بمنطقة جودائم
0.014±0.041[b]	0.075±0.505[b]	0.071±0.282[b]	0.086±1.142[b]	محجر ومكب نفايات بجودائم
0.012±0.040[b]	0.060±0.486[de]	0.061±0.218[e]	0.082±1.209[e]	المتوسط العام لكل المواقع
0.010±0.023[a]	0.052±0.329[a]	0.037±0.109[da]	0.081±1.016[a]	منطقة زراعية بالحشان بالزاوية

ملاحظة: الاحرف المتشابه في العمود الواحد لا توجد بيها فروق معنوية عند مستوى معنوية 5%

هناك دراسات تجاوز فيها تركيز الرصاص النتائج المتحصل عليها بهذه الدراسة مثل الدراسة التي أجراها (الهدار ، 2006) علي 20 عينة عسل من مناطق مختلفة من ليبيا فكان تركيز الرصاص في بعض العينات وصل إلى 50.0 مغ/كغ وكذلك الدراسة التي أجراها كل من (Rashed & Soltan, 2004) في عينات من العسل المصري فكان تركيز الرصاص في العسل في مدى ما بين 4.2-9.3 مغ/كغ. بذلك يمكن ان يتم استخدام نتائج هذه الدراسة كمؤشر بيولوجي لتلوث البيئة بالمعادن الثقيلة مثلما أوضح أيضاً (Matin et al., 2016) من خلال نتائج دراستهم لعسل النحل في بعض المناطق الصناعية بتركيا.

تركيز الكادميوم في عينات العسل: تبين من خلال نتائج التحليل الإحصائي لعنصر الكادميوم في عينات العسل بالمناطق المختلفة بأن أعلى تركيز لعنصر الكادميوم في

العينات القريبة من مصفاة تكرير النفط بالزاوية 0.062±0.293 مغ/كغ وأقل تركيز كان بالقرب من محطة توليد الكهرباء جنزور 1000م 0.033±0.177 مغ/كغ وبمتوسط عام لجميع المواقع 0.061±0.218 مغ/كغ مقارنة بالمنطقة الزراعية 0.037±0.109 مغ/كغ. اتضح أيضاً بأنه توجد فروق معنوية بين المواقع عند مستوى معنوية 5% وبمقارنتها بالمنطقة الزراعية كعينة شاهد كما يشير الجدول رقم (1). تبين من نتائج التحليل الإحصائي أن العينات التي كانت قريبة من مصادر التلوث مجتمعة أعلي تركيز من العينات التي كانت في المنطقة الزراعية وهذا مؤشر على أن هذه المصادر لها تأثير مباشر على التلوث البيئي للمنطقة المحيطة بها مما نتج عنه زيادة تركيز هذا العنصر بالعسل، ومن الممكن ايضاً زيادة تركيزه بالمحاصيل الزراعية المنتجة بالقرب من بتلك المناطق. كانت جميع العينات أعلى من المعيار الأوربي المسموح به وهو 0.1 مغ/كغ (Byrne,

2000) حيث أن احتمال وصول التلوث للعسل من خلال المناطق المختلفة ناتج عن تلوث تلك المناطق بعوادم السيارات، انبعاثات عوادم عمليات الحرق في المكب وعملية التجوية بالمحاجر القريبة وانبعاثات عوادم فرن الحرق ومداخن محطة توليد الكهرباء ومصفاة تكرير النفط. أما المنطقة الزراعية كانت بعيدة عن مصادر التلوث فمن المتوقع أن يكون تركيز هذا العنصر منخفض إلى حد ما إلا أن احتمال وجود عنصر الكادميوم في عينات العسل بهذه المنطقة قد يكون ناتج عن عمليات رش المحاصيل الزراعية بالمبيدات الزراعية المختلفة والتي قد تحتوي على مركبات عنصر الكادميوم.

يلاحظ أن جميع النتائج المتحصل عليها من خلال هذه الدراسة كانت متوافقة مع الدراسة التي أجراها كل من (Čelechovská & Vorlová, 2001)، في العسل المنتج في التشيك حيث كان تركيز الكادميوم ما بين 0.003-0.500 مغ/كغ. كما أنها كانت في نطاق الدراسة التي أجراها كل من (Rashed & Soltan, 2004)، في عينات من العسل المصري فكان تركيز الكادميوم ما بين 0.01-0.43 مغ/كغ، وكانت جميع النتائج المتحصل عليها من هذه الدراسة متوافقة مع الدراسة التي أجراها (Adebiyi et al., 2004) في عينات من العسل المنتج بنيجيريا فكان تركيز الكادميوم في العسل 0.01-0.430 مغ/كغ. كانت نتائج الدراسة التي أجراها (الهدار، 2006) على عينات من العسل من مناطق مختلفة بليبيا أغلبها أعلى من النتائج التي تم الحصول عليها في هذه الدراسة فكان تركيز الكادميوم ما بين 0.475-1.275 مغ/كغ. أيضا كانت أغلب نتائج هذه الدراسة في نطاق الدراسة التي أجراها كل من (Forte et al., 2001) في العسل فكان تركيز الكادميوم ما بين 0.07-0.592 مغ/كغ. كانت نتائج بعض الدراسات السابقة أقل من النتائج المتحصل عليها من هذه الدراسة وذلك بالنسبة لعنصر الكادميوم مثل الدراسة التي أجراها كل من (Tuzen & Soylak, 2005) في عسل منطقة الأناضول المتوسطية بتركيا فكان تركيز الكادميوم ما بين 0.31-0.34 ميكرو غرام/كيلوغرام (مكغ)/كغ وأيضا

الدراسة التي أجراها كل من (Staniškienė et al., 2006) في عسل لتوانيا فكان تركيز الكادميوم ما بين 0.004-0.014 مغ/كغ، ما عدا ثلاث عينات فكانت في نطاق الدراسة.

تركيز النحاس في عينات العسل: يتبين من خلال النتائج المتحصل عليها من هذه الدراسة بإن جميع العينات احتوت على تركيزات من عنصر النحاس كما هو موضح بالجدول رقم (2). حيث كان متوسط تركيز النحاس في عينات المناطق المختلفة 0.486±0.060 مغ/كغ وعند مدى 0.454-0.505 مغ/كغ. أن جميع نتائج العينات كانت اقل بكثير من الحد المسموح به وفق معيار هيئة دستور الأغذية وهو (5.0) مغ/كغ. كان أعلى تركيز 0.521±0.084 مغ/كغ في العينات القريبة من محطة توليد الكهرباء جنزور وأقلها في عينات المنطقة الزراعية 0.329±0.052 مغ/كغ. تبين أيضاً بأنه توجد فروق معنوية ما بين المنطقة الزراعية والمناطق المختلفة الأخرى عند مستوى معنوية 5%. إن هذه التركيزات من النحاس احتمال إن تكون ناتجة بفعل المصدر النباتي الذي يتغذى عليه النحل أو من مصادر التلوث المختلفة القريبة من خلايا النحل والتي من شانها إن تزيد من تركيز عنصر النحاس في العسل. أيضا من نتائج التحليل الإحصائي تبين بأن العينات التي كانت قريبة من مصادر التلوث مجتمعة أعلى تركيز من العينات التي كانت في المنطقة الزراعية وهذا مؤشر على أن هذه المواقع قد تزيد من مستوى تركيز عنصر النحاس من خلال المناطق الجغرافية المحيطة بخلايا النحل وعليه يعتبر النحل مؤشر أو دليل حيوي يمكن من خلاله معرفة المناطق الملوثة المحيطة والذي يظهر من خلال منتجات النحل المختلفة ومن بينها العسل. نجد إن النتائج المتحصل عليها من هذه الدراسة كانت متوافقة مع نتائج الدراسة التي أجراها كل من (Demirezen & Aksoy, 2005) في عسل منطقة القيصري بتركيا والتي كان فيها تركيز النحاس عند مدى 0.15-0.66 مغ/كغ. كذلك كانت نتائج هذه الدراسة في نطاق مدي نتائج الدراسة التي أجراها كلاً من (Tuzen & Soylak, 2005)، في منطقة الأناضول المتوسط بتركيا والتي كان تركيز النحاس بها عند

مدى 0.25-1.1 مغ/كغ. أيضا كانت في نطاق الدراسة التي أجراها كلاً من (Osman et al., 2007) في عسل منطقة القسيم بالسعودية والتي وجد فيها تركيز النحاس ما بين 0.203-0.389 مغ/كغ. كما كانت النتائج المتحصل عليها من خلال هذه الدراسة أعلى من بعض نتائج الدراسة التي أجراها كلاً من (Forte et al., 2001) في عسل الصنوبر والخروب حيث كان تركيز عنصر النحاس ما بين 0.005-0.067 مغ/كغ وأيضا كانت أعلى من الدراسة التي أجراها كلاً من (Tuzen & Soylak, 2005) لعينات من العسل التي تم تجميعها من مناطق مختلفة من تركيا والتي كانت في حدود 0.008-0.011 مغ/كغ. كما إن نتائج هذه الدراسة كانت أعلى من نتائج الدراسة التي أجراها كلاً من (Staniškienė et al., 2006) في عسل لتوانيا والتي كانت في مدي 0.119-0.347 مغ/كغ.

تركيز الزنك في عينات العسل: تبين من خلال الجدول رقم (1) بأن جميع النتائج المتحصل عليها تحتوي على تركيزات متفاوتة من عنصر الزنك. كان المتوسط العام لجميع المواقع من عنصر الزنك 0.040±0.012 مغ/كغ، وكان تركيزه في المنطقة الزراعية 0.023±0.010 مغ/كغ. كما كان اعلى تركيز في عينات المنطقة القريبة من مصفاة تكرير النفط بالزاوية 0.068±0.010 مغ/كغ وأقل تركيز بعينات العسل بالمنطقة القريبة من محطة توليد الكهرباء بالزاوية. تبين وجود فروق معنوية عند مستوى معنوية 5% فيما بين عينات المناطق المختلفة والمنطقة الزراعية. اتضح ايضاً بأن جميع نتائج العينات كانت اقل بكثير من الحد المسموح به وفق معيار هيئة دستور الأغذية (5.0) مغ/كغ. إن احتمال وجود هذه التركيزات المنخفضة من عنصر الزنك في العسل قد يرجع الى وجود هذا العنصر أساساً في النباتات المختلفة والأزهار الذي يجمع منها النحل الرحيق وبالتالي تصل إلى العسل أو قد يرجع ذلك إلى مصادر التلوث المختلفة بالمنطقة والقريبة من خلايا النحل. تبين أيضا من نتائج التحليل الإحصائي أن العينات التي كانت قريبة من مصادر التلوث

مجتمعة كانت أعلي تركيز من العينات التي كانت في المنطقة الزراعية.

نجد أن جميع النتائج المتحصل عليها من هذه الدراسة كانت في نطاق مدي نتائج الدراسة التي أجراها كل من (Bogdanov et al., 2007) في العسل السويسري حسب الظروف الجغرافية والنباتية والتي كانت في مدي ما بين 0.016-3.317 مغ/كغ وكذلك كانت نتائج تركيز الزنك في عينات العسل في نطاق مدي الدراسة التي أجراها كل من (Fredes & Montenegro, 2006) في عسل التشيلي وكان تركيز الزنك في مدي ما بين 0.01-4.93 مغ/كغ وكانت نتائج الدراسات التي أجراها كل من (Bratu & Georgescu, 2005; Osman et al., 2007; Rashed & Soltan, 2004; Staniškienė et al., 2006 والهدار، 2006) لتقدير تركيز الزنك في العسل أعلي من النتائج التي تم الحصول عليها في هذه الدراسة.

تركيز الرصاص في حبوب اللقاح، الشمع والصمغ: تبين من خلال النتائج بالجدول رقم (2)، بأن جميع العينات احتوت على كميات متفاوتة من عنصر الرصاص تراوحت بين 1.166-3.543 مغ/كغ فيما يخص حبوب اللقاح، شمع النحل وصمغ النحل حيث كان تركيز الرصاص بهذه العينات جميعاً أعلي من العسل. كما تبين وجود فروق معنوية عند مستوى معنوية 5% ما بين نتائج المنطقة الزراعية كعينة شاهد وموقع محطة كهرباء جنزور 250م حيث كان متوسط تركيز مستوى الرصاص في عينات حبوب اللقاح بهذه المنطقة 1.555±0.076 مغ/كغ مقارنة بالمنطقة الزراعية 1.166±0.029 مع/كغ، واتضح أيضاً بأن أعلى تركيز كان في عينات صمغ النحل بموقع محطة كهرباء جنزور 250م وهو 3.543±0.097 مع/كغ كما هو موضح بالشكل (1، 2، 3). وهذا يؤيد الفرضية التي تقول إن النحل يعمل على تصفية وترشيح العسل من الملوثات (Fakhimzadeh & Lodenius, 2000) فبالتالي كان تركيز عنصر الرصاص

في عينات العسل أقل بكثير من عينات حبوب اللقاح، الشمع والصمغ.

تركيز الكادميوم في حبوب اللقاح، الشمع والصمغ: تبين من خـلال النتائـج الـواردة بالجدول رقم (2) بـأن كميـة عنصـر الكادميوم في عينات حبوب اللقاح، شمع النحل وصمغ النحل في منطقة محطة كهرباء جنزور 250م أعلى من المنطقة الزراعية حيث كان تركيز عنصر الكادميوم 0.279±0.063، 0.375±0.072 و0.504±0.081 مغ/كغ في حبوب اللقاح، شمع النحل وصمغ النحل على التوالي وهي تركيزات عالية مقارنـــة بعينـــات المنطقـــة الزراعيـــة، حيـــث كانـــت 0.108±0.045، 0.207±0.092 و0.355±0.085 مغ/كـغ على التوالي. كما هو موضح بالشكل (1،2،3). كانت هناك

فروق معنوية عالية عند مستوى معنوية 5% ما بين المناطق المختلفة. هذا قد يؤكد بأن النحل يعمل علي ترشيح العسل من الملوثات (Fakhimzadeh & Lodenius, 2000) وبالتالي يكون تركيز العسل مـن العناصر المعدنية أقل مـن حبوب اللقاح والشمع والصمغ المنتج، وهذا يتوافق مع ما وجدهما كلاً من (Thakur & Nanda, 2020) بأن حبوب اللقاح كمكون غذائي طبيعـي مـن منتجـات النحل يحتوي على العديد من الخصائص والمركبات الوظيفية ومكونات غذائية جيدة وأملاح معدنية وغيرها وقد تتأثر هذه الخصائص والمكونات بالمناطق الجغرافية المختلفة لخلايا النحل، الأمر الذي قد يعكس مستوى الجـودة ومعاييـر السـلامة لمنتجـات النحـل المختلفـة وتلوثهـا بالعناصر المعدنية من خلال البيئة المحيطة.

جدول (2). كمية العناصر المعدنية (الرصاص، الكادميوم، النحاس والزنك) مغ/كغ في حبوب اللقاح، شمع النحل وصمغ النحل بالمنطقة الزراعية ومحطة كهرباء جنزور 250م

مصدر التلوث (المنطقة)	نوع العينة	كمية العناصر المعدنية مغ/كغ			
		الرصاص	الكادميوم	النحاس	الزنك
محطة كهرباء جنزور 250م	حبوب اللقاح	1.555±0.076[a]	0.279±0.063[a]	7.854±0.298[a]	0.851±0.095[a]
منطقة زراعية	حبوب اللقاح	1.166±0.029[b]	0.108±0.045[b]	4.652±0.145[b]	0.606±0.124[b]
محطة كهرباء جنزور 250م	شمع النحل	1.764±0.090[c]	0.375±0.072[c]	0.934±0.087[c]	0.754±0.075[c]
منطقة زراعية	شمع النحل	1.244±0.085[d]	0.207±0.092[d]	0.583±0.162[d]	0.283±0.075[d]
محطة كهرباء جنزور 250م	صمغ النحل	3.543±0.097[e]	0.504±0.081[e]	11.212±1.340[e]	7.325±0.684[e]
منطقة زراعية	صمغ النحل	2.033±0.011[f]	0.355±0.085f	3.308±0.125[f]	1.703±0.086[f]

ملاحظة: الاحرف المتشابه في العمود الواحد لنفس نوع العينات لا توجد بيها فروق معنوية عند مستوى معنوية 5%

تركيز النحاس في حبوب اللقاح، الشمع والصمغ: يلاحظ من خلال نتائج الدراسة وفق الجدول رقم (2) بأن عينات حبوب اللقاح، الشمع والصمغ تحتوي على تركيزات مختلفة مـن عنصـر النحـاس حيـث كـان متوسـط تركيـز عنصـر النحاس في عينات حبوب اللقاح، شمع النحل وصمغ النحل 7.854±0.298، 0.934±0.087 و11.212±1.340 مغ/كغ على التوالي في منطقة محطة كهرباء جنزور 250م، كمـا هـو موضـح بالشـكل (1،2،3). كـان تركيـز عنصـر

النحاس في هذه العينات مرتفع مقارنة بنتائج عينات المنطقة الزراعية ويلاحظ ارتفاع تركيزه في صمغ النحل أكثر من العينات الأخرى، كم تبين وجود فروق عالية المعنوية بينها عند مستوى معنوية 5%. وهذا يؤكد فرضية إن النحل يعمل على ترشيح العسل من الملوثات (Fakhimzadeh & Lodenius, 2000).
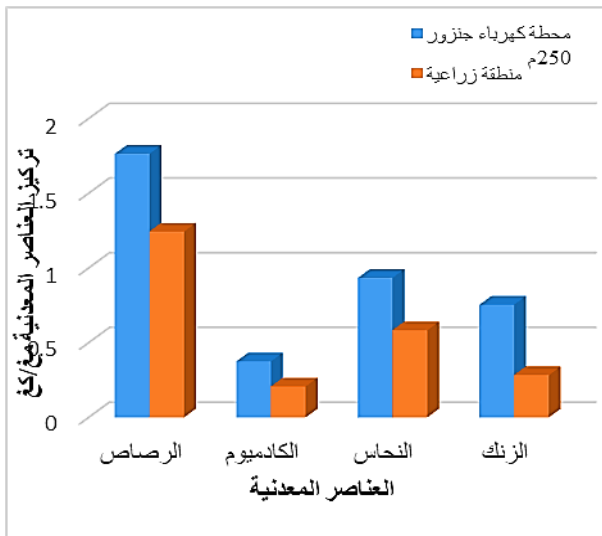
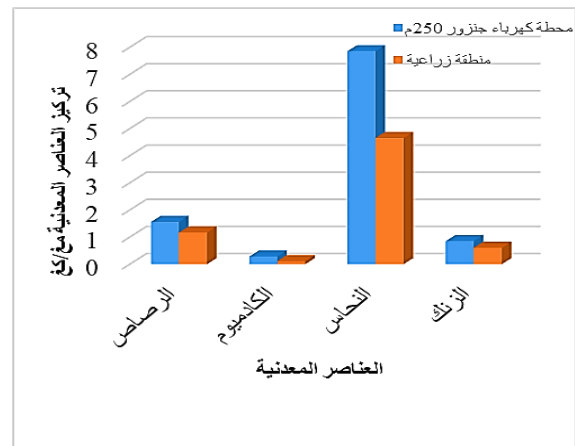**شكل (3).** تركيز العناصر المعدنية (مغ/كغ) لعينات شمع النحل

الاستنتاج

يستنتج من خلال هذه الدراسة وجود كميات مرتفعة من بعض العناصر المعدنية الثقيلة وخاصة عنصري الرصاص والكادميوم في عسل النحل ومنتجاته المختلفة (حبوب اللقاح، شمع النحل وصمغ النحل) في بعض المناطق التي تحتوي على مصادر مختلفة لتلوث البيئة. حيث إنه من الممكن إرجاع تلك التركيزات إلى مصادر التلوث المختلفة مثل انبعاثات عوادم السيارات، مداخن المصانع، محطات توليد الكهرباء، أو من عملية التجوية في المحاجر، أو من المبيدات والأسمدة الزراعية المستخدمة، والتي بدورها قد تنتقل عبر الهواء، الماء والتربة إلى النبات والأزهار ومنها إلي العسل من خلال الرحيق. كما تبين بأنه هناك علاقة مباشرة ما بين مصادر التلوث المختلفة وزيادة تركيز العناصر المعدنية الثقيلة بعسل النحل ومنتجاته. بذلك يمكن أن يعتمد على النحل من خلال منتجاته المختلفة كمؤشر حيوي لتحديد مدى تلوث البيئة ببعض العناصر المعدنية في المناطق المحيطة بخلايا النحل.
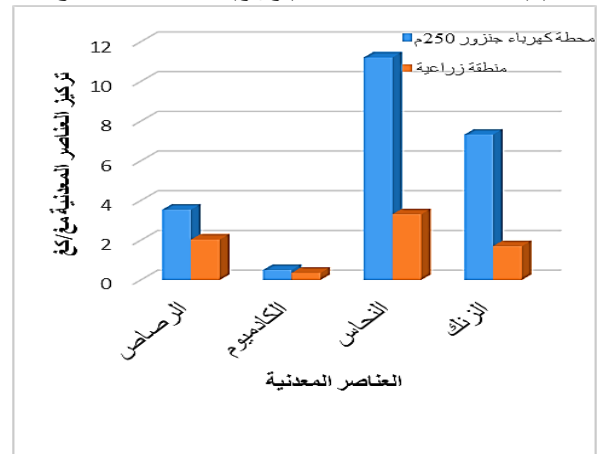
ازدواجية الاهتمام: يعلن المؤلفان أنه ليس لديهما ازدواجية في الاهتمام مرتبطة بهذه الدراسة.

مساهمات المؤلف: قام المؤلف الأول بوضع مخطط

تركيز الزنك في حبوب اللقاح، الشمع والصمغ : يتبين من خلال نتائج تحليل عينات حبوب اللقاح، الشمع والصمغ لعنصر الزنك بالجدول رقم (2) وجود تركيزات متباينة من بين المناطق المختلفة، حيث كان اعلى تركيز لعنصر الزنك في العينات القريبة من محطة كهرباء جنزور وهي صمغ النحل 0.684±7.325 مغ/كغ يليها عينات حبوب اللقاح 0.095±0.851 مغ/كغ ثم عينات شمع النحل 0.075±0.754 مغ/كغ مقارنة بعينات المنطقة الزراعية، كما هو موضح بالشكل (1،2،3). كما تبين وجود فروق معنوية بينها عند مستوى معنوية 5%. تعتبر هذه التركيزات أعلى من كمية الزنك بعينات العسل وهذا قد يؤيد الفرضية التي تقول بأن النحل يعمل على ترشيح وتصفية العسل من بعض الملوثات (Fakhimzadeh & Lodenius, 2000).

شكل (1). تركيز العناصر المعدنية (مغ/كغ) لعينات حبوب اللقاح

شكل (2). تركيز العناصر المعدنية (مغ/كغ) لعينات صمغ النحل

الدراسة، تجميع بعض المراجع العلمية وتعليل النتائج والمساهمة في إعداد مسودة الدراسة ومراجعة النسخة النهائية. كما قام الباحث الثاني بتجميع وتحليل العينات والبحث عن بعض المراجع العلمية وكتابة المسودة الأولى.

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