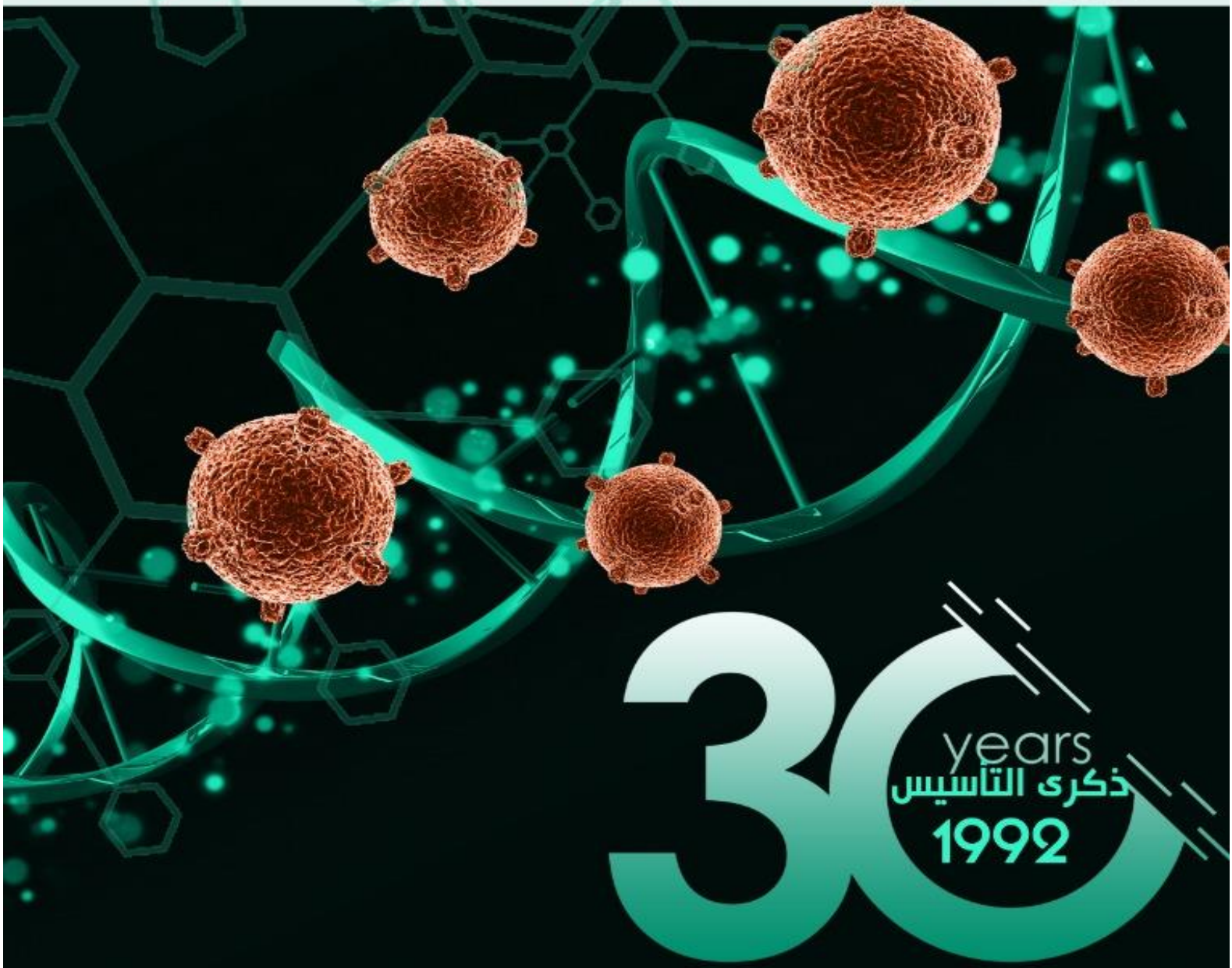
# مجلة المختار للعلوم

# AL-Mukhtar Journal of Sciences

Volume: 37 Issue: 3

2022




ISSN:26-17-2178 (Print)

SSN:26-17-2186 (Online)

دار الكتب الوطنية - رقم الإيداع القانوني 280-2013

# مجلة المختار للعلوم


**جامعة عمر المختار**

**البيضاء، ليبيا**

**مجلة علمية محكّمة، المجلد السابع والثلاثون، العدد الثالث، 2022**

**تصدر عن جامعة عمر المختار، البيضاء، ليبيا.**

**مجلة المختار للعلوم**

**رقم الايداع في المكتبة الوطنية 2013/280/بنغازي**



**جامعة عمر المختار – البيضاء – ليبيا**

**مجلة محكّمة تصدر عن جامعة عمر المختار، البيضاء، ليبيا**

**مجلة علمية محكّمة، المجلد السابع والثلاثون، العدد الثالث، 2022**

بريد إلكتروني: omu.j.sci@omu.edu.ly

ص.ب. 919 البيضاء – ليبيا، فاكس: 7053 463 69 218+

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**مجلة المختار للعلوم 37:(3)، 2022**

**Al-Mukhtar Journal of Sciences 37: (3), 2022**

*Al-Mukhtar Journal of Sciences* 37 (3): 185-193, 2022
*ISSN: online 2617-2186 print 2617-2178*
**Journal Homepage** https://omu.edu.ly/journals/index.php/mjsc/index

# The Allelopathic Effects of Two Species of *Cistus* Genus on Germination and Root Length of *Ceratonia Siliqua* L

**Mohamed A. Omar*, Moussa Masoud and Abdulsalam A. Albukhari**

*Department of Forestry and Rangeland, Faculty of Natural Resources, Omar Al-Mukhtar University, Al-Bayda, Libya*

Received: 13 November 2021/ Accepted: 07 August 2022
Doi: https://doi.org/10.54172/mjsc.v37i3.384

**Abstract:** This study examines the allelopathic effects of *Cistus incanus (synonym C. villosus)* L. and *Cistus salviifolius* L. on the germination and the root length of *Ceratonia siliqua*. Six aqueous extracts were prepared for both species by soaking fresh leaves in cold water, boiling leaves and leaving for 24 hours, and grinding dried leaves. The result suggests that the germination percentage was slightly affected by extract concentration regardless of the type of extraction since F-value and P-value at 0.05 confidence level were 2.93 and 0.043 respectively. However, root length showed a significant response to the type of extraction, extract concentration, and interaction between both factors as F-values reached 6.3, 12.4, and 2.4 respectively. LSD test showed the response of seeds germination was inverse with the increase of concentration. In addition, it showed that the extracts of dried leaves were higher in root inhibition than the extracts of fresh leaves. The interaction effect reached its highest values when comparing the dried leaves' extract to the freshly soaked ones, even for the same concentrations. In conclusion, seeds of *c. siliqua* were able to easily start germination because the nutrition compounds needed were available in the endosperm, and the media supported them just by moisture for establishment. While for root length, the root tissue absorbed extra quantities of the inhibiting agents from media leading to failure or weakness in root development.

**Keywords:** Allelopathy; Aqueous extracts; *Cistus incanus*; *Cistus salviifolius*; Germination percentage; Root length.

## INTRODUCTION

The term (Allelopathy) refers to the process of producing and releasing chemical substances by a plant into the environment (Rice, 1984; Hussain & Khan, 1988; Mallik, 2008). It seems that numerous sources of chemical substances exist in plants in different forms (Masoud and Omar, 2018). Some species depend on roots' ability to exert these chemicals. Or by dropping leaves or other areal parts to the adjacent soil (Thompson, 2005; Scognamiglio; 2013, Salhi et al., 2011; Idris and Omar, 2018). During the decomposition of the dropped matter, or by roots, and in the presence of water, some chemical compounds would reach to seed banks or the recently established seedlings. In many cases, the germination or seedlings' growth and development may fail or be inhibited (Idris and Omar, 2018).

The affected species would not respond to the allelopathic agent to the same extent. Herranz

***Corresponding author:** :* Mohamed A. K. Omar, *Mohamed.omar@omu.edu.ly*, Department of Forestry, Faculty of Natural Resources, Omar Al-Mukhtar University, Al-Bayda, Libya.

et al., (2006) reported that some Mediterranean species show reasonable levels of resistance towards the aqueous extracts of common allelopathic species. What is more interesting is the response of species to the chemical concentration. According to Omar et al (2017), the aqueous extracts of *Artemisia herba-alba* Asso have no significant effects on the germination of *C. siliqua* seeds, while the root length of established seedlings was significantly affected. Therefore, the natural regeneration in a given plant community may be affected by such allelochemicals. Such an influence may contribute to the shaping of forest ecosystems on the levels of composition, structure, and density (Caboun and Jacob, 2015).

In the Mediterranean region, many shrubs and annual plants produce some chemical substances in the act of competing against other species, especially in summertime (Muller, 1969). In Libya, natural vegetation covers about 1% of the country area, and 95% of it exists in Al-Jabal Akhdar in the northeast consisting of 100 - 140 species, subspecies, or varieties endemic to Al Jabal Al Akhdar (Radford et al., 2011). In such environments, allelopathy may, to some extent, influence biodiversity (Omar et al., 2017).

*Cistus ladanifer* L. has been reported as a fundamental allelopathic species to the extent that it may influence plant diversity in the Mediterranean (Chaves and Escudero, 1997; Chaves et al., 2001; Herranz et al., 2006). The current paper aims to investigate whether other species of the *Cistus*, which belong to the family of Cistaceae, could have any similar effect. Two native species were chosen: 1) *Cistus incanus* L. It is a shrub, erect or spreading with a stem up to 1 meter in length with white-villous. Leaves are ovate, obovate or elliptic, or almost flat. Pubescent or tomentose with stellate hairs. Flowers 4-6 cm in diameter, purplish-pink. Capsules densely adpressed-villous. The seeds are small and blackish (Jafri and El-Gadi, 1977). Figure 1 (A). 2) *Cistus salvifolius* L. It is an evergreen native small shrub, 60 - 90 cm in length. Leaves are green spreading and hairy, mostly ovate-elliptic, rounded at base, green, scabrid, and rugose above, with short stellate hairs on both surfaces, petiolate. The flowers are predominately white. Five sepals, manifestly cordate at the base, are scabrid with stellate hairs usually (Jafri and El-Gadi, 1985) Figure 1(B).

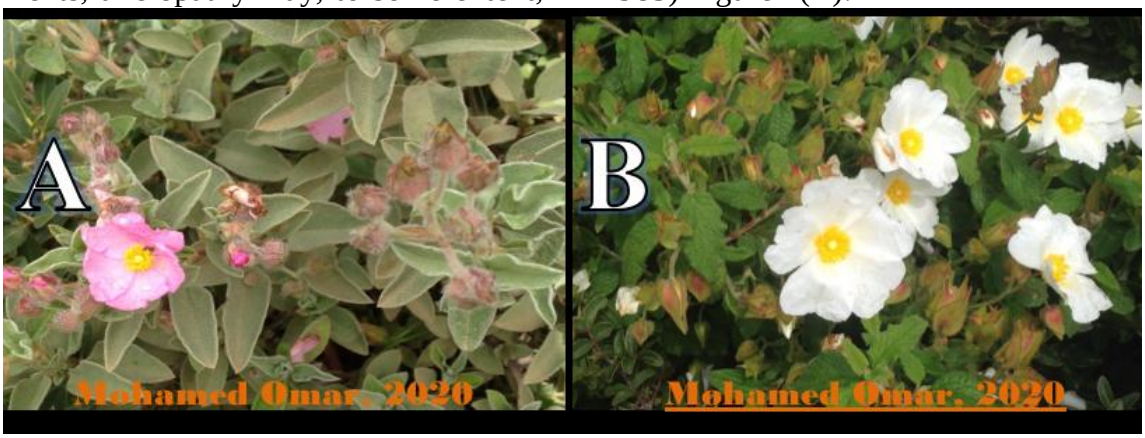


**Figure (1).** A) *Cistus incanus*, B) *Cistus salvifolius.*

The targeted species is *Ceratonia siliqua* L. which belongs to the Caesalpinioideae subfamily of the legume family, Fabaceae. It is an evergreen sclerophyllous tree or shrub, with a height of up to 9 meters. It can live up to 200 years (Masoud and Omar, 2018). Leaves are oval or elliptical, leathery, with a length of 10 – 20 cm. Flowers are greenish with red inflorescence, and inflorescence axis is catkin-like (Jafri and El-Gadi, 1985). *C. siliqua* seeds are known to have a high percentage of germination if they are subject to

pre-sowing treatment; otherwise, they rarely exceed 10% (Bostan and Kilic, 2014). According to the same study, it seems that soaking the seeds for 30 minutes in sulfuric acid 95%, then immersing them in water for 48 hours, would increase the germination percentage to more than 90%.

## MATERIALS AND METHODS

**Preparing the aqueous extracts** :Samples of *Cistus incanus* and *Cistus salvifolius* were collected in October 2019 from the forest of Werdama, located just about five kilometres east of Albayda city. Leaves were present in both species. Six aqueous extracts were prepared as follows: 1) Two extracts were prepared by collecting 100 gm of fresh leaves (on the day of samples collection) from both species, then soaked in cool distilled water for 24 hours at room temperature (Herranz et al., 2006). 2) Two extracts were prepared by collecting 100 gm of fresh leaves from both species (on the same day of collection), then added to boiling distilled water and left on a heat source for ten minutes, and kept for 24 hours at room temperature (Herranz et al., 2006). 3) Two extracts were prepared by drying fresh leaves for 30 days in room enrapture and then grinding dried leaves for both species with (Glen Creston Ltd, Stanmore, UK) mill. The powder of each species was diluted into distilled water 500 g to1000 ml (W/V %). The two mixtures were placed on a flask orbital laboratory shaker (mrc laboratory instruments, Cambridge, UK) for 24 hours at a speed of 120 rpm at room temperature (Ghorbani et al., 2008). Four-folded cotton fabric were used as a filter to separate rough solid particles from solutions. The solutions were then centrifuged with (ELE International, UK) at a speed of 2000 rpm for 15 minutes (Bajalan et al., 2013). All the above six mentioned extracts were considered main stocks. 25% and 50% solutions based on volume/volume percent (*v/v.* %) were prepared by adding distilled water from each main stock. All the prepared solutions and the main stocks were tested against control and among each other on *C. siliqua* seeds. The control is saline water to deviate from the osmotic-potential effect since the prepared solutions have a level of salinity. The salinity was l% (l g NaCl/1000ml distilled water), and the osmotic potential is equal to that in the high-concentration hot extract (Herranz et al., 2006).

**Seeds preparation and testing :**The seeds of *C. siliqua* were randomly selected and then subjected to a floating test. To break seed dormancy, a chemical treatment was applied. We then treated *C. siliqua* seeds with Sulphuric acid (95 %) for 20 minutes before soaking them in regular water for two days (Bostan and Kilic, 2014).

**Experimental design and statistical analysis:** A randomized complete block with a replication design was implemented. The six extract types were considered treatments, while the four solutions of different concentrations, from 0 % (control) to 100% main stocks, are considered blocks within each treatment. In every block, we prepared three replicants. Every replicant is a Petri dish (90 mm diameter) that contains five seeds of *c. siliqua* placed on a piece of filter paper, ensuring that seeds don't touch each other (Omar et al., 2017). The total number of dishes was 72. The Petri dishes were then incubated for ten days in a controlled cabinet at 20ºC in darkness (Memmert, Schwabach, Germany). Observations of germination percent for each dish and root length, as an average for every dish, were recorded. Concerning the statistical analysis, R (CRAN) software package was used. ANOVA test was applied, and the LSD test was carried out for the comparison between means. Some of our experimental units did not show any germination, meaning that the germination percentage was (zero). In such cases, data transformation is recommended. A logarithm-based transformation has been applied by replacing the value of each germination percentage ($x$) with $\log(x+1)$, as has been suggested by (Robert and Casella, 1999).

## RESULTS

**Seeds Germination:** The germination started in the control block 48 hours from the beginning of the trial, reaching 95% in all treatments. Observations were recorded every 24 hours and the germination was tested in eight days (Abd El-Fattah et al., 2011; and Al-Watban & Salama 2012; Omar et al., 2017). The analysis of variance showed that there are no significant differences between the effects of aqueous extracts types, either the cold extract, the hot extract, nor the powder-based extract, on the germination of *C. siliqua* and for both species as the F-value was (1.61) as depicted in Table 1.

**Table (1).** Analysis of variance (ANOVA) for the extracts type and concentration on the Germination percentage.

| S.O.V | D.F | S.S | M.S | F-value | P-value |
|---|---|---|---|---|---|
| Extract type | 5 | 28.67 | 5.73 | $1.61^{ns}$ | 0.177 |
| Concentration | 3 | 31.41 | 10.47 | $2.93^{*}$ | 0.043 |
| Interaction | 15 | 40.76 | 2.718 | $0.76^{ns}$ | 0.711 |
| Error | 48 | 171.4 | 3.571 | | |

Similarly, the interaction between the extract types and concentration has not shown any significance in its effect on the germination of seeds. However, the clear significance appeared among concentrations in a somewhat gradual inverse relation between concentration level and germination percentage, as shown in Table 2.

**Table (2).** Effects of *C. incanus* and *C. salviifolius* aqueous extracts on germination of *C. siliqua* seeds. $\alpha = 0.05$

| Concentration (%) | Means of germination percentage |
|---|---|
| Control | $100^{a}$ |
| 25% | $60^{ab}$ |
| 50% | $45^{b}$ |
| 100% | $20^{c}$ |
| F-Value | $2.93^{*}$ |
| LSD | 0.386 |

Means within columns followed by different letters are significantly different according to LSD, $P<0.05$.

**Root length:** Root length is a significant indicator for understanding phytotoxicity, that is, the extent to which chemical compounds affect the growth and development of plant cells. Moreover, it gave a better idea than the germination percentage to understanding allelopathies. The variance analysis showed a significant difference between the type of extract, the concentration, and the interaction on the root length (Table 3). The F value of concentration reached 12.4, which was the highest value, followed by the type of extract, which was 6.299. The F value of the interference was low; however, it was significant and reached 2.4.

**Table (3):** Analysis of variance (ANOVA) for the extracts type and concentration on the Root length

| S.O.V | D.F | M.S | F-value | P-value |
|---|---|---|---|---|
| Extract type | 5 | 30.72 | $6.299^{**}$ | 0.177 |
| Concentration | 3 | 60.33 | $12.369^{**}$ | 0.043 |
| Interaction | 15 | 11.86 | $2.439^{**}$ | 0.711 |
| Error | 48 | 4.87 | | |

The LSD test showed (Table 4) that *C salvifolius* powder-based extract at a concentration of 100% has the highest significant effect on root length where the length of the roots exceeded 4 cm. Also, the interaction between type of extract and concentration is significant with a value ranging from 5 to 7.5 cm. This result was indicated whenever the type of extract differed and the concentration decreased. It can be concluded that the powder of the leaves gives a stronger effect than the soaked. The effect increases by rising the concentration except when treating the seeds with cold soaking for both types. At the same time, the length was from 7.5 cm to 8.5 cm, which is considered very close to the length of the root under the influence of control.

**Table (4).** Paired-wise comparisons between type of extracts, concentrations and interactions. Combinations that have same letters indicate to non-significant effect among them comparing to LSD. (A) Refers to shortest root lengths obtained ranged from (2.98 – 3.98) cm. (B) refers to longest root lengths obtained (3.99 – 5.22) cm.

| | | *Cistus incanus* aqueous hot extract | | | | *Cistus incanus* aqueous cold extract | | | | *Cistus salvifolius* aqueous hot extract | | | | *Cistus salvifolius* aqueous cold extract | | | | *Cistus incanus* powder-based extract | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | c | 25 | 50 | 100 | c | 25 | 50 | 100 | c | 25 | 50 | 100 | c | 25 | 50 | 100 | c | 25 | 50 | 100 |
| *Cistus incanus* aqueous cold extract | c | NS | | | | | | | | | | | | | | | | | | | |
| | 25 | NS | NS | | | | | | | | | | | | | | | | | | |
| | 50 | NS | NS | NS | | | | | | | | | | | | | | | | | |
| | 100 | | NS | NS | NS | | | | | | | | | | | | | | | | |
| *Cistus incanus* hot extract | c | NS | | | | NS | | | | | | | | | | | | | | | |
| | 25 | A | NS | | | NS | NS | | | | | | | | | | | | | | |
| | 50 | A | NS | NS | | NS | NS | NS | | | | | | | | | | | | | |
| | 100 | A | A | A | A | A | A | A | NS | | | | | | | | | | | | |
| *Cistus salvifolius* aqueous cold extract | c | NS | | | | NS | | | | NS | | | | | | | | | | | |
| | 25 | NS | NS | | | NS | NS | | | NS | NS | | | | | | | | | | |
| | 50 | NS | NS | NS | | NS | NS | NS | | NS | NS | NS | | | | | | | | | |
| | 100 | A | A | A | NS | A | A | A | NS | A | A | A | NS | | | | | | | | |
| *Cistus incanus* powder-based extract | c | NS | | | | NS | | | | NS | | | | NS | | | | | | | |
| | 25 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | | | | | | |
| | 50 | A | NS | B | | A | NS | B | | A | NS | B | | NS | NS | NS | NS | | | | |
| | 100 | A | A | A | NS | A | A | A | NS | A | A | A | NS | A | A | A | NS | | | | |
| *Cistus salvifolius* powder-based extract | c | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | | | |
| | 25 | NS | B | | | NS | | | | NS | B | | | NS | NS | | | NS | NS | | |
| | 50 | A | NS | NS | | A | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | | NS | NS | NS | |
| | 100 | A | A | A | NS | A | A | B | B | A | A | A | NS | A | A | A | 3 | A | NS | NS | NS |
| LSD | | 3.79 | | | | | | | | | | | | | | | | | | | |

## DISCUSSION

Regarding the germination initialization of *C. siliqua*, the key factor is the concentration of the active substance regardless of the species and the extraction method. The interpretation of such a difference in means has been reported by Chaves et al. (2001). Several chemical compounds are responsible for the inhibitory effect on the species that belong to the legume species. Among these substances are ferulic and p-hydroxybenzoic acid, which is the main substance that inhibits seed germination (Chung et al., 2001). It is unclear whether the other phenolic compounds have a direct inhibitory effect since (Herranz *et al.*, 2006) reported that some legume species such as *Lupínus hispanicus* did not show a significant response in terms of seed germination. However, Krogmeier and Bremner, 1989 reported that (caffeic acid, chlorogenic acid, and p-coumaric acid) have a clear effect when they reach soil on the seed germination of many species' seeds.

Root length is a significant indicator for understanding phytotoxicity, that is, the extent to which chemical compounds affect the growth and development of plant cells. Moreover, it gave a better idea than the germination percentage in understanding allelopathies. The result indicated that, whenever the type of extract differed and the concentration decreased. It can be concluded that the powder of the leaves gives a stronger effect than those soaked.

The effect increases by rising the concentration except when treating the seeds with cold soaking for both types. Masoud et al. (2018) concluded that the allelochemicals may be reducing cell division or auxin-induced growth of roots.

The aerial contains high inhibitory allelochemicals, and the extracts have inhibitory effects on the growth of roots and shoot some species. Also, the constituents of the aqueous extract have been reported to be potent inhibitors of seed germination and root seedling elongation (Zunino & Zygadlo, 2004; Masoud et al, 2018).

## CONCLUSION

Root length is a significant indicator for understanding phytotoxicity which chemical compounds affect the growth and development of plant cells. These studies should be conducted further in more depth at the anatomical level, examining the toxic effect on tissues. This could represent a great opportunity to understand some variables in the plant environment, especially to understand the phenomenon of competition for limited resources in the ecosystem.

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# التأثيرات الأليلوباثية لنوعين من جنس نبات البربش *Cistus Genus* على بذور الخروب *Ceratonia Siliqua L.*

**محمد علي عمر*، موسى مسعود وعبدالسلام عبد القادر البخاري**

*قسم الغابات والمراعي، كلية الموارد الطبيعية وعلوم البيئة، البيضاء، ليبيا*

تاريخ الاستلام: 13 نوفمبر 2021/ تاريخ القبول: 07 أغسطس 2022

Doi:https://doi.org/10.54172/mjsc.v37i3.384

**المستخلص** : أجريت هذه الدراسة لبحث التأثيرات الأليلوباثية لـ *Cistus incanus (synonym C. villosus) L. Cistus salviifolius L*، على نسبة الإنبات وطول الجذر لـ *Ceratonia siliqua*. تم تحضير ست مستخلصات مائية للنوعين بنقع أوراق طرية في ماء مقطر بارد لمدة 24 ساعة، ومستخلص لأوراق طرية مغلية، ومستخلص عن طريق طحن الأوراق المُجففة ثم إذابتها في ماء مقطر. تُشير النتائج إلى أن النسبة المئوية للإنبات تأثرت بشكل طفيف بتركيز المُستخلص بغض النظر عن نوع الاستخلاص حيث أن قيمة F وقيمة P عند مستوى ثقة 0.05 كانت 2.93 و0.043 على التوالي. كما أظهر طول الجذر استجابة معنوية لنوع الاستخلاص، وتركيز المستخلص، والتداخل بين كلا العاملين حيث بلغت قيم F 6.3 و12.4 و2.4 على التوالي. أظهر اختبار LSD أن استجابة إنبات البذور كانت في علاقة عكسية مع زيادة التركيز، بالإضافة إلى ذلك تبين أن مستخلصات الأوراق المجففة كانت أعلى في تثبيط الجذور من مستخلصات الأوراق الطازجة. وصل تأثير التداخل إلى أعلى قيمة عند مقارنة مستخلصات الأوراق المجففة مع المستخلصات المنقوعة حديثًا، حتى مع نفس التركيزات. نستنتج من الدراسة أن بذور *C. Siliqua* تمكنت من بدء الإنبات بسهولة؛ لأن مركبات التغذية اللازمة كانت متوفرة في السويداء، وكانت البيئة تدعمها بالرطوبة فقط للتأسيس. بينما بالنسبة لطول الجذر امتصت أنسجة الجذر كميات إضافية من العوامل المثبطة من الوسائط مما أدى إلى فشل أو ضعف في نمو الجذر.

**الكلمات المفتاحية** : Allelopathy، *Cistus incanus*، *Cistus salviifolius*، طول الجذر، مستخلصات مائية، نسبة الإنبات.

*[1] محمد علي خليفة: *Mohamed.omar@omu.edu.ly* ، قسم الغابات والمراعي، كلية الموارد الطبيعية وعلوم البيئة، البيضاء، ليبيا.

*Al-Mukhtar Journal of Sciences* 37 (3): 194-208, 2022
*ISSN: online 2617-2186 print 2617-2178*
**Journal Homepage** https://omu.edu.ly/journals/index.php/mjsc/index

# Knowledge of Food Safety Rules among Women and Their Perception and Attitude Towards Their Application in the Celebration Halls in Tripoli, Libya

**Thuraya A. Abuhlega*, Dorsaf A. Al turki**

*Department of Food Sciences and Technology, Faculty of Agriculture, University of Tripoli, Tripoli, Libya*

Received: 24 December 2021/ Accepted: 14 July 2022
Doi: https://doi.org/10.54172/mjsc.v37i3.581

**Abstract:** The government through its respective institutions and centers is responsible for protecting the consumers by assuring the safety of food supplies until consumption. The study sought to assess the knowledge of food safety, discover the incidence of food poisoning, evaluate the perception of meals and waitresses, and know the attitude towards the meals served among a sample of women who attended celebrations in the celebration halls in Tripoli city, Libya. A total of 410 women were selected randomly and data were collected through face-to-face interviews using questionnaire forms. A chi-square test was used for independence. The majority of the participants (41.7%) were between the age of 18 and 25 years. The results showed that the majority of the participants (95.4%) had a good level of food safety knowledge. Also, the results showed a significant association between age and occupation of the participants and food safety knowledge scores ($P<0.05$). Twenty-four percent of the participants reported that they were exposed to food poisoning after eating dinner in the celebration halls. Only 15% of women always washed their hands before eating the meal in the celebration halls. A significant association was found between hand washing practice and food poisoning exposure ($p <0.05$). Only 22.4% of participants agreed that the food served inside the celebration hall was safe. It is very clear that the meals served at celebration halls need to be regulated by the repective authorities. In addition, the respective public authorities should be working hard to eliminate the negative perception of individuals about food safety practices in celebration halls.

**Keywords:** Food Safety; Food poisoning; Knowledge; Women; Celebrations hall.

## INTRODUCTION

Governments are intensifying their efforts to raise the level of food safety and to make their citizens aware of the importance of food safety for their health and the state economy. Food safety incidents and consequent consumer notification and disposal of food from shelves, as well as compensation for lawsuits, cost the US economy about $ 7 billion (Hussain & Dawson, 2013).

Food, in addition to water and air, is the priority for human life on the earth. However, food is also a source of different hazards causing disease and death (El Sheikha, 2015). Food-borne diseases have been defined as any infectious or toxic disease caused by food consumption (WHO, 2008). The percentage of people in developed countries who

***Corresponding author:** Thuraya Ahmed Abuhlega *t.abuhlega@uot.edu.ly* , Department of Food Sciences and Technology., Faculty of Agriculture, University of Tripoli, Tripoli, Libya

are exposed to food-borne diseases caused by pathogenic microorganisms is up to 30% per year and is likely to be higher in developing countries (WHO, 2002). The factors responsible for food-borne disease outbreaks which have been mentioned in the literature were cross-contamination, improper cooling, improper storage or holding foods at room temperature for long-durations, contaminated raw food/ingredient, and poor personal hygiene by persons who handle food, despite continuing progress made in food quality and safety (Angelillo et al., 2000).

Unsafe food containing microorganisms such as bacteria, viruses, parasites, or harmful chemicals causes more than 200 diseases and can lead to health problems ranging from diarrhea to cancer (WHO, 2015). There are many reasons making food unsafe such as improper practices of food handlers. Food handlers have a major role in maintaining food safety at all stages of food handling, such as production, processing, storage, and preparation. Mishandling and lack of compliance with food hygiene measures by food handlers can lead to food contamination and thus health and economic problems (Sousa, 2008).

They were the cause of approximately 10 to 20% of outbreaks of food-borne diseases (Yasuda, 2010). Most of the problems of food-borne diseases could be controlled by the efforts of the food handlers, whether in a processing plant, a restaurant, or otherwise (Sousa, 2008). Time, temperature abuse, inadequate heat treatment, and unhygienic handling were causes of an outbreak among guests at a wedding ceremony (Aljoudi et al., 2010). At parties in Libya, meals are cooked on a large scale. Cooking large amounts of food at the same time requires many food handlers. Employing a large number of food handlers increases the chances of contamination of the final food and the spread of food-borne diseases, causing health and economic burdens on the countries (Akabanda et al., 2017).

According to the available information, there have been no studies on the level of knowledge of food safety for women attending celebrations in commercial halls. In recent decades, Libyan families have been conducting celebrations of various kinds, such as birthday celebrations and wedding ceremonies, etc., in commercial halls instead of doing them in homes and using ready-made meals rather than home-cooked ones. However, this cultural advancement was not accompanied by a strategy by the respective authorities for monitoring and regulating commercial kitchens preparing meals for these celebrations, as well as controlling the serving of meals in the halls in terms of the application of good handling practices and the availability of appropriate conditions in meal handlers. In addition to the lack of documentation of food poisoning occurring in Libyan society, especially in celebration halls, despite the occurrence of food poisoning cases inside them.

Therefore, the study sought to assess the knowledge of food safety, discover the incidence of food poisoning, evaluate the perception of meals and waitresses, and know the attitude towards the meals served among a sample of Libyan women who attended celebrations in the celebration halls in the city of Tripoli.

## MATERIALS AND METHODS

**Experiment design and data collection:** Four hundred and ten women who attend parties in celebration halls were selected randomly from different areas in Tripoli city, Libya. The study was carried out in the period from January to June 2019. A face-to-face questionnaire was used in the Arabic language containing three demographic questions, 11 questions about the knowledge of food safety, four questions about exposure to food poisoning, 12 questions about the perception of meals and waitresses, and nine questions about the attitude towards meals safety. The interview did not exceed 20

minutes. Knowledge scores were calculated by giving one point for the correct answer and 0 points for the incorrect answer. Food safety knowledge scores range from 0 to 11; 0 indicates that there is no correct answer to any question, while 11 indicates that all answers are correct. Results from 0 to 5 represent poor knowledge of food safety, while 6 to 11 represent good knowledge of food safety.

**Pilot study:** The Primary study was conducted by presenting questionnaire forms to 27 women to ensure the validity and reliability of the questionnaire.

**Statistical analysis:** Data were analyzed using SPSS (Statistical Package Social Sciences, version 22). Descriptive statistics were conducted to determine frequencies, percentages, mean and standard deviation. Chi-square test was used to determine the association between variables of sample demographic characteristics and food safety knowledge scores of the participants and between variables of sample demographic characteristics and exposure to food poisoning of the participants, and also between variables of sample demographic characteristics and handwashing practice of the participants. The association between food safety knowledge scores and exposure to food poisoning, as well as the association between handwashing practice and exposure to food poisoning were determined. A significance level of $P \leq 0.05$ was used to establish significance.

## RESULTS

**Demographic characteristics of a study sample:** Table (1) presents the demographic characteristics of the sample. Approximately 42% of the participants in this study were between the ages of 18 and 25, and a high percentage of them (44.4%) were employees. The highest educational level of the participants (69.8%) was a university education.

**Table (1).** Demographic characteristics of a sample of women in Tripoli, Libya (N= 410).

| Demographic characteristics | Category | Number of participants | % of participants |
|---|---|---|---|
| Age | | | |
| | 18- 25 | 171 | 41.7 |
| | 26-35 | 119 | 29.0 |
| | 36-45 | 73 | 17.8 |
| | 46-55 | 36 | 8.8 |
| | ›55 | 11 | 2.7 |
| Occupation | | | |
| | Student | 142 | 34.6 |
| | Laborer | 37 | 9 |
| | Employee | 182 | 44.4 |
| | Retired | 2 | 0.5 |
| | Housewife | 47 | 11.5 |
| Education level | | | |
| | Uneducated | 3 | 0.73 |
| | Basic education | 10 | 2.43 |
| | Secondary education | 67 | 16.34 |

**Knowledge of food safety*:*** The results show that the majority of the participants have a good level of food safety knowledge scoring between 6-11 of the total score, with a percentage of 95.4% (391). Only 4.6% (19) of participants have a poor level of food safety knowledge, with total food knowledge scores between 0-5, as in Table (2). The mean of the food safety knowledge score of the participants was calculated to be 8.5±0.71, with the scores ranging from 0 -11.

**Table (2).** Food safety knowledge levels among the women's sample in Tripoli, Libya (N=410)

| Scores | Level | N* | % |
|---|---|---|---|
| 0 –5 | Poor | 19 | 4.6 |
| 6 – 11 | Good | 391 | 95.4 |
| Total | - | 410 | 100 |

*N = number of participants

Table (3) presents the knowledge of a sample of women in Tripoli, Libya regarding food safety. Results show that the majority of the participants (83.2%) were aware that food poisoning occurs due to pathogenic microorganisms. More than 80% of participants in

this study are aware of the correct answer that food poisoning can occur by spraying insecticides at meal preparation locations. A total of 62.0% of the participants were knowledgeable that food poisoning could occur due to the detergents used in the kitchen. More than 68 % of the participants knew that food poisoning could occur due to cookware used to cook meals and packages used to store them in the freezer. A high percentage of the sample (89%) were aware that food poisoning causes serious illnesses that end in the hospital and sometimes in death. More than 64 % of women participating in the study correctly answered that the number of pathogenic bacteria rapidly increases at room temperature. More than half of the participants (52.0 %) knew that any food must be disposed of if left at room temperature for more than two hours or one hour at a temperature higher than 32 $^{O}$C. A high percentage of participants (91.0%) realized that food poisoning could cause health and economic effects on society. Over eighty-six percent of participants were aware that children, pregnant women, and the elderly are likely more prone to the risk of food poisoning. The results showed that the majority of the participants (94.4%) gave correct answers that you should always wash your hands after coughing or sneezing. The results also showed that 86% were aware that handwashing with water only is not sufficient to remove the bacteria before touching the food.

**Table (3).** Food safety knowledge among women's sample in Tripoli, Libya (N=410).

| Statement | Correct answer N* (%) | Incorrect answer N* (%) | Do not know N* (%) |
|---|---|---|---|
| Food poisoning occurs due to pathogenic microorganisms. | 341 (83.2) | 12 (2.9) | 57 (13.9) |
| Food poisoning can occur by spraying insecticides at meal preparation locations. | 338 (82.4) | 32 (7.8) | 40 (9.8) |
| Food poisoning can occur due to the detergents used in the kitchen. | 254 (62.0) | 100 (24.4) | 56 (13.6) |
| Food poisoning can occur due to cookware used to cook meals and packages used to store them in the freezer. | 280 (68.3) | 79 (19.3) | 51 (12.4) |
| Food poisoning causes serious illnesses that end in the hospital and sometimes in death. | 365 (89.0) | 21 (5.1) | 24 (5.9) |
| The number of pathogenic bacteria rapidly increases at room temperature. | 264 (64.4) | 52 (12.7) | 94 (22.9) |
| Any food must be disposed of if left at room temperature for more than two hours or one hour at a temperature higher than 32C. | 215 (52.0) | 80 (20.0) | 115 (28.0) |
| Food poisoning can cause health and economic effects on society. | 373 (91.0) | 8 (2.0) | 29 (7.1) |
| Children and pregnant women and the elderly are likely more prone to the risk of food poisoning. | 356 (86.8) | 30 (7.3) | 24 (5.9) |
| You should always wash your hands after coughing or sneezing. | 387 (94.4) | 7 (1.7) | 16 (3.9) |
| Handwashing with only water is sufficient to remove the bacteria before touching the food. | 313 (76.3) | 86 (21.0) | 11 (2.7) |

*N = number of participants.

The association between demographic characteristics and level of food safety knowledge among women was examined by using the $X^2$ chi-square test. The results showed (Table 4) that there was a significant association between food safety knowledge scores and age and also between food safety knowledge scores and occupation ($P<0.05$). The findings also disclosed there was no statistically significant association between food safety

knowledge scores and education level (P› 0.05).

**Table (4).** Association between demographic characteristics and food safety knowledge level among the women's sample in Tripoli, Libya (N= 410).

| Variable | Total Knowledge scores | | P-value |
|---|---|---|---|
| | Poor | Good | |
| Age | | | |
| 18- 25 | 10 | 161 | 0.012 |
| 26-35 | 3 | 116 | |
| 36-45 | 1 | 72 | |
| 46-55 | 5 | 31 | |
| ›55 | 0 | 11 | |
| Occupation | | | |
| Student | 5 | 137 | 0.012 |
| Laborer | 5 | 32 | |
| Employee | 5 | 177 | |
| Retired | 0 | 2 | |
| Housewife | 4 | 43 | |
| Education level | | | |
| Uneducated | 0 | 3 | 0.169 |
| Basic education | 0 | 10 | |
| Secondary education | 6 | 61 | |
| University education | 13 | 273 | |
| High education | 0 | 44 | |

**Exposure to food poisoning:** The results in Figure (1) showed that 24% of the participants in the study reported that they were exposed to food poisoning after dinner in the celebration halls.

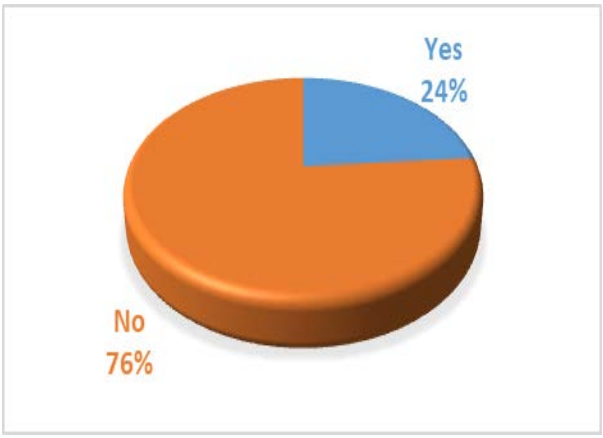


**Fig (1).** Exposure to Food poisoning as reported by the women's sample in Tripoli, Libya (N= 410).

Table (5) shows the symptoms of food poisoning reported by the women sampled in this study. More than half of participants who were exposed to food poisoning said that they developed diarrhea with other common symptoms, including vomiting (36%), abdominal pain (36%), nausea (25%), and fever (7%). While 20% of participants suffered from all the symptoms previously mentioned.

**Table (5).** Symptoms of food poisoning were reported by the women's sample in Tripoli, Libya (N=97).

| Symptoms | Number of participants | % of participants |
|---|---|---|
| Vomiting | 36 | 37.1 |
| Nausea | 25 | 25.8 |
| Abdominal pain | 36 | 37.1 |
| Diarrhea | 51 | 52.6 |
| Fever | 7 | 7.2 |
| All mentioned above | 20 | 20.6 |

Table (6) displays the type of food that likely caused food poisoning as mentioned by the women's sample. More than half, 54.6% and 51.5% of the participants, reported that chicken and meat were the likely food that caused poisoning, respectively. While 8.2% and 4.1% of the sample mentioned vegetables and rice, respectively. The statistical results showed an insignificant association between exposure to food poisoning of the participants and age (P=0.916), occupation (P=0.541), and education level (P=0.348).

**Table (6).** The type of food likely caused poisoning as mentioned by women's sample in Tripoli, Libya (N=97).

| Food | Number of participants | % of participants |
|---|---|---|
| Chicken | 53 | 54.6 |
| Meat | 50 | 51.5 |
| Vegetable | 8 | 8.2 |
| Rice | 4 | 4.1 |
| Others | 3 | 3.1 |
| Do not know | 7 | 7.2 |

Figure (2) illustrates the practice of handwashing of participants in the study before eating the meal in the celebration halls. Only 15% of women washed their hands always

before eating the meal in the celebration halls while 40% of them did not wash their hands. Using a chi-square test, there was no significant association (P› 0.05) between the hand-washing practice of participants and age (P=0.495), occupation (P=0.178), and education level (P=0.651). Also, the statistical results showed that there was a significant association (P‹0.05) between hand wash practice and exposure to food poisoning (P=0.007).

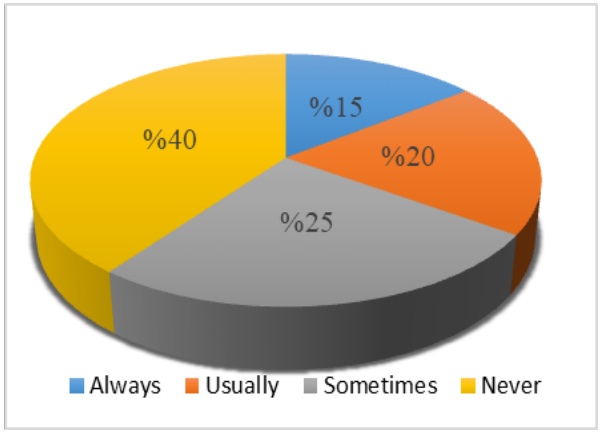


**Fig (2).** Washing hands practice before eating the meal in the celebration halls among the women's sample in Tripoli, Libya (N= 410).

**Perception of meals and waitresses:** Figure (3) shows a source of meals served at celebration halls as reported by participants. About 85% of participants indicated that commercial home kitchens were the source of meals at celebration halls.

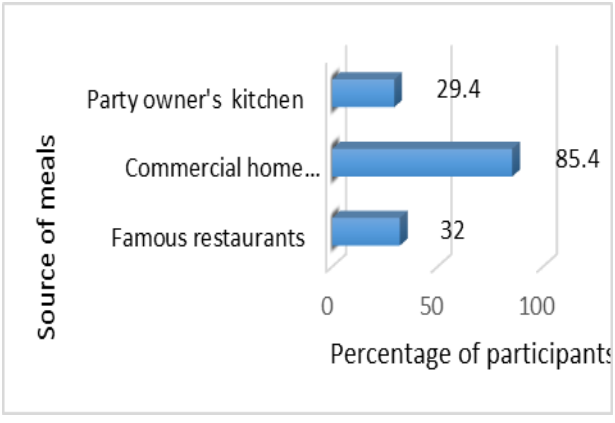


**Fig. 3.** Source of meals served at the celebration halls (N= 410).

Figure (4) shows how meals are served in the celebration halls as reported by participants. The responses by the women in the study showed that direct serving within dishes was the usual way of serving meals in the celebration halls (96.6%) while using a buffet was lower (29.8%).

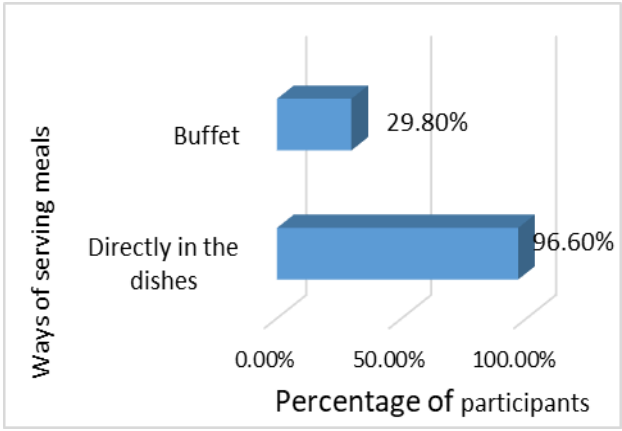


**Fig (4).** Ways of serving meals at celebration halls (N= 410).

As shown in Table (7), more than 13 % of the participants knew that the meals served in the halls were frozen and not fresh. In this study, 14.6% of participants knew that meals were heated sufficiently and appropriately before serving. A low percentage (7.8%) of the participants knew that meals were heated directly without thawing them. Also, a low percentage of a sample (6.1%) knew how much time that the meal is left after heating before serving it in the hall. More than the third (35.4%) of participants confirmed that in the case of a buffet, hot food was available on food warmers to keep it hot. Less than a quarter (21.7%) of participants also confirmed that in the case of the buffet, the cold food was inside bowls with ice to keep it cool. The majority of participants (96.7%) responded that they knew if dishes were washed before using or not. Of all participants, around 64% reported that the waitresses were wearing a special uniform. While 28.5% of participants affirmed that the waitresses wore headcovers. A low percentage (18.5%) mentioned that the waitresses wear gloves.

**Table (7).** Women's perception of meals and waitresses at the celebration halls in Tripoli, Libya (N=410).

| Statement | Yes N*(%) | No N*(%) | Some-times N*(%) | Do not know N*(%) |
|---|---|---|---|---|
| Meals served in the halls were frozen and not fresh. | 55 (13.4) | 71 (17.3) | 138 (33.7) | 146 (35.6) |
| Meals were heated sufficiently and appropriately before serving. | 60 (14.6) | 106 (25.9) | 170 (41.5) | 74 (18.0) |
| Meals heated directly without thawing them. | 32 (7.8) | 62 (15.1) | 95 (23.2) | 221 (53.9) |
| You knew how much time the meal left after heating before serving it in the hall. | 25 (6.1) | 146 (35.6) | 53 (12.9) | 186 (45.4) |
| In the case of a buffet, hot food was available on food warmers to keep it hot. | 145 (35.4) | 81 (19.7) | 102 (24.9) | 82 (20.0) |
| In the case of a buffet, the cold food was inside bowls with ice to keep it cool. | 89 (21.7) | 120 (29.3) | 99 (24.1) | 102 (24.9) |
| You know if dishes were washed before using or not. | 78 (19.0) | 119 (29.0) | 48 (12.0) | 165 (40.0) |
| Waitresses wore a special uniform. | 262 (63.9) | 35 (8.5) | 97 (23.7) | 16 (3.9) |
| Waitresses wore a head cover. | 117 (28.5) | 124 (30.0) | 142 (35.0) | 27 (6.50) |
| Waitresses wore gloves. | 76 (18.5) | 188 (45.9) | 109 (26.6) | 37 (9.0) |

*N = number of participants

**Attitude towards meal safety:** Table (8) illustrates the attitude of a sample of women towards meals safety served in celebration halls. The results show that only 22.4% of participants agreed that the food served inside the celebration halls is safe. Also, 21.5% of participants agreed that the meal served in the celebration halls is regulated by the respective authorities in the country. More than a quarter of participants (26.1%) agreed that the kitchens inside the celebration halls are monitored by the competent authorities in the country. A high percentage of participants (71.0%) agreed that the celebration owner is responsible for the safety of the meal served inside the celebration halls. While more than half of the participants (57.0%) agreed that the celebration hall owner is responsible for the safety of the meal served in the hall. From the answers above, participants need to be educated about laws relating to food safety responsibility. Less than the third (32.0%) of participants agreed that the celebration hall owner employs workers in the kitchens and waitresses without health certificates. Approximately 43 % of the tested sample agreed that commercial kitchens have licenses to prepare ready-to-eat meals for celebrations. It should also be noted that 44.0 % of participants agreed that private commercial kitchens owners use temporary laborers. Also, 30.5% of the participants agreed that private commercial kitchens use workers without health certificates.

**Table 8.** Attitude towards meals safety served in celebration halls among women's sample in Tripoli, Libya (N=410).

| Statement | Agree | Neutral | Disagree |
|---|---|---|---|
| Food served inside the celebration hall is safe. | 92 (22.4) | 241 (58.8) | 77 (18.8) |
| Meal served in the celebration hall is controlled by the competent authorities in the country. | 88 (21.5) | 105 (25.6) | 217 (52.9) |
| The Kitchens inside the celebration hall are monitored by the competent authorities in the state. | 107 (26.1) | 89 (21.7) | 214 (52.2) |
| The celebration owner is responsible for the safety of the meal served inside the celebration hall. | 291 (71.0) | 47 (11.4) | 72 (17.6) |
| The celebration hall owner is responsible for the safety of the meal served in the hall. | 233 (57.0) | 69 (17.0) | 108 (26.0) |
| The celebration hall owner employs workers in kitchens and waitresses without health certificates. | 131 (32.0) | 120 (29.0) | 159 (39.0) |
| Commercial kitchens have licenses to prepare ready-to-eat meals for celebrations. | 175 (42.7) | 126 (30.7) | 109 (26.6) |
| Private commercial kitchens owners use temporary laborers. | 181 (44.0) | 142 (35.0) | 87 (21.0) |
| Private commercial kitchens use workers without health certificates. | 125 (30.5) | 120 (29.3) | 165 (40.2) |

*N = number of participants.

## DISCUSSION

**Knowledge of food safety:** The study aimed to assess the knowledge of food safety, discover the incidence of food poisoning, evaluate the perception of meals and waitresses, and to know the attitude towards the meals served among a sample of women who attended celebrations in the celebration halls in Tripoli city, Libya. The results showed that the majority of the participants (95.4%) had a good level of food safety knowledge. Similarly, 77.7% and 14.1% of university students who participated in the study had a good and excellent level of food safety knowledge, respectively (Abuhlega et al., 2020).

The mean of the food safety knowledge score of the participants was 8.5±0.71, with the scores ranging from 0 -11. While the mean of the food safety knowledge score of the university students was a little lower (20.14±3.761 with scores ranging from 0 -29) (Abuhlega et al., 2020). The results of this study showed that the majority of the participants (83.2%) were aware that food poisoning occurs due to pathogenic microorganisms. Similarly, a high percentage of the sample of schools' boys (69.1%) were aware that food poisoning occurs due to pathogenic microorganisms (Almansour et al., 2016). While only 39.9% of the sample of consumers were aware of the role played by microorganisms (Langiano et al., 2012). Food poisoning is caused by the consumption of food or water contaminated with bacteria, viruses, or toxins of a biochemical or chemical nature such as ciguatera or pesticides. Biochemical or chemical agents in food can cause transient neurological symptoms or lead to death (CHP, 2017). More than 80% of participants in this study are aware of the correct answer that food poisoning can occur by spraying insecticides at meal preparation locations. A total of 62.0% of the participants were knowledgeable that food poisoning could occur due to the detergents used in the kitchen. More than 68 % of the participants knew that food poisoning could occur due to cookware used to cook meals and packages used to store them in the freezer. According to the information available, there are no studies carried out about the individuals' knowledge of the previous three questions. The result of this study also showed that a high percentage of the sample (89%) were aware that food

poisoning causes serious illnesses that end in the hospital and sometimes in death. Similarly, most of the food handlers and dietetic students (89.6%) knew that food poisoning can lead to death (Yusof et al., 2018). Also, 70.7% of the sample gave a correct answer to a similar question (Almansour et al., 2016). More than 64 % of women participating in the study correctly answered that the number of pathogenic bacteria rapidly increases at room temperature. Similarly, 75% of the U.S. department of agriculture's expanded food and nutrition education Program participants gave a correct answer to a question that bacteria responsible for causing foodborne illness grows at room temperature (Meer & Misner, 2000).

While only 56.3% of participants in the study conducted among school students correctly answered the same question (Meer & Misner, 2000). More than half of the participants (52.0 %) knew that any food must be disposed of if left at room temperature for more than two hours or one hour at a temperature higher than 32C. In a study carried out by (Association & Foods, 1999), 69% of a sample were knowledgeable that keeping food at room temperature or contamination of food after cooking causes food poisoning. A high percentage of participants (91.0%) realized that food poisoning could cause health and economic effects on society. Similarly, 97.8% of the sample of veterinary medicine students also realized the previous fact (Stratev et al., 2017). In their study, over eighty-six percent of participants were aware that children, pregnant women, and the elderly are more likely prone to the risk of food poisoning. Similarly, a high percentage of correct answers (95.6%) was also found among a sample of veterinary medicine students (Stratev et al., 2017), while approximately 70% of respondents of institutional food handlers knew that the elderly are likely more prone to the risk of food poisoning (Akabanda et al., 2017). In the current study, the results showed that the majority of the participants (94.4%) gave correct answers that you should always wash your hands after coughing or sneezing. Similarly, 92.0 % of a sample of school students gave a correct answer to a similar question (KojoPrah et al., 2017). In this study, the results also showed that 86 % were aware that handwashing with water only is not sufficient to remove the bacteria before touching the food. Similarly, 78.2% of a sample of school students gave correct answers to a similar question (Norazmir et al., 2012), while the percentage was lower (45.4%) among a sample in a study conducted on the same category (KojoPrah et al., 2017). The outcome of this study also showed that a significant association was found between food safety knowledge scores and age and also between food safety knowledge scores and occupation, while there was no statistically significant association between food safety knowledge scores and education level. On the contrary, in the study conducted on a sample of mothers, a significant association was found between food safety knowledge and the education level of mothers (Ayaz et al., 2018).

**Exposure to food poisoning:** The result of this study showed that 24% of the participants reported that they were exposed to food poisoning after having dinner in the celebration halls. Outbreaks of food poisoning at wedding parties or other parties are not rare events and occur in developing and developed countries (Al-Bassam et al., 2001; Ayaz et al., 2018; Gaulin et al., 2002; RE, 1996) The result also showed that more than half of the participants who were exposed to food poisoning mentioned they developed diarrhea with other common symptoms, including vomiting (36%), abdominal pain (36%), nausea (25%), and fever (7%), while 20% of participants suffered from all the symptoms previously mentioned. Similar symptoms were reported by (Jiang et al. 2003), who found that 85 of the 280 guests (45 males and 40 females) developed symptoms of food poisoning after eating lunch at the wedding party, including watery diarrhea (100.0%), ab-

dominal pain (83.5%), vomiting (32.9%), nausea (29.4%), dizziness (16.5%), and fever (10.6%). In a similar study, it was mentioned that 88 guests became sick after having a meal at the wedding ceremony (21.6% males and 78.4% females) and they developed diarrhea, with other common symptoms of colicky abdominal pain (94.3%), fever (86.4%), vomiting (64.8%), headache (48.9%), and nausea (30.7%) (Aljoudi et al., 2010).

In this study, more than half, 54.6% and 51.5% of the participants mentioned that chicken and meat were the likely food that caused poisoning, respectively, while 8.2% and 4.1% of participants reported that vegetables and rice were the cause, respectively. In another study, the cold dish (wine preserved chicken, slices of pork tongue, slices of abalone, shrimp balls, and shellfish) was considered a likely cause of the food poisoning outbreak (Jiang et al., 2003), while others reported that the cause of food poisoning at the wedding party was eating meat and rice contaminated with salmonella (Aljoudi et al., 2010). The results of this study also showed that only 15% of women always washed their hands before eating the meal in the celebration halls. The outcome of this study also showed that there was a significant association between handwashing practice and exposure to food poisoning of the participants. The preparation and eating of food with contaminated hands cause transmission of pathogenic microorganisms from the food to the human body and therefore the development of diseases (Iyasu et al., 2017). Forty-four of the respondents consistently forgot to wash their hands properly before meal preparation (Association & Foods, 1999). In another study that assessed the practice of handwashing, 43% of respondents in the study reported they did not always wash their hands before preparing food (Food and Drink Federation, 2001).

**Perception of meals and waitresses:** The results showed that about 85% of participants indicated that commercial home kitchens were the source of meals at celebration halls. The previous point should be taken into account, as these kitchens are mostly out of the authorities' control. It was reported that around 10-20% of all food poisoning outbreaks in celebrations were related to catering services because they very often work outdoors and their facilities are relatively poor (Jiang et al., 2003). To prevent food poisoning outbreaks, caterers and restaurateurs need to ensure that food handlers have received adequate training and use appropriate practices of food handling when serving food to large groups of people (Sloan-Gardner et al., 2014). The results also showed that the responses of the women in the study showed that serving directly in the dishes was the dominant way of serving meals in the celebration halls (96.6%) while using a buffet was lower (29.8%). Catering buffet meals need special care. Therefore, attention should be paid to controlling food temperature so that hot food should be kept at an internal temperature of 60 °C or warmer, and cold food should be kept at 4 °C or colder as well as the safe handling of food in terms of cleanliness, separation, cooking and chilling. It was reported that 56% of people (125/225) became ill following the buffet lunch at the restaurant which was significantly associated with the consumption of curried prawns and Caesar salad (Sloan-Gardner et al., 2014).

In the present study, more than 13 % of the participants knew that meals served in the halls were frozen and not fresh. In this study, 14.6% of participants knew that meals were heated sufficiently and appropriately before serving. A low percentage (7.8%) of the participants knew that meals were heated directly without thawing them. Also, a low percentage of a sample (6.1%) knew how much time the meal was left after heating before distributing it in the hall. It is clear from the above results that the lack of knowledge of the previous details about meals may be due to embarrassment or lack of attention to these details.

More than a third (35.4%) of participants confirmed that in the case of a buffet, hot food was available on food warmers to keep it hot. Less than a quarter (21.7%) of participants also confirmed that in the case of a buffet, the cold food was inside the bowls with ice to keep it cool. The majority of participants (96.7%) responded that they knew if dishes were washed before they use or not. Of all participants, around 64% reported that the waitresses wore a special uniform. While 28.5% of participants affirmed that the waitresses wore a headcover. A low percentage of a sample (18.5%) mentioned that the waitresses wore gloves. From the results above, it is very clear that the meals served in celebration halls need to be regulated and monitored by responsible sectors besides, the results show that the improper practices of waitresses related to uniforms, head cover, and gloves may reflect a lack of their knowledge about food safety rules. In line with these results, it was reported that catering employees did not receive enough education about food safety rules that qualify them to apply them in the workplace (Hertzman & Barrash, 2007).

**Attitude towards meal safety:** The results showed that only 22.4% of participants agreed that the food served inside the celebration halls is safe. Also, only 21.5% of participants agreed that the meal served in the celebration halls is regulated by the respective authorities in the country. More than a quarter of a sample (26.1%) agreed that the kitchens inside the celebration halls are monitored by the respective authorities in the country. It is worth mentioning that intensive efforts should be made to raise the confidence level of an individual in the food available anywhere served to the public. Similarly, it was found that the confidence of people in food safety systems began to be lower (Shen et al., 2015).

The results also showed that a high percentage of participants (71.0%) agreed that the celebration owner is responsible for the safety of the meal served inside the celebration halls. While more than half of the participants (57.0%) agreed that the celebration hall owner is responsible for the safety of the meal served in the hall. From the answers above, participants need to be educated about laws relating to food safety responsibility. Educating people, including women, about all food safety issues is one of the responsibilities of food safety agencies (Zeeshan et al., 2017).

The outcome of this study also showed that less than a third (32.0%) of participants agreed that the celebration hall owner employs workers in the kitchens and waitresses without health certificates. Approximately 43 % of the participants agreed that commercial kitchens have licenses to prepare ready-to-eat meals for celebrations. It should also be noted that 44.0 % of participants agreed that private commercial kitchen owners use temporary laborers. Also, 30.5% of the participants agreed that private commercial kitchens use workers without health certificates. The answers above illustrate the important role that the respective authorities should play in eliminating the negative perception of individuals about food safety practices in the celebration halls by preventing all negative practices related to the conditions of employment of workers, and the license of the catering profession.

## CONCLUSION

In conclusion, the study found that the majority of women possessed sufficient knowledge of food safety rules. The study also found that twenty-four percent of them reported that they were exposed to food poisoning after eating dinner in the celebration halls, and a high percentage of them considered that the food served inside the celebration hall was unsafe. A low percentage of them always washed their hands before eating the meal in the celebration halls. A significant association was found between handwashing practice and food poisoning exposure. Therefore, to prevent food poisoning in the celebration halls, some steps should be achieved as fol-

lows: (I) Meals source; famous restaurants and commercial home kitchens must be under the control of responsible authorities. (II) Meal handlers in the kitchens which prepare food must be trained on good food safety practices and not allowed to work without a certificate of attending a food safety course. (III) Raise the awareness level of the celebration owner so that he/she brings meals from a place that has a catering license by various means, such as distributing guidance leaflets in the celebration halls. (IV) In the case of a buffet, the hall should be provided with equipment to keep hot food at 60°C or more and cold food at 4°C or less until eaten by guests. (V) Waitresses in the celebration halls must have a health certificate as well as a certificate of attending a course in food safety, especially how to deal with frozen and fresh food ready to eat. (VI) Women who attend the celebrations in the halls should be educated about the importance of washing hands before eating. (VII) Raise the level of awareness of women about the importance of asking officials in the halls and waitresses about the meal in terms of its source, ingredients, and handling conditions.

**Future studies**

- Assessment of the availability of health requirements in commercial kitchens in terms of the building, employees, source of meal ingredients, cooking methods, type of cooking utensils and cookware, storage conditions, and packaging.
- Exploring knowledge and practices of food safety among food handlers in commercial kitchens.
- Evaluation of kitchens in the celebration halls in terms of their readiness to serve meals inside the hall.
- Evaluation of food safety knowledge and practices among waitresses in the celebration halls.

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# معرفة قواعد سلامة الغذاء لدى النساء وتصورهن وموقفهن من تطبيقها في قاعات الاحتفالات في طرابلس، ليبيا

**ثريا أحمد أبو حليقة*، درصاف علي التركي**

قسم علوم وتقنية الأغذية، كلية الزراعة، جامعة طرابلس، طرابلس، ليبيا

تاريخ الاستلام: 24 ديسمبر 2021/ تاريخ القبول: 14 يوليو 2022
Doi:https://doi.org/10.54172/mjsc.v37i3.581

**المستخلص** : الحكومة من خلال مؤسساتها ومراكزها المعنية مسؤولة عن حماية المستهلكين م ن خ لال ض مان س لامة الإمدادات الغذائية حتى الاستهلاك. سعت الدراسة إلى تقييم المعرفة بسلامة الغذاء، واكتشاف حالات التسمم الغ ذائي، وتقي يم تصور الوجبات والنادلات، ومعرفة الموقف من الوجبات المقدمة لدى عينة من النساء اللاتي حضرن الاحتفالات في قاع ات الاحتفالات بمدينة طرابلس، ليبيا. تم اختيار ما مجموعة 410 امرأة بشكل عشوائي، وجُمعت البيانات عن طري ق المق ابلات وجهاً لوجه باستخدام استمارات الاستبيان. تم استخدام مربع كاي(Chi-square) من أجل اختبار الاستقلالية. غالبية المشاركات (41.7٪) تتراوح أعمارهن بين 18 و25 سنة. أظهرت النتائج أن غالبية المشاركات (95.4٪) لديهن مستوى جيد من المعرفة بسلامة الغذاء. كما أظهرت النتائج وجود ارتباط معنوي ($P<0.05$) بين العمر ونوع التوظيف للمشاركات، ودرجات المعرفة بسلامة الغذاء. أفادت 24% من المشاركات أنهن تعرضن للتسمم الغذائي بعد تناول العشاء في قاعات الاحتفال. خمسة عش ر بالمئة فقط من النساء المشاركات يغسلن أيديهن دائمًا قبل تناول الوجبة في قاعات الاحتفال. بينت النتائج وجود ارتباط معنوي ($P<0.05$) بين ممارسة غسل اليدين والتعرض للتسمم الغذائي. وافقت 22.4٪ فقط من المشاركات على أن الغذاء المقدم داخل قاعة الاحتفال آمن. من الواضح جدًا أن الوجبات المقدمة في قاعات الاحتفال تحتاج إلى رقابة من قبل السلطات المسؤولة. إلى جانب ذلك، يجب على الجهات المختصة أن تعمل جاهدة لإزالة التصور السلبي للأفراد حول ممارسات س لامة الغ ذاء ف ي قاعات الاحتفال.

**الكلمات المفتاحية:** سلامة الغذاء؛ التسمم الغذائي؛ المعرفة؛ النساء؛ قاعة الاحتفالات.

* ثريا أحمد أبوحليقة: t.abuhlega@uot.edu.ly قسم علوم وتقنية الأغذية، كلية الزراعة، جامعة طرابلس، طرابلس، ليبيا.

*Al-Mukhtar Journal of Sciences* 37 (3): 218-225, 2022
*ISSN: online 2617-2186 print 2617-2178*
**Journal Homepage** https://omu.edu.ly/journals/index.php/mjsc/index

# Measurement of Radium and Radon Exhalation Rate in Marble Samples used in Al-Bayda City Market-Libya

**Jemila Mussa Ali*, Suhayla Abdelhady Ibrahim**

*Department of Physics, Faculty Of Science Omar AL-Mukhtar University, Al-Bayda, Libya*

Received: 16 February 2022/ Accepted: 10 August 2022
Doi: https://doi.org/10.54172/mjsc.v37i3.626

**Abstract:** The aim of the present study is to measure the activity concentrations of $^{226}$Ra, $^{222}$Rn, the mass exhalation rate of $^{222}$Rn, and the annual effective dose of radon in marble samples collected from Al-Bayda city local market –Libya. Samples were measured by using a low-background NaI (Tl) detector. The average activity concentrations of $^{226}$Ra and $^{222}$Rn were 72.57 Bq.kg$^{-1}$ and 597.85 Bq.m$^{-3}$.The radon exhalation rate in marble samples vary from 0.05-0.30 Bq.kg$^{-1}$.S$^{-1}$ with an average of 0.13 Bq.kg$^{-1}$.S$^{-1}$. The annual effective dose of radon was calculated in samples under investigation. For most samples, the values were lower than the maximum permissible dose limits. It can be concluded that marble samples under investigation do not pose any radiological hazard to the dwellers of buildings used in their construction.

**Keywords:** Marble, NaI (Tl) Detector, Annual Effective Dose of Radon, Radon Exhalation Rate.

## INTRODUCTION

The human body is naturally exposed to ionizing radiation, which can be found in soils, rocks, and water (Abo-Elmagd, 2014). In addition, artificial radiation was added to this background radiation. The background radiation arises from natural sources present in natural ores, such as some building materials. This radiation is due to primordial radionuclides of the natural radioactive series of Thorium-232 ($^{232}$Th) and uranium-238 ($^{238}$U) series and their decay products(Ghose et al., 2012). These radionuclides are widely distributed and their concentrations depend on the geological conditions. Therefore, it is important to measure the natural activity of all building materials. This step will help to assess the possible radiological risks to human health (Kumara et al., 2018). This radioactive isotope results from the disintegration of radium-226 ($^{226}$Ra), a decay product of the $^{238}$U series and responsible for the largest source of natural radiation to which the population is exposed (Kama et al., 2011). Radon and Thoron are both generated from radium decay in the solid grains, then migrated to a significant distance from the site of generation in rock, soil, and building materials into the atmosphere (exhalation) before undergoing radioactive decay (Bala et al., 2017). Radon-222 ($^{222}$Rn) concentration can reach high levels in buildings depending on exhalation from the building material used, such as concrete, marble, or granite. Marble is one of the metamorphic rocks found to occur on the earth's surface. The colors of the marble depend on the mineral composition and metamorphism. Marbles are used commonly as floor laying material (Kaiser et al., 1999).

***Corresponding author:** Jemila Mussa Ali: *Jemila.mussa@omu.edu.ly* , Department of Physics, Faculty Of Science Omar AL-Mukhtar University, Al-Bayda, Libya

In this study, the gamma radiation has been measured in marble samples collected from the Al-Bayda local market in Libya to obtain an activity concentration of $^{226}$Ra, radon exhalation rate, and the annual effective dose of radon. Because of health risks caused by exposure to indoor radiation, many international organizations, such as the International Commission on Radiological Protection (ICRP,. 1993), the World Health Organization (WHO,. 2021), and UNSCEAR, have adopted strong measures in order to reduce such exposure.

## MATERIALS AND METHODS

Samples Collection and Preparation: Nine marble samples were collected from the local market in Al-Bayda city – Libya, to measure the radioactivity concentrations of $^{226}$Ra and $^{222}$Rn (Marble available in Libya, some local and some imported.). All samples were brought to the laboratory and properly cataloged, washed, and dried (at 110C$^{o}$ for 2h) for complete removal of moisture. Then, all samples were crushed to a fine powder with a particle size ≈1 mm (This process took place in the laboratory of the Faculty of Engineering- Omer Al-Mukhtar University, Al-Bayda, Libya). Samples were packed and sealed in radon impermeable airtight cylindrical plastic containers, then stored for four weeks before counting to ensure $^{226}$Ra and its short-lived daughters reached secular equilibrium (Sroor, 2013). Table (1) shows the description of the samples.

Gamma-Ray Detection System: A gamma-ray NaI(Tl) scintillation detector contains a 3"×3" crystal, with a multichannel analyzer (MCA) used for the spectral measurements of naturally occurring radionuclides. The detector was placed in the center of a two-layered shield made from stainless steel of 10 mm thickness, and lead of 30 mm thickness. The shield must be used to reduce the radioactive background, as well as the detector from unwanted background radiation, and reduce the contribution of scattered radiation. After that, the sample was placed on a detector for 7200 S. The spectra were analyzed using a software program. The samples were prepared and measured in the Advanced Nuclear Lab-Department of Physics-Faculty of Science - Omer Al-Mukhtar University, Al-Bayda, Libya.

**Table (1).** Description of marble samples.

| Samples code | Trade Name | Country of manufacture |
|---|---|---|
| M1 | Brak El-Shati | ( made in Libya ) |
| M2 | Classic Paradiso | ( made in India ) |
| M3 | Galaxy | ( made in India ) |
| M4 | Monty Clad | (made in India ) |
| M5 | Ten Brown | ( made in India ) |
| M6 | Oriental Cream | (made in Oman ) |
| M7 | Self-Marble | (made in Egypt ) |
| M8 | Miral Lacquer Marble | (made in Turkey ) |
| M9 | Mirwan Marble | (made in Spain) |

**Activity Concentration:** The activity concentration (A) of a radionuclide for a peak at energy, is given by the relation (Al-Sewaidan, 2019):

$$A = \frac{N}{\varepsilon I_{\gamma} t m} \quad (1)$$

Where: ε is the absolute efficiency at photopeak energy, t is the time of the sample spectrum collection in seconds, $I_{\gamma}$is the intensity of emitted gamma-ray (gamma abundance), m is the mass of the sample in (kg), N is the number of count in a given peak area corrected for background peaks of a peak at energy.

**Radon Mass Exhalation Rate:** The emanation rate coefficient and factor of $^{222}$Rn that can diffuse through the raw and building materials is known as the emanation coefficient. The emanation coefficient (CRn) is a very important radiological index that can be used to determine the amount of the $^{222}$Rn emanated fraction released from building raw mate-

rials and products containing naturally occurring radionuclides such as $^{226}Ra$ in radioactivity equilibrium with parents. The emanation rate is estimated by measuring gamma-rays from the radon decay daughter products, $^{214}Pb$ and $^{214}Bi$. Assuming an equilibrium state:

$$C_{Rn} = (A_{Ra} - A_D) \times \rho \quad (2)$$

Where $A_{Ra}$ is the measured activity of $^{226}Ra$, $A_D$ is the measured activity of daughter element $^{214}Pb$ (or $^{214}Bi$), which escapes into the surrounding environment. ρ is the density of radon (9.73 $kg.m^{-3}$). The introduction of the radon emanation factor F, which is defined as:

$$F_{Rn} = \frac{A_{Ra} - A_D}{A_{Ra}} \quad (3)$$

The radon exhalation rate $E_{Rn}$ ($Bq.kg^{-1}.S^{-1}$) is the product of the emanation factor, and the $^{222}Rn$ production rate was determined by used relation (Turhan & Gündüz, 2008):

$$E_{Rn} = F_{Rn} \cdot A_{Ra} \cdot \lambda_{Rn} \quad (4)$$

Where: $\boldsymbol{\lambda_{Rn}}$ is the decay constant of $^{222}Rn$ ($2.1x10^{-6}$ $S^{-1}$).

**Annual Effective Dose of Radon:** Radon concentration was converted into an effective dose, as the long-standing exposure to a high concentration of radon, and its progenies, may lead to pathological effects like lung cancer. The annual effective dose, received by workers and residents due to inhalation of radon gas and its decay products, where calculated by relation (Abd El-Halim, 2019):

$$AED_{Rn} = \frac{C_{Rn \times 0.4 \times K \times H}}{3700\ Bq.m^{-3} \times 170h} \quad (5)$$

Where $AED_{Rn}$ is the annual effective dose ($mSv.y^{-1}$), $C_{Rn}$ is the emanation coefficient of radon ($Bq.m^{-3}$), K is the ICRP dose conversion factor (5 mSv $WL.M^{-1}$ for occupational workers, and 3.88 $mSvWLM^{-1}$ (effective dose per unit Work Limit in Month) for the general public), H is the annual occupancy at the location, 2160 h for workers and 7000 h for residents (80% of total time) and 170 is exposure hours taken for $WL.M^{-1}$ (ICRP, 1993).

## RESULTS

The present values in Table (2) show, the activity concentration of $^{226}Ra$, $^{222}Rn$, radon emanation factor $F_{Rn}$, Radon mass exhalation rate $E_{Rn}$, and the annual effective dose from radon ($AED_{Rn}$). The activity concentrations of $^{226}Ra$ ranged between (26.03-148.58) $Bq.kg^{-1}$, and the average value was 72.57 $Bq.kg^{-1}$. For $^{222}Rn$, the activity concentrations varied between (240.33 – 1392.85) $Bq.m^{-3}$, with an average 597.85 $Bq.m^{-3}$, as shown in Figure (1).

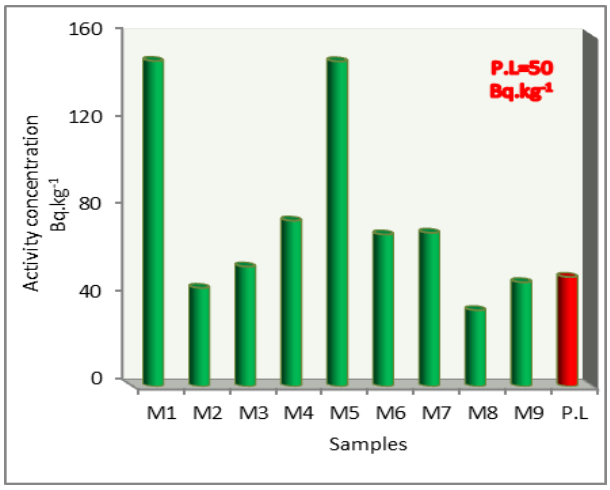


**Figure (1)**. The activity concentrations of $^{226}Ra$ in marble samples (P.L: Permissible Level).

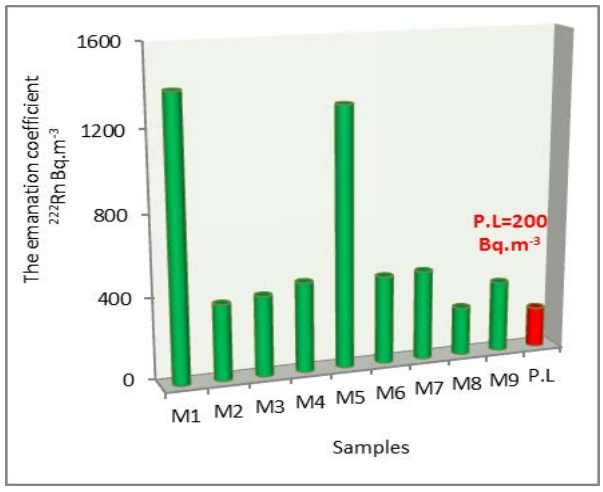


**Figure (2)**. The emanation coefficient of $^{222}Rn$ $Bq.m^{-3}$ in marble samples (P.L: Permissible Level).

The results show that there is a variation in radon exhalation rate from one sample to another, depending on the geological formation of the region from which the sample is taken. The variation in values of radon exhalation rate may be due to the differences in radium content and porosity of the marble (Frutos-Puerto et al., 2020).

**Table (2):** The values of activity concentrations of $^{226}$Ra Bq.kg$^{-1}$, emanation coefficient $^{222}$Rn Bq.m$^{-3}$, $E_{Rn}$ and $AED_{Rn}$ in marble samples.

| Samples Code | $A_{Ra}$ Bq.kg$^{-1}$ | $C_{Rn}$ Bq.m$^{-3}$ | Ern*10$^{-3}$ Bq.kg$^{1}$.S$^{-1}$ | $AED_{Rn}$ mSv.y$^{-1}$ | |
|---|---|---|---|---|---|
| | | | | for worker | for residents |
| M1 | 148.58 | 1392.85 | 0.30 | 9.57 | 24.06 |
| M2 | 26.03 | 379.76 | 0.08 | 2.61 | 6.56 |
| M3 | 32.44 | 399.90 | 0.09 | 2.75 | 6.91 |
| M4 | 75.69 | 446.60 | 0.10 | 3.07 | 7.71 |
| M5 | 148.39 | 1286.89 | 0.28 | 8.84 | 22.23 |
| M6 | 69.407 | 437.82 | 0.09 | 3.01 | 7.56 |
| M7 | 70.17 | 445.05 | 0.10 | 3.06 | 7.69 |
| M8 | 34.84 | 240.33 | 0.05 | 1.65 | 4.15 |
| M9 | 47.60 | 351.45 | 0.08 | 2.41 | 6.07 |
| Max | 148.58 | 1392.85 | 0.30 | 9.57 | 24.06 |
| Min | 26.03 | 240.33 | 0.05 | 1.65 | 4.15 |
| Average | 72.57 | 597.85 | 0.13 | 4.11 | 10.33 |

The present values of the radon exhalation rate observed in the marble ranged between 0.05-0.30 Bq.kg$^{-1}$.S$^{-1}$ (Turkey -Libya) with a 0.13 Bq.kg$^{-1}$.S$^{-1}$ average, as shown in Figure (3).

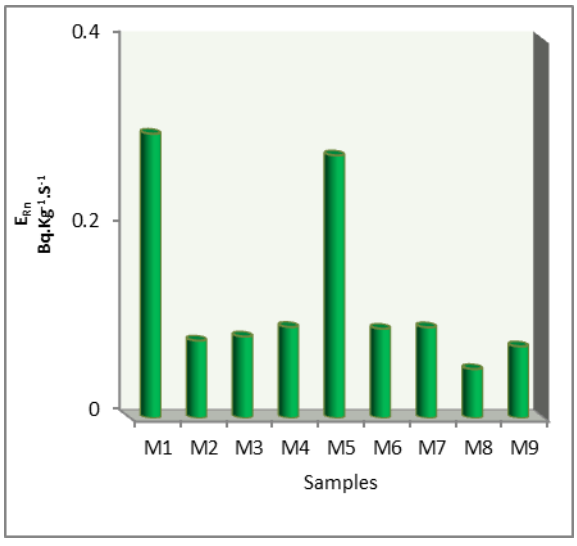


**Figure (3):** The mass exhalation of radon in marble samples.

From Figure (4), the variation of radon mass exhalation rate with $^{226}$Ra activity concentrations shows a correlation between them. Therefore, it can be concluded that it is possible to predict the radon exhalation rate from the activity concentration of radium.

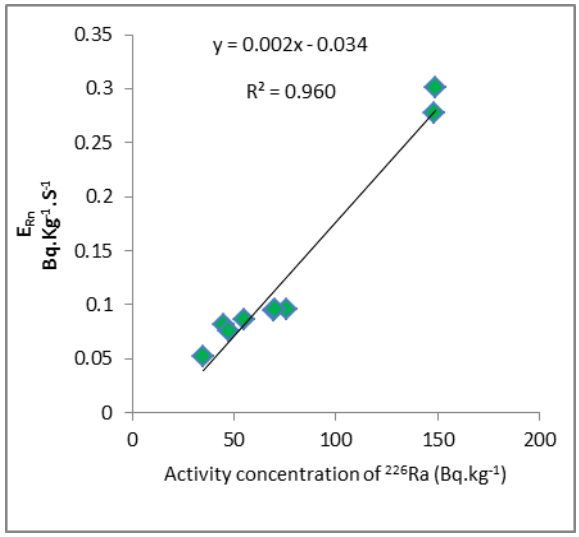


**Figure (4):** Correlation between $^{226}$Ra activity concentration and radon exhalation rate in the marble samples.

The results indicate low levels of an annual effective dose from radon in marble samples for workers and residents, except samples M1 and M5 (made in Libya and India) for residents. This result is due to the increased exposure time for residents, as shown in Figure (5).

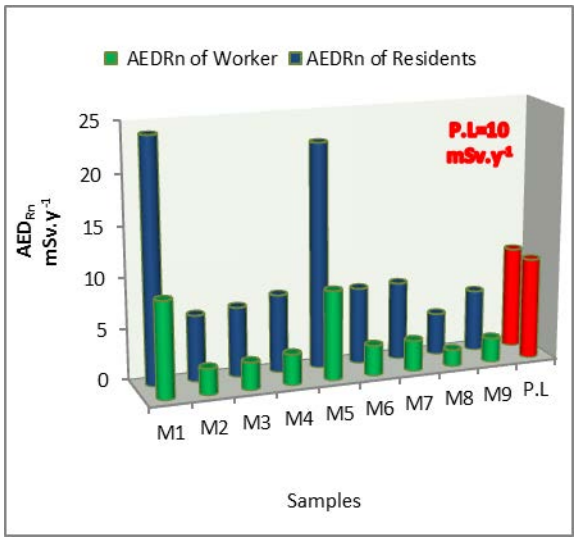


**Figure (5):** The annual effective dose of radon in marble samples (P.L: Permissible Level).

## DISCUSSION

The results have shown that the activity concentration of $^{226}Ra$ for most samples is higher than the world value of 50 $Bq.kg^{-1}$(WHO, 2021), and for $^{222}Rn$, the values are higher than the average permissible level of 200 $Bq.m^{-3}$ (Jasaitis & Girgždys, 2007; Sharma et al., 2016). When comparing values of the radon exhalation rate between the different samples of marble, we found that the present value of the radon exhalation rate in sample M1 is higher than the values of other samples. The results indicate that low levels of an annual effective dose from radon in most marble samples were lower than the maximum permissible dose limits (10 $mSv.y^{-1}$) recommended by (ICRP,. 1993).

**Table (3).** Comparison values of radium concentration, radon exhalation rate and of marbles samples used in different countries.

| Countries | Radium concentration $Bq.kg^{1}$ | Radon exhalation rate × $10^{-3}$ $Bq.kg^{1}.S^{-1}$ | References |
|---|---|---|---|
| Egypt | 17.60-120.03 | 0.015-0.14 | (Sroor, 2013) |
| India | 31.90-44.60 | 53.75- 66.78 | (Bala et al., 2017) |
| Morocco | 18 | $1.66\times10^{-3}$ | (Kassi et al., 2018) |
| Spin | 4.9–40.7 | $(2.8\text{-}6)\times10^{-3}$ | (Frutos-Puerto et al., 2020) |
| Presents work | 26.03-148.58 | 0.05-0.30 | |

## CONCLUSION

The obtained results showed that the average radium and radon concentration in investigation samples varies from 72.57 $Bq.kg^{-1}$ and 597.85 $Bq.m^{-3}$. Also, the obtained value of radon mass exhalation rate varies between 0.05-0.30 $Bq.kg^{-1}.S^{-1}$ (Turkey -Libya) with an average of 0.13 $Bq.kg^{-1}.S^{-1}$. It is recommended that the radon exhalation rate should be measured for all building materials and a standard code placed on all products. The annual effective dose of radon in marble samples for workers and residents is lower than the maximum permissible dose limit $10mSv.y^{-1}$ recommended by (ICRP, 1993), with the exception of samples M1 and M5 (made in Libya and India) for residents. The variation in obtained results depends on the geological formation of the region and the increased exposure time. The annual effective dose limit and the activity concentration index show that the investigated samples are

within the recommended safety limit and do not pose any source of radiation hazard. Therefore, the use of these materials in the construction of dwellings is considered to be safe for inhabitants.

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# قياس الراديوم ومعدل الزفير للرادون في عينات الرخام المستخدمة في سوق مدينة البيضاء – ليبيا

**جميلة موسى علي *، سهيلة عبد الهادي إبراهيم**

*قسم الفيزياء، كلية العلوم، جامعة عمر المختار، البيضاء، ليبيا*

تاريخ الاستلام: 16 فبراير 2022 / تاريخ القبول: 10 أغسطس 2022

Doi: https://doi.org/10.54172/mjsc.v37i3.626

**المستخلص** : الهدف من هذه الدراسة هو قياس تراكيز نشاط الراديوم-226 والرادون-222، ومعدل الزفير للرادون-222، والجرعة الفعالة السنوية من غاز الرادون في عينات الرخام التي تم جمعها من السوق المحلي لمدينة البيضاء – ليبيا ، تم قياس العينات بواسطة باستخدام كاشف ايوديد الصوديوم المنشط بالثاليوم NaI (Tl) منخفض الخلفية. كان متوسط تركيزات النشاط للراديوم-226 والرادون- 222 72.57 بيكرل/ كجم و 597.85 بيكرل/ م3 ويتراوح معدل الزفير الشامل في عينات الرخام من 0.05-0.30 بيكرل/ كجم. ث بمتوسط 0.13 بيكرل/ كجم. ث. تم حساب الجرعة الفعالة السنوية من الرادون في العينات قيد الدراسة، وكانت القيم بالنسبة لمعظم العينات أقل من الحد الأقصى للجرعات المسموح بها. يمكن الاستنتاج أن عينات الرخام في هذه الدراسة لا تشكل أي خطر إشعاعي على سكان المباني التي استخدم في تشيدها.

**الكلمات المفتاحية** : الرخام، كاشف ايوديد الصوديوم، الجرعة الفعالة السنوية للرادون، معدل الزفير للرادون.

*[1] جميلة موسى علي: Jemila.*mussa@omu.edu.ly*، قسم الفيزياء، كلية العلوم، جامعة عمر المختار، البيضاء، ليبيا

*Al-Mukhtar Journal of Sciences* 37 (3): 226-250, 2022
*ISSN: online 2617-2186 print 2617-2178*
**Journal Homepage** https://omu.edu.ly/journals/index.php/mjsc/index

# Adverse Effects of Organochlorine Pesticide Residues on Biochemical Parameters and Oxidative Stress in Libyan Agricultural Workers

**Khaled S. Al salhen[1*], Omalsaad E. I. Omar[2]**

[1] *Department of Biochemistry, Faculty of Medicine, Derna University, Derna, Libya*
[2] *Department of Biochemistry, Faculty of Medicine, Omar Al-Mukhtar University, Al-Bayda, Libya*

Received: 06 April 2022/ Accepted: 21 July 2022
Doi: https://doi.org/10.54172/mjsc.v37i3.742

**Abstract:** Agricultural workers are in danger of being exposed to toxic substances such as pesticides. To estimate the individual danger of handling pesticides, the bio-monitoring of effects on agricultural workers is required. There has been no such research previously conducted among Libyan agricultural workers. This research was designed to study the biochemical parameters impacts of the pesticide contamination among Libyan agricultural workers at Aljebal Alakhtar, Libya. 45 blood samples were taken from male agriculture workers at Aljebal Alakhtar who had been exposed to pesticides in crop fields for a long time, while 25 blood samples were taken from a group of people who had not been exposed to pesticides (control). Kits were used to assess plasma ALT, AST, ALP, GST, SOD, total protein, albumin, globulin, total bilirubin, total cholesterol, triglycerides, HDL-C, LDL-C, VLDL-C, urea, and creatinine. The thiobarbituric acid reactive substances (TBARS) assay was used to evaluate lipid peroxidation in serum. Using a gas chromatography-electron capture detector, the blood samples were tested for organochlorine pesticide residues (GC-ECD). In comparison to controls, workers had significantly higher SOD, GST, ALP, AST, and ALT activities, as well as higher levels of lipid profile, total bilirubin, creatinine, and urea, as well as significantly higher TBARS concentrations. Furthermore, long-term pesticide exposure was also related to reduceing total protein, albumin, and globulin, as well as reduced HDL-C levels. Pesticide exposure seems to influence various biochemical markers in general. These biomarkers appear to be indicative of pesticide-related deleterious effects in agricultural workers, indicating that they should be used for routine monitoring of impacts.

**Keywords:** Lipid profile; Liver function; Kidney function; Oxidative stress biomarkers.

## INTRODUCTION

The negative effects of persistent organic pollutants (POPs) on the environment have been a prominent topic of debate around the world. These chemicals are poisonous, and because of their propensity to be transported long distances and released by water or wind, they have a variety of effects on biota and people (Buccini, 2003). Due to their omnipresent nature, lipophilic properties, and persistence within the environment, they tend to accumulate in the tissues of living organisms (Olisah, Adeniji, et al., 2019) Pesticide use has risen in recent years around the world. Farmers and agricultural workers are fre-

***Corresponding author:** Khaled S. Al salhen: *Khaled.alsalhen@omu.edu.ly* , Department of Biochemistry, Faculty of Medicine, Derna University, Derna, Libya

quently the most exposed individuals among those involved in the formulation and final distribution of pesticide combinations (Alves et al., 2016). Because of their versatility, chemicals in this group, such as organochlorine pesticides (OCPs), are employed for pest management (Olisah, Okoh, et al., 2019).

These include insecticides such as aldrin, dieldrin, endrin, chlordane, dichlorodiphenyltrichloroethane (DDT), mirex, toxaphene, heptachlor, fungicides, and hexachlorobenzene (HCB). Other chemicals in this group that are inadvertently delivered are polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The high quantity of pesticides detected in the atmosphere, water bodies, and soils is due to inappropriate treatment of these chemicals by farmers and other end users, posing a hazard to human health and the ecosystem as a whole. Pesticides prevalent in the environment (particularly organochlorine kinds) have caused a variety of difficulties in Asia, America, Europe, and Africa (Ali et al., 2013; Elibariki & Maguta, 2017; Olisah et al., 2020). These incorporate DDTs, endosulfans, HCB, and Drins (aldrin, endrin, and dieldrin).

Despite being banned, most African countries continued to use OCPs as of 2009. Researchers in Africa and other regions of the world have found extremely high amounts of these pollutants in a variety of matrices, including biota, sediment, soil, water, and food products.(Bempah & Donkor, 2011; Kolani et al., 2016) . Pesticide poisoning affects around 25 million agricultural laborers in underdeveloped nations each year, according to the World Health Organization (WHO, 2009). Low-level pesticide exposure can cause a range of biochemical changes, some of which may be responsible for the documented negative biological effects in humans and animals (Banerjee et al., 1999; Hernández et al., 2006). Some metabolic changes, on the other hand, may not always result in clinically detectable symptoms.

The study of molecular markers of human pesticide exposure has received much interest in recent years. These markers are used to detect the effects of pesticides before they have a negative impact on human health. Biological markers must be present in conveniently accessible and ethically acceptable tissues in humans, such as blood or urine. Pesticide poisoning may have an impact on biological factors related to organ function in humans. Hepatic or renal cytotoxicity could be the cause of the biochemical dysfunctions. A mild nephrotoxic alteration in pesticide-exposed workers has been described (Hernández et al., 2006; Olisah et al., 2020). Pesticide workers exposed to various pesticides have reported altered liver enzyme activity, such as serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Altuntas et al., 2002; Khan et al., 2008).

Some biological parameters can be utilized to identify preclinical changes or negative health consequences caused by a compound's external exposure and absorption. These biomarkers may indicate an early stage of illness development and thus may be predictive of future disease (Benford et al., 2000). Pesticides may produce damaging and degenerative alterations in numerous organs, including the kidneys, resulting in these biochemical abnormalities (Gangemi et al., 2016; Khan et al., 2013). In Africa, there hasn't been much research done on detecting these pollutants in human fluids (Olisah et al., 2020). This study was carried out to evaluate the effects of organochlorine pesticides on biochemical parameters in agricultural workers chronically exposed to these compounds, because of the limited research in Libya related to human monitoring of organochlorine pesticides' exposure.

## MATERIALS AND METHODS

**Sample collections and experimental design**: A total of 70 people were chosen as a sample size from various locations of El-Jabil

Al Kadar, with 45 blood samples collected from male agriculture workers who had been frequently exposed to pesticides for a long time but had no previous history of infectious diseases or other environmental exposures. Individuals for the study were enrolled in February 2015, with a mean age of 61.24 ± 5.12 and an average of 25 years of experience working in agricultural fields. Similarly, 25 healthy volunteers were recruited from the same location, but had no current or recent history of infectious illnesses, pesticide exposure, or other environmental exposures (as a control). The age distribution of the control subjects (59.14 ± 3.21 years) was nearly identical to that of the patients.

**Data collection**: Filling out the questionnaire, which was specified for matching the study need, was done through a meeting interview. The researcher performed all interviews in person. During the study, the interviewer clarified any of the participants' questions that were unclear to them. The majority of the questions were yes/no questions, which provided a binary option. After informing the respondents about the purpose of the study and getting informed consent, the individuals were given a questionnaire with questions about their age, smoking habits, sex, and sickness duration. The questionnaire was also used to collect information regarding medical history, such as the presence of kidney disease, cardiovascular disease, liver disease, and recurring infection signs and symptoms.

**Sampling**: By numbering them and the blood samples taken from them, all subjects were made anonymous. Blood samples (7 mL) were drawn from each person and collected in anticoagulant sterile vacutainer tubes. Within 2 hours of blood donation, the blood specimens were preserved in an ice-cold chamber, transported, and delivered to the laboratory for processing.

**Oxidative stress biomarkers:** Lipid peroxidation was estimated in serum by thiobarbituric acid reactive substances (TBARS) assay, through a malondialdehyde (MDA) reaction with 2-thiobarbituric acid (TBA), which was optically measured. TBARS levels were expressed as nmol MDA/mg protein according to (Buege & Aust, 1978). The level of plasma MDA was determined spectrophotometrically with a thiobarbituric acid (TBA) solution. In brief, to a 150 µl plasma sample, the following were added: 1ml (17.5%) trichloroacetic acid (TCA) and 1ml of 0.66% TBA, mixed well by vortex, incubated in boiling water for 15 minutes, and then allowed to cool. One ml of 70% TCA was added, and the mixture allowed to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, and the supernatant was taken out for spectrophotometer assay. The concentration of MDA was calculated as follows:

$$MDA\ (\mu mol/l) = \frac{Absorbance\ at\ 532\ nm}{L \times E_0} \times D \times 10^6$$

L: light path (1cm).
$E_0$: Extinction coefficient 1.56 x105 $M^{-1}$. $cm^{-1}$
D: Dilution factor.

**Biochemical assays:** Total protein, albumin, globulin, total bilirubin, total cholesterol, triglycerides, high-density Lipoprotein concentration (HDL-C), low-density Lipoprotein concentration (LDL-C), very low-density Lipoprotein concentration (VLDL-C), urea and creatinine were all measured using kits (Vitro Scient, Germany) (Tolman & Rej, 1999).

**Extraction of pesticide residues from the whole blood:** The procedure used by (Agarwal et al., 1976) was used for extraction. Blood (5 ml) was diluted with 25 ml distilled water and 2 mL saturated brine solution, then transferred to a separatory funnel with a capacity of 125 ml. It was extracted three times with hexane: acetone (1:1) (20 ml) by vigorously shaking the separatory funnel for 2-3 minutes, intermittently releasing the pressure. As a result, the layers were given the opportunity to separate. Using a rotary vacuum evaporator, the three mixed extracts were passed through anhydrous sodi-

um sulfate and condensed to around 1-2 ml. As a result, whole blood was used.

**Sample analysis:** The samples were analyzed for organochlorine using gas chromatography at Cairo University's Faculty of Science. Organochlorine pesticide standard solutions were prepared in n-hexane (Clarke, 1986).

**Statistical analysis:** Student's t-test was used to assess and compare data from agriculture workers and healthy controls from several experiments. The data was presented as a mean standard deviation. The significant test was used with a p-value of less than 0.05.

## RESULTS

The current study was carried out to determine the effects of pesticides on the health of farmers in Aljebal Alakhtar, north-east Libya, by determining their levels of biochemical markers. For this reason, the individuals missing any history of disease were examined after conducing preparatory investigations. Table 1 shows the characteristics of the population under consideration. Mean age of the control and spraying peoples were 59.14 ± 3.21 and 61.24 ± 5.12 years, respectively, which were not significantly different. All farm workers were exposed to pesticides for an average of 21 ± 3.7 years. Personal protection equipment was not used by any of the populations surveyed (PPE).

**Table (1).** Characteristics of the study population (Mean ± SD)

| Characteristic | Control group [n=25] | Exposed farm workers [n=45] |
|---|---|---|
| Gender | Male | Male |
| Age (year) | 59.14 ± 3.21 | 61.24 ± 5.12 |
| Wt (Kg) | 76.36 ± 6.75 | 74.76 ± 7.34 |
| Years of exposure | - | 21 ± 3.7 |
| Personal protective equipment | - | - |

There has been no research on blood levels of organochlorine pesticides in the Libyan population. Organochlorines and their isomer residue levels in the whole blood of agriculturalists and non-agriculturalists were quantified. An analytical method of GC-electron-capture detection using a capillary column was implemented to determine dichlorodiphenyltrichloroethane (DDT) and its metabolites (p,p-DDD and p,p-DDE), as well as other organochlorine pesticides in whole blood samples from 45 farmers and 25 non-occupationally exposed workers. As shown in Table 2, we detected four (o,p-DDE, o,p-DDD, o,p-DDT, and p,p-DDT) of the 16 organochlorine compounds tested for in the whole blood samples (Table 2).

**Table (2).** The distribution concentrations (ppm) of organochlorine pesticide residues detected in whole blood samples collected from Libyan farm workers (Mean ± SD)

| Pesticides detected in farmers group | Concentrations of pesticide residues (ppm) |
|---|---|
| Dichlorodiphenyltrichloroethane DDT/metabolites | |
| p,p`-DDT | 0.169 ± 0.001 |
| o,p`-DDT | 0.129 ± 0.003 |
| Dichlorodiphenyldichloroethylene DDE total | |
| o,p`-DDE | 0.328 ± 0.007 |
| Dichlorodiphenyldichloroethane DDD total | |
| p,p`-DDD | 0.510 ± 0.01 |

Quantitative examination of serum total bilirubin, total protein, albumin, globulin, ALP, ALT, and AST, which were used as biochemical markers of liver damage, was used to evaluate hepatotoxicity (Table 3).

**Table (3).** Effects of chronic exposure to a mixture of pesticides on liver function parameters measured in plasma of controls and exposed group (Mean ± SD)

| Parameters | Control (n= 25) | Exposed farm workers (n = 45) |
|---|---|---|
| AlP (U/L) | 59.38 ± 6.32 | 98.21 ± 19.84* |
| ALT (U/L) | 29.76 ± 2.11 | 41.02 ± 6.72* |
| AST (U/L) | 31.12 ± 3.54 | 53.27 ± 6.91* |
| Total protein (g/dl) | 7.19 ± 0.28 | 5.43 ± 0.71* |
| Albumin (g/dl) | 5.41 ± 0.23 | 3.90 ± 0.18* |
| Globulin (g/dl) | 1.78 ± 0.08 | 1.53 ± 0.17* |
| Total bilirubin (mg/dl) | 0.99 ± 0.03 | 1.39 ± 0.07* |

*$p<0.05$ for exposed group compared to control group.

The values of biochemical parameters determined for the exposed workers and the control group are shown in Table 4.

**Table (4).** Effects of chronic exposure to a mixture of pesticides on kidney function parameters measured in plasma of control and exposed group (Mean ± SD

| Parameters | Control (n= 25) | Exposed farm workers (n = 45) |
|---|---|---|
| Urea (mg/dl) | 26.32 ± 1.03 | 33.58 ± 3.61* |
| Creatinine (mg/dl) | 0.64 ± 0.02 | 0.97 ± 0.06* |

*$p$<0.05 for exposed group compared to control group.

The effects of chronic exposure to a mixture of pesticides on biochemical plasma lipid parameters are also shown in Table 5.

**Table (5).** Effects of chronic exposure to a mixture of pesticides on the biochemical plasma lipid parameters measured in plasma of controls and exposed group (Mean ± SD)

| Parameters | Control (n= 25) | Exposed farm workers (n = 45) |
|---|---|---|
| Total Cholesterol (mg/dl) | 185.16 ± 11.09 | 219.02 ± 21.17* |
| Triglycerides (mg/dl) | 160.72 ± 11.13 | 199.0 ± 25.98* |
| HDL-C (mg/dl) | 39.61 ± 1.16 | 21.10 ± 3.93* |
| LDL-C (mg/dl) | 101.25 ± 10.23 | 148.85 ± 19.87* |
| VLDL-C (mg/dl) | 32.14 ± 2.22 | 39.85 ± 7.19* |

*$p$<0.05 for exposed group compared to control group.

Table 6 shows the effects of chronic pesticide exposure on enzymatic antioxidants and plasma thiobarbituric acid reactive substances (TBARS) concentrations.

**Table (6).** Effects of chronic exposure to a mixture of pesticides on antioxidant enzymes and TBARS measured in plasma of control and exposed group (Mean ± SD)

| Parameters | Control (n= 25) | Exposed farm workers (n = 45) |
|---|---|---|
| TBARS (nmol/ml) | 2.14 ± 0.03 | 3.97 ± 0.29* |
| GST (µmol/hr) | 0.52 ± 0.005 | 0.68 ± 0.009* |
| SOD (U/ml) | 0.69 ± 0.004 | 1.21 ± 0.01* |

*$p$<0.05 for exposed group compared to control group.

## DISCUSSION

During their work, pesticide sprayers in this study are exposed to various pesticides (insecticides, fungicides, and herbicides). All agricultural workers in the exposed group were regularly exposed to complicated pesticide mixes (2-3 times per week). Our research farmers used a wide range of pesticides, generally in the "moderately dangerous" to "somewhat hazardous" categories. World Health Organization (WHO) (very toxic) pesticides should not be used in developing countries, according to the Food and Agriculture Organization (FAO). It also recommended that pesticides classified as class II (moderately dangerous) be avoided. Individuals are routinely exposed to a variety of pesticides or pesticide mixtures, either concurrently or sequentially, making it difficult to distinguish between their effects. The link between pesticide-related cytotoxicity and overt clinical organ dysfunction remains unknown. In this case, biomarkers could be used to detect pesticide impacts before they cause adverse clinical health effects (Hassanin, 2009). Organochlorines have long been a source of concern for ecologists due to their resistance to biodegradation. Organochlorine deposition in biolipids is caused by their low water solubility due to their high lipophilicity. These characteristics explain why organochlorine accumulates to amounts that are regarded as significant organochlorine residual loads in human adipose tissue (Kreiss et al., 1981). Despite the fact that organochlorines are extremely long-lasting, some biodegradation can occur, yielding the metabolites indicated in Figure 1.

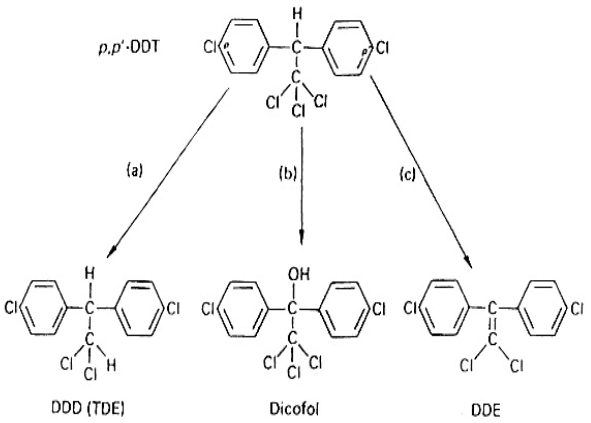


**Figure (1).** Metabolites formed from DDT, (a) reductive dechlorination, (b) oxidation, (c) dehydrochlorination (Kresis et al., 1981).

Because different analytical methods were used, the concentration of dichlorodiphenyldichloroethylene (DDE) in whole blood samples from Libyan farm workers was 0.328 ppm. Other authors used packed columns, while we used capillary column separation and GC/MS (El Zorgani et al., 1994; Manal, 1997). Despite the limitations mentioned above, it can be concluded that the blood concentrations of Libyan farm workers were extremely high when compared to values determined for workers in other countries (Bouwman et al., 1991; Dogheim et al., 1996; Mazzarri B & Lauschner, 1988). All Egyptian and international published data, such as those of (Barkatina et al., 1998; Cok et al., 1999; Pardio et al., 1998; Saleh et al., 1996; Salem & Ahmed, 2002; Waliszewski et al., 2001), showed that p, p'-DDE was the most prevalent compound among DDT. This could be related to DDT's high solubility and tendency for accumulating and being stored within fatty tissues (Waliszewski et al., 2001). DDT is normally converted into its more stable metabolite DDE in natural conditions in a living organism (Waliszewski et al., 2001). The study found that DDT and DDE measured levels were lower than those previously reported by (Dogheim et al., 1996; Saleh et al., 1996; Salem & Ahmed, 2002) in several Egyptian governorates. In countries where DDT is still used, the DDT level is usually greater. As a result, DDT has been prohibited in most Western countries since the 1970s (Travis & Arms, 1988). Despite the fact that DDT has been banned in Libya for over a decade, its traces can still be found in the environment. This is owing to the high level of persistence. The persistence and long-range transport characteristics of DDT and its metabolite DDE, as well as their capacity to bioaccumulate and biomagnify in the food chain, make this concentration useful in detecting the source of contamination (Zhang et al., 2018).

This disparity is thought to be attributable to various pesticide spraying practices in the fields. Pesticides are frequently administered manually in Libya, particularly in the Aljebal Alakhtar area, without any protective devices. DDT is a nonsystemic insecticide with a lot of influence. 2,2-bis (p-chlorophenyl)-1,1-dichloroethylene (p, p'-DDE), a significant DDT metabolite, was found at mean levels of 0.328 ppm in whole blood samples taken from Libyan farm workers. DDE has a longer half-life than DDT. p, p`-DDD In whole blood samples, another DDT metabolite was found at mean levels of 0.510 ppm. DDT was found in blood samples, probably due to its long-lasting nature. Because DDT is known to undergo metabolic conversion and dehydrochlorination, the detection of DDT metabolites such as DDD and DDE in our study could be the result of these metabolic processes. Biochemical alterations are essential biological indicators since they are the body's sensitive response to hazardous exposures. These modifications enable you to keep track of the level of exposure and prevent irreversible consequences. AST and ALT are liver enzymes that are utilized as biological indicators of liver disease (Machado et al., 2021). Cirrhosis, biliary obstruction, infectious and toxic hepatitis, and ischemia all raise AST levels, as can cirrhosis, biliary obstruction, and infectious and toxic hepatitis (Wang et al., 2006). Furthermore, the increase in ALT occurs solely as a result of hepatic alterations (Murphy et al., 2018).

Table 3 shows that the mean values of the liver enzymes ALT, AST, and ALP, as well as serum total bilirubin, were significantly higher in pesticide-exposed workers than in the control group ($P<0.05$). Pesticide-exposed workers, on the other hand, had considerably lower serum total protein, albumin, and globulin concentrations than the control group, with 95.0 percent significance (Table 3). Air Force veterans who were involved in aerial herbicide spraying in Vietnam had a higher risk of liver dysfunction, which was attributed to increased AST and ALT levels (Michalek et al., 2001). Glyphosate and paraquat have been shown to suppress the activity of two enzymes in vitro: ALT and AST (Yousef et al., 2003).

(Azmi et al., 2006) found that pesticide exposure caused a considerable increase in enzyme levels (ALT, AST, and ALP) in several fruit and vegetable farm-station workers. The activity of serum transaminases may be enhanced in many diseases due to increased release from non-liver tissue sources (Kobayashi et al., 2020). A high level of impaired liver function in agricultural workers could indicate pesticide toxicity and pesticide residues in the blood. Occupational workers exposed to organophosphorus insecticides alone or in combination with organochlorines have been shown to have altered liver enzyme activity (Muñoz-Quezada et al., 2016). According to (Awad et al., 2014), agriculture workers had a significant increase in serum liver enzymes (AST, ALT, and ALP) as compared to controls (Awad et al., 2014). Other researchers have documented high levels of AST, ALT, and ALP in pesticide-exposed people (Azmi et al., 2006; Khan et al., 2013). The end product of heme catabolism, total bilirubin, possesses antioxidant and anti-inflammatory effects (Maines, 1988). (Vitek et al., 2019) identified bilirubin as a compound with high antioxidant and anti-inflammatory effects. As a result, it's not surprising that bilirubin levels have risen in this study because it has protective benefits against oxidative stress and associated repercussions. A study by (Fahimul-Haq et al., 2013) found that total bilirubin levels in both groups were not only within the normal range, but also comparatively near the upper normal limit among pesticide industrial workers. It could be related to long-term pesticide exposure, which disrupted normal red blood cell metabolism, producing hepatic dysfunction and raising bilirubin levels in the blood, resulting in hyperbilirubinemia, which could be caused by the creation of more bilirubin than the typical liver can eliminate (Awad et al., 2014). When pesticide-exposed workers were compared to healthy controls, blood total protein albumin and globulin levels were found to be lower. The decrease in serum protein was thought to be mostly attributable to a decrease in albumin rather than the globulin fraction (Yousef et al., 2003). Furthermore, it was revealed that protein deficiency in the blood was primarily caused by severe nephrosis loss (Yousef et al., 2003). Furthermore, a decline in blood protein could be attributable to protein loss, which could be caused by decreased protein synthesis, increased proteolytic activity, or breakdown (Shakoori et al., 1990). The decrease in serum protein was mostly owing to lower serum albumin and serum globulin levels (Table 3).

The farmers' lower serum albumin levels could be due to their dietary situation and/or liver synthetic function. The farmers appeared to be in good health and were not starving. The farmers' reduced albumin levels could be the result of a slight alteration in liver expression levels (Aroonvilairat et al., 2015). Other authors, on the other hand, found lower serum total protein and albumin levels in pesticide sprayer farmers (Singh & Singh, 2014). Nevertheless, the current findings show that pesticide exposure causes changes in protein metabolism. Pesticide toxicity can change serum protein concentrations by impairing protein synthesis in hepatocytes and disrupting kidney function (Mostafalou & Abdollahi, 2013). Also, as evidenced by the increased activities of serum AST, ALT,

and ALP, the observed decrease in serum protein could be attributable in part to the damaging effect on pesticide-exposed workers' liver cells (Table 3). These are the hepatocellular injury markers that are employed in primary screening (Giannini et al., 2005). The serum urea and serum creatinine values are used to determine whether or not there is a concern with renal function (Kanwar et al., 2015). When there are issues with renal filtration, blood levels of creatinine and urea rise, while total protein levels are reduced (Murphy et al., 2018). Furthermore, creatinine, urea, and total protein are used as biomarkers of renal changes, assessing the ability of the kidneys to filter plasma in the glomeruli by measuring the clearance of the same (Murphy et al., 2018). As a result, these markers can be used to determine whether renal function has been affected by agricultural pesticide exposure (Calvert, 2016).

The values of biochemical parameters determined for the exposed workers and the control group are shown in Table 4. In pesticide-exposed farm workers, mean levels of urea and creatinine were considerably ($P<0.05$) higher than in controls (Table 4). An increase in serum urea may be attributed to a decrease in its synthesis as a result of impaired hepatic function and/or a disruption in protein metabolism (Elfowiris & Banigesh, 2022; Idonije et al., 2011) Creatinine is a waste product filtered from the blood and discharged in the urine. Higher creatinine levels in exposed agricultures workers may be related to changes in kidney function (Hassanin et al., 2018). Furthermore, the observed elevations in urea and creatinine could be explained by glomerular hyper-filtration caused by increased creatinine clearance from the circulation (Palatini, 2012). Glomerular Filtration Rate is measured by serum creatinine and urea (GFR). Though, when compared to urea level, serum creatinine is a more sensitive indicator of renal function. This is because creatinine meets the majority of the criteria for an ideal filtration marker (Palatini, 2012). Previous research has found a considerable increase in serum urea and creatinine concentrations in pesticide-exposed workers, which has been linked to renal injury and kidney dysfunction (Hassanin et al., 2018; Mahmoud Abdul_Aal & Mahmoud, 2019; Yassin, 2015). Elevated blood urea and creatinine levels in response to pesticide exposure may be explained by: 1) increased urea and creatinine production as a result of reduced hepatic function, as seen in the current investigation. 2) A disruption in protein metabolism, as evidenced by the current findings, and 3) a decrease in renal filtration rate, as evidenced by the current findings. Pesticide toxicity could explain the observed elevations in urea and creatinine levels as a measure of renal function in the exposed group compared to the controls (Haghighizadeh et al., 2015). Lipids serve as messengers and regulators of inflammation, as well as being precursors for hormones and employed for energy storage (Watson, 2006). One of the most vulnerable targets for free radicals is lipids (Rajani & Ashok, 2009). The liver is a metabolically diverse organ that regulates the chemical environment inside the body (Behl et al., 2011). It is particularly important in the synthesis and regulation of circulating lipids, lipoproteins, triglycerides, cholesterol, cholesterol esters, and in the degradation of cholesterol and steroids. The liver is the primary location for detoxification and promotes clearance by excreting water-soluble compounds, as well as being the key organ of the antioxidant defense system (Arulmozhi et al., 2010). Organochlorine, like other pesticides, remains in the environment and bioaccumulates in human tissues (Botella et al., 2004).

Table 5 shows the serum lipid profile of pesticide-exposed farmers and controls, including cholesterol, triglycerides, HDL-C, LDL-C, and VLDL-C. The average levels of serum cholesterol, triglycerides, LDL-C, and VLDL-C were found to be higher in pesticide-exposed farmers (219.02 ± 21.17, 199.0 ± 25.98, 148.85 ± 19.87, and 39.85 ± 7.19 mg/dl, respectively) compared to controls (185.16 ± 11.09, 160.72 ± 11.13, 101.25 ±

10.23, and 32.14 ± 2.22 mg/dl, respectively). The difference was statistically significant ($P<0.05$). Pesticide-exposed farmers, on the other hand, had considerably lower blood HDL-C levels than healthy controls (21.10 ± 3.93 vs. 39.61 ± 1.16 mg/dl). Pesticide-exposed farmers had a significant increase in cholesterol and triglyceride levels, followed by a significant decrease in phospholipid levels, compared to controls, indicating severe pesticide-induced hyperlipidemia. (Sharma et al., 2010) previously reported that organochlorine insecticides cause hepatotoxicity. Previously, rats and mice fed an organochlorine pesticide-contaminated diet had higher serum levels of triglycerides, cholesterol, and phospholipids (Boll et al., 1995; Ravinder et al., 1990).

The findings of this study (Table 5) are consistent with the reports mentioned above. Excessive synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS), among other reactive species, causes oxidative stress. Such chemicals are found in a variety of physiological conditions and constitute an important part of human metabolism (Marrocco et al., 2017). Through oxidative and antioxidant indicators, researchers can monitor the prevalence of oxidative stress. The amounts of Thiobarbituric acid reactive substances (TBARS) and protein carbonyls are measured to determine oxidative indicators. Considering that, lipid peroxidation (LPO), which is the leading cause of cell death, as well as DNA damage, enzyme inactivation, and other factors, Oxidation of hormones and DNA are both markers of oxidative cell damage (Ruas et al., 2008). LPO, in particular, has been proposed as one of the pesticide-induced toxicity pathways (Santi et al., 2011). Pesticide-induced oxidative stress has been the subject of toxicological research for more than a decade as a potential toxicity mechanism. The direct measurement of lipid peroxidation by-product malondialdehyde (MDA; the end result of lipid peroxidation) can demonstrate the toxic effects of pesticides on humans, especially by eliminating radical generation (Muniz et al., 2008). When compared to control subjects, there was a significant rise in TBARS or MDA in the exposed population (Table 6). The increased generation of MDA or TBARS in the sprayer population could be attributable to enhanced membrane peroxidation. The basic process by which any and related radicals, including superoxide and hydroxy radicals, produce membrane damage in lipid peroxidation is documented in the literature (LPO). LPO can be triggered by free radical intermediates as well as superoxide hydroxy radicals. LPO causes bio-membrane and subcellular organelle degradation. The oxidation of microsomal membranes high in polyunsaturated fatty acids is a concern (Freeman & Crapo, 1982). However, pesticides were found to enhance lipid peroxidation in the liver and kidneys of experimental animals (Pawar & Kachole, 1978). After eight weeks of therapy, oral administration of DDT (100 and 200 ppm) and lindane (40 and 80 ppm) dose-dependently raised TBARS levels in serum (Koner et al., 1998). DDT is shown to have severe acute effects on male Wistar rats, and TBARS levels were found to be considerably greater (Li et al., 2017).

The toxicity of pesticides has been linked to reactive oxygen species (ROS). Organophosphates cause oxidative stress alterations that are unique to them (Abdollahi et al., 2004). Organophosphates induce characteristic changes in oxidative stress (Abdollahi et al., 2004). When compared to controls, sprayers exposed to organophosphate, carbamate, and organochlorine pesticides had significantly higher MDA (TBARS) levels, suggesting that oxidative stress may play a role in pesticide toxicity (Prakasam et al., 2001). (Varga & Matkovics, 1997). Our findings are in agreement with other research that suggested pesticides cause oxidative stress in humans, showing that MAD, the last product of lipid peroxidation, was found to be significantly higher in sprayers compared to controls, and that serum MDA or TBARS levels were 1.8 times higher in farm workers compared to

controls. These findings suggest that free radicals may play a role in organochlorine-induced immunotoxicity (Li et al., 2017). Organisms have evolved mechanisms to minimize the impacts of radicals produced in the cellular membrane. The antioxidant system, such as superoxide dismutase, is involved in this mechanism (SOD). Antioxidant systems work by converting highly reactive oxygen species into less reactive intermediates that are no longer harmful to the cell (Birben et al., 2012). However, for healthy biological integrity to be maintained, there must be a balance between oxidation and antioxidant levels in the system. SOD is one of the most active enzymes in the body, with sufficient activity to dismutate superoxide anions formed during oxidative stress in cells (Birben et al., 2012). SOD and Glutathione-S-transferase activity were used to assess the antioxidant profile (GST).

The exposed group's SOD activity was substantially higher than the control group's (Table 6). SOD is an important antioxidant enzyme that converts superoxide anion to peroxide. The enhanced activity of this enzyme indicates a higher production of superoxide anion as a result of pesticide exposure. When the amount of reactive oxygen species (ROS) produced in a cell or tissue exceeds the cell's antioxidant capacity, oxidative stress occurs (Sevanian & Peterson, 1984). Chronic pesticide exposure has already been linked to increased SOD activity, according to (Shadnia et al., 2005). Other studies have found that pesticide exposure to various categories, such as organophosphates, carbamates, or pyrethroids, causes oxidative stress in pesticide sprayers (Da Silva et al., 2012; Prakasam et al., 2001). Glutathione S-transferase (GST) is a detoxification gene family that plays a key role in the detoxification of exogenous chemicals (Gui et al., 2009). We observed increased oxidative stress in this study as a result of several pesticides used by farmers. As a result of the oxidative stress, GST enzyme gene expression may have risen, resulting in increased GST activity in our study (Table 6). The addition of glutathione to endogenous xenobiotics is catalyzed by GST (Satheesh et al., 2010). GSH and GST have been used as markers of toxic effects of exposure to diverse xenobiotics due to their high sensitivity to environmental contaminants (Manno et al., 1985) In the current study, GST levels were found to be significantly higher ($P<0.05$) in pesticide-exposed individuals (Table 6). GST activity was significantly increased (p0.05) in serum workers exposed to pesticides. In rats exposed to the insecticide, (Otitoju & Onwurah, 2007) found an increase in plasma GST activity. Increased GST activity has been associated with pesticide resistance in all of the major classes. Increased GST activity in tissues could signal the development of a defense mechanism to counteract pesticide effects, as well as the prospect of more effective pesticide toxicity prevention (Ranson & Hemingway, 2005).

## CONCLUSION

Some of the organochlorine pesticides assessed in this study have lower and higher levels than previously reported. The prevalence and levels of several pesticides, particularly p, p'-DDT, o, p'-DDT, o, p'-DDE, and p, p'-DDD, remain high, requiring more effective and continuous defenses. Food of animal origin, fish, vegetables, fruits, and cereals from polluted areas will be monitored frequently and continuously, allowing consumers to take precautions while consuming these pesticides. The findings of this study revealed that some organochlorine pesticide residues are still present in the environment. 4 out of 16 pesticides were detected in blood samples from Aljebal Alakhtar, indicating that each person is exposed to and carries a body burden of multiple pesticides, which could be due to a combination of direct and indirect pesticide exposure. Organochlorine pesticides make up the majority of total pesticide concentrations in blood samples from Aljebal Alakhtar. The presence of organochlorine pesticides in the blood indicates that they stay in the body for a long period. Long-term

exposure to various pesticides causes cytotoxicity, resulting in biochemical changes in particular, according to this study. Several biomarkers could be used to monitor the early impacts of pesticides on agricultural workers' health.

## ACKNOWLEDGEMENT

All the volunteers who participated in the collection of samples were thanked by the authors.

## ETHICS

The Libyan National Committee for Biosafety and Bioethics gave their clearance to this retrospective study.

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# الآثار الضارة لبقايا المبيدات العضوية الكلورية على العوامل البيوكيميائية لدى العمال الزراعيين الليبيين

**خالد سالم الصالحين [1*]، أم السعد عمر [2]**

*[1] قسم الكيمياء الحيوية، كلية الطب البشري، جامعة درنه، ليبيا*

*[2] قسم الكيمياء الحيوية، كلية الطب البشري، جامعة عمر المختار، البيضاء، ليبيا*

تاريخ الاستلام: 06 أبريل 2022 / تاريخ القبول: 21 يوليو 2022

Doi: https://doi.org/10.54172/mjsc.v37i3.742

**المستخلص :** يتعرض عمال الزراعة لخطر التعرض للمواد السامة مثل مبيدات الآفات. ولتقدير الخطر الفردي لمناولة مبيدات الآفات، يلزم الرصد البيولوجي لتأثيراتها على العمال الزراعيين. لم يتم إجراء أي بحث بين عمال الزراعة الليبيين. لذلك صممت هذه الدراسة لدراسة تأثير المعايير البيوكيميائية للتلوث بالمبيدات بين عمال الزراعة الليبيين في الجبل الأخضر، ليبيا. وفي منطقة الجبل الأخضر، تم أخذ 45 عينة دم من عمال زراعيين ذكور تعرضوا للمبيدات في حقول المحاصيل لفترة طويلة، فيما تم أخذ 25 عينة دم من مجموعة لم يتعرضوا للمبيدات (مجموعة المقارنة). تم استخدام المحاليل القياسية لتقدير البلازما AST،ALT ، GST،ALP ، SOD والبروتين الكلي، والألبومين، والجلوبيولين، والبليروبين الكلي، والكوليسترول الكلي، والدهون الثلاثية وHDL-C، LDL-C، VLDL-C، واليوريا، والكرياتينين أيضًا كالكوليسترول الكلي، الدهون الثلاثية، HDL-C، LDL-C، VLDL-C، اليوريا، والكرياتينين .تم استخدام المواد التفاعلية المنتجة من حمض ثيوباربيتوريك (TBARS) لتقدير بيروكسيد الدهون في مصل الدم. باستخدام كروماتوغرافيا الغاز (GC-ECD) تم تحليل عينات الدم من بقايا المبيدات العضوية الكلورية. بالمقارنة مع مجموعة التحكم، كان لدى العمال أنشطة SOD، GST، AST،ALP ، ALT أعلى بشكل ملحوظ، بالإضافة إلى مستويات أعلى من إجمالي الدهون، والبيليروبين، والكرياتينين، واليوريا، بالإضافة إلى تركيزات أعلى بشكل ملحوظ من TBARS. علاوة على ذلك، كان التعرض لمبيدات الآفات على المدى الطويل مرتبطًا أيضًا بتقليل البروتين الكلي، والألبومين، والجلوبيولين ، فضلاً عن تقليل مستوى HDL-C. يبدو أن التعرض لمبيدات الآفات يؤثر على مختلف العوامل البيوكيميائية بشكل عام. وهذه المؤشرات الحيوية تشير إلى الآثار الضارة المرتبطة بمبيدات الآفات على العمال الزراعيين، مما يشير إلى أنه ينبغي استخدامها للرصد الروتيني لتأثيراتها.

**الكلمات المفتاحية :** مستوى الدهون؛ وظائف الكبد؛ وظائف الكلى؛ مؤشرات الاجهاد التأكسدي.

*[1] خالد سالم الصالحين: Khaled.alsalhen@omu.edu.ly قسم الكيمياء الحيوية، كلية الطب البشري، جامعة درنه، ليبيا.

*Al-Mukhtar Journal of Sciences* 37 (3): 251-260, 2022
*ISSN: online 2617-2186 print 2617-2178*
**Journal Homepage** https://omu.edu.ly/journals/index.php/mjsc/index

# The Design and Implementation of a Libyan Salary Mobile Application using Flutter

**Aeyman M. Hassan**
*School of Computer Engineering, University of Zawia, Zawiay, Libya*

Received: 05 May 2022/ Accepted: 17 August 2022
Doi: https://doi.org/10.54172/mjsc.v37i3.802

**Abstract:** Mobile applications have become widespread in many aspects of our daily lives. Many people do most of their work via their phones, such as online banking, shopping, etc. Due to many individuals being unaware of their salary details, this paper presents a design and implementation of a Libyan salary application" الراتب" using Flutter (cross-platform). The application allows users to know details of their salaries, including bonuses and deductions according to the salary schedule of each ministry or sector. The application currently contains salary schedules for ten ministries. The implementation consists of the following four steps: data collection of laws and payroll tables for each ministry to use as a local database for the salary application, design of friendly-use application screens using Flutter, integration of the mobile application with Firebase (backend platform by Google), and finally, preparing and deploying an Android and web version of the salary application. The results that were obtained from reviewers in the Google Play Store were satisfactory. There is an increasing local demand to add other ministries to the application. The citizens of the local community encourage providing such a service, which is considered the first of its kind. The application reached more than 100k downloads in less than a year, and the average rating is 4.5 out of 5 in the Google Play Store at the time of writing this paper.

**Keywords:** Mobile Application; Flutter; Cross-platform; Salary Application.

## INTRODUCTION

Nowadays, smartphones are the most used device in the world. Therefore, almost each company needs to build a mobile application for its business to reach as many audiences as possible. Developers tend to target mobile operating systems that are widely used, which are Android and iOS. These mobile operating systems are used in total by more than a billion mobiles. Android is an open-source system acquired by Google in 2005, while iOS is a mobile operating system created and developed by Apple Inc for iPhone and iPod Touch. There are a significant number of mobile apps published in the Google Play Store for Android mobiles and the App Store for iOS mobiles. The Android apps are written in Java and Kotlin, while iOS apps are written in Swift and Objective-C. Many companies currently work in mobile application programming. They usually have two teams or even more. One team focuses on building Android applications, and another team works on iOS applications. In addition, they might have some developers who work on web applications. It might be a better solution to have several teams designing and building apps for different platforms. However, small companies cannot afford to pay many employees.

***Corresponding author:** Aeyman M. Hassan: *a.hassan@zu.edu.ly*, School of Computer Engineering, University of Zawia, Zawia, Libya.

With the existence of software development kits (SDKs) that have a cross-platform feature, this platform is based on the concept of building once and running everywhere. One of those SDKs used in this work is called Flutter. It is an open-source SDK released by Google in 2016. Flutter is based on Dart programing language, which is an Object-Oriented Programming (OOP) language. In Flutter, each component that appears on mobile phone screens is called a widget. Therefore, it's considered the principal item in this SDK. Flutter also has a hot-reload feature, which boosts the coding time because any change during writing code will be updated directly in the dart virtual machine. Consequently, we can notice these changes on a real device or an emulator once we run the code. Furthermore, the code written in Flutter can deploy to multiple platforms such as Android, iOS, desktop, web, etc.

Flutter has been used to build various apps. The Billing and Reward App was built using Flutter. Users gain points for shopping, and those awarded points can be used for purchases after reaching a specific amount. The app consists of a client-side which is running on Android or iOS-based smartphones. They also built a server part on a Windows OS for system management (Tashildar et al., 2020). Ghusoon Arb and Kadhum Al-Majdi designed a freight app based on Dart and Flutter. They published their app on Android and iOS devices. On the client side, customers who use the app were able to track the details of their packages, including the cost and location. On the server side, charging staff uses a framework to modify dispatch states (Arb & Al-Majdi, 2020). The Evecurate app also uses the QR for event management at universities. Event details such as the date and time can be announced via the app (Juliana et al., 2021). Due to the COVID-19 pandemic, much research has been carried out to help people in different life aspects. One of those useful works was developing a healthcare app that provides many services. Many patients can be diagnosed via this app. In addition, you can easily ask for an ambulance vehicle. The app also enables patients to order their medicines online (Mamoun et al., 2021). Praveen et al. used Flutter to build a conference room booking app. People can use this app to book conference rooms based on availability (Praveen et al., 2020). A food services app was also developed using Flutter and MySQL (Structured Query Language) to provide food to clients who order by the app (Riyadi & Cahyono, 2021). A student attendance app was built using Flutter for Android mobiles. The app uses a Quick Response code (QR) to register students during the class. Opening and closing sessions are managed by lecturers, and students and lecturers use the app as clients. Users get all necessary data from a web service by a HTTPS (Hypertext Transfer Protocol Secure) request (Wiriasto et al., 2020). A salary management system was built for a factory, using a biometric scanner to collect the attendance of employees, which is uploaded to a cloud database. The employees use a mobile app to see their salary details (Patra et al., 2021). An employee salary report application was developed for Android devices. The app uses Firebase to send employee salary reports (Dinata & Risawandi, 2021). Firebase is also used in the Libyan salary app to send notifications to users. Although, the last two research have similar applications to the Libyan salary app, the app in this paper is entirely different because it does not rely on any attendance system. In addition, it displays salaries in a general way according to salary tables for each ministry, not for a specific employee. Although Flutter has been used to solve many problems in different countries, each country has issues related to its government and individuals. The local community in Libya might need a specific mobile app that helps people in their daily life. There are many salary schedules for Libyan employees. The ministry of finance of Libya does not provide a public database for payroll tables and laws, where individuals can access this information smoothly and easily. In the current situation, for instance, someone who

works at a university knows that his salary belongs to the ministry of higher education. He might spend a couple of hours browsing on the internet until he finds what the name of the statute and payroll table for universities as a Word document or Portable Document Format file (PDF). This inconvenience also applies to other ministries and sectors as well. It is exhausting work finding this information. Therefore, all these scattered salary schedules on the internet need to be organised and displayed in a suitable manner. This was the motivation for building the Libyan salary app. The app aims to help local citizens to know all exact details about their salary in a few seconds.

## MATERIALS AND METHODS

The process of building the Libyan salary app contains four main stages. The first stage involves collecting the payroll schedule of each ministry which will be stored in a local database. The second stage was to build the graphical user interface for the application screens, which were implemented using Flutter and Dart programing language. In the third stage, the app was integrated with Firebase to use for notifications. The last stage is releasing the salary app on Android and the web. Figure 1 shows the block diagram for the building process of the salary application.

**Data Collection:** The app currently consists of ten ministries. For each ministry, a payroll table was obtained by searching the internet for the existing employment laws of that ministry. The payroll tables were formatted to Comma-Separated Values (CSV) files in order to use in the next stage. This procedure was repeated for all other ministries. Even though this kind of work takes a long time, it is performed only once. Table 1 shows an example of the salary table of the Libyan ministry of interior. At this point, the app database had been created successfully, and the app will be able to load any of those salary tables smoothly. The first column in Table 1 demonstrates the occupation grade, which starts from 450 Libyan Dinner (LD) for level one until 1276 LD for level sixteen. The second column shows the annual increment that corresponds to each row in Table 1. The value of the annual increment starts from 3 LD until 15 LD. As previously mentioned, this is only one instance, and these steps were applied to all ten ministries in the Libyan salary app.

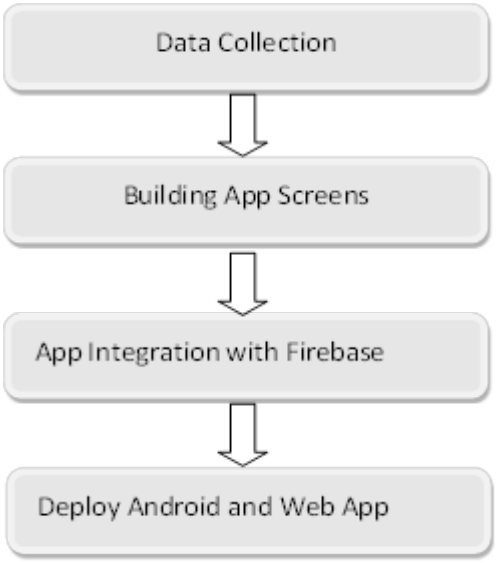


**Figure: (1).** The building process of the App

**Table (1).** The Libyan Ministry of Interior

| Job Ranking | Annual Bonuses |
|---|---|
| 450 | 3 |
| 475 | 3 |
| 500 | 6 |
| 532 | 6 |
| 550 | 8 |
| 582 | 9 |
| 615 | 9 |
| 662 | 9 |
| 745 | 12 |
| 820 | 12 |

The app uses the local database created in the first stage. As a result, getting information from any salary schedule and demonstrating it in the mobile app is performed quickly.

**Graphical User Interface (GUI):** The Libyan salary app might be used by many users from different backgrounds. In addition, they have different education levels. Therefore,

the app was designed to be a user-friendly mobile app. In general, a user only needs to select his occupation grade and annual increment from a dropdown button. Furthermore, there are more options for some sectors like the General Electricity Company of Libya. Its employees have overtime hours and night shifts on many days each month. Visual Studio Code was chosen as an integrated development environment (IDE) for programming the mobile app. Contrary to Android Studio, this IDE can work even with limited hardware. Visual Studio Code has an option called extensions to enable the addition of many programming languages. Flutter and Dart have been added for building this app. Figure 2 illustrates the working app architecture. As you can notice, the Android and web versions of the salary app acquire data from the local database, which contains payroll tables for all ministries. Firebase sends a notification message which contains either an update or news about salaries.

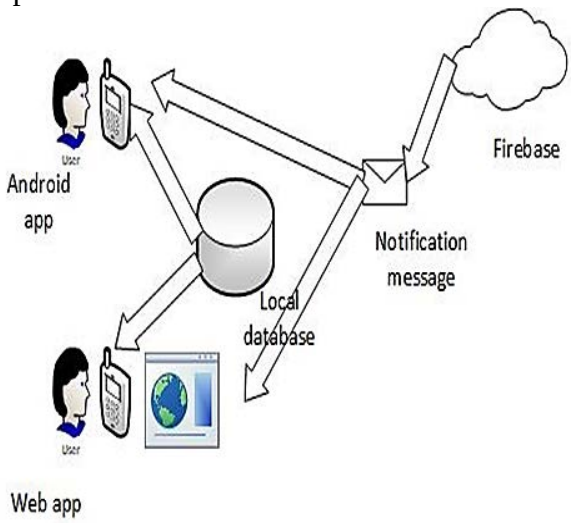


**Figure: (2).** Working App architecture

For the design of the GUI, as almost every app contains a login screen, it was the first screen programmed in the salary app. However, for now, to make the app as simple as possible, users will not be asked for any information. Therefore, they do not create a user profile to enter the app. App users must have an internet connection to use the app. The login screen for this app is shown in Figure 3. When the user clicks the button "دخول", the code will run a code routine to check if there is an internet connection. If so, it will then take you to the Homepage screen as shown in Figure 4. For instance, if a user works at a university, he will click the fourth option on the Homepage screen, which is the ministry of higher education and scientific research, as shown in Figure 5. Presuming the user works as a lecturer, he will click on the first choice in the Staff screen. Consequently, the salary screen will be shown as in Figure 6. The user chooses his scientific grade and annual increment from two dropdown buttons at the top of the current screen. There is also a checkbox for teaching hours. After that, he can click the button "أحسب" to display all salary details.

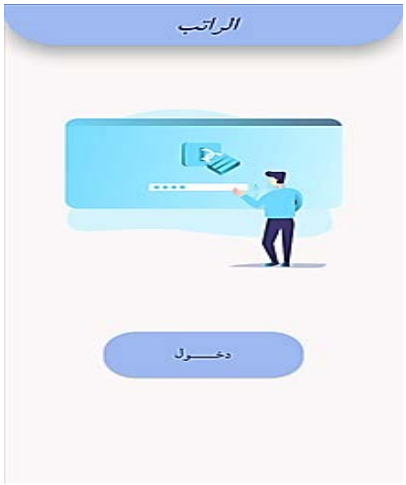


**Figure: (3).** Login Screen

**Figure: (4).** Homepage screen


**Figure: (5).** Staff Screen


**Figure: (6).** Salary Screen

**App integration with Firebase:** Firebase was acquired by Google in 2014. Many developers used Firebase as backend development programming language to build high-performance apps. Firebase is not limited to hosting websites as it provides many services. You can use it to store databases, which can be used later by mobile apps or websites. Nevertheless, this service is not used by the Libyan salary app because of the small size of its database. Firebase presents another backend service which is called authentication. The benefit of the service is to identify the user and allow him to use the app. It is also not used in this work because there is no registration procedure for the Libyan salary application (Khawas & Shah, 2018).

**Application Deployment:** The salary app is released to work for Android mobiles. There are two formats for releasing apps. Either an App bundle (preferred by the Google play store) or APK (Android Package Kit). The following Flutter command is for the first option. After that, the app bundle will be ready to publish to the google play store.

**VS Code Terminal**

```
flutter build appbundle
```

The salary app was also deployed as a web app, which is achieved by the first command using Flutter on the Terminal window of Visual Studio Code (VS Code), as follows:

**VS Code Terminal**

```
flutter build web
flutter run -d chrome
```

The second command is to test the web app using the Google Chrome browser. Flutter supports Progressive WebApp (PWA). Therefore, the web app can be installed similarly to other web-based apps. Even though the salary web app was released successfully, upto this step, it only works locally. In order to make this web app available to all users, a hosting service should be used. A Firebase platform was used to host the web app version. All users can access the web salary app through the following web link:
https://salary-webapp.web.app

By providing a web app for this work, users will not be limited to any operating system. They can open the app from Android and iOS mobiles. The web app also can be used by a personal computer (PC) because all we need is a web browser to open the previous web app link. Firebase has a powerful feature called Firebase Cloud Messaging (FCM). It enables sharing information and sending notification messages to a specific user or group of users who use the same app, which might be an Android, iOS, or web app. FCM was

used in (Heryandi, 2018). They used FCM at university to keep parents engaged by sending them performance progress reports as a notification message. Parents can receive those messages quickly via an app installed on their smartphones without any cost. In this paper, FCM was used to send notifications to all users, as shown in Figure 7. It consists of a few steps. Firstly, choose a notification title. Secondly, write a notification text. Finally, you choose the scheduling by which you want to send the notification and click the Publish button. In the Libyan salary app, there are two kinds of notifications that have been sent. The first kind is to inform app users about any new updates.

The second kind of notification is news. Especially the news that is related to salaries, as shown in Figure 8. It displays a notification message about the central bank of Libya regarding the salary for January. Firebase also enables you to know the number of active users in the last thirty minutes via the Firebase Console. In addition, you can use Google Analytics to obtain valuable information about the application's performance. Google Admob" advertising on mobile" was used in the app for mobile advertising, where a small banner at the bottom of the app was specified for advertisements after blocking or filtering unwanted advertisement categories.

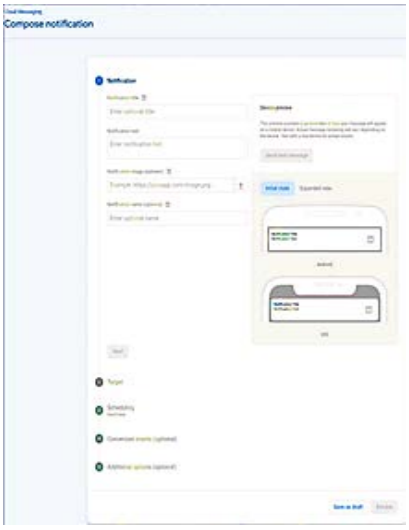


**Figure: (7).** Salary Screen


**Figure: (8).** App Notification

## RESULTS AND DISCUSSION

The app has gained interest from many individuals. It has more than 50k downloads. The app has also many positive reviews. The average rating of the Libyan salary app currently is 4.5, as shown in Figure 9. This rating is a result of 403 reviewers who contributed by rating the app. The rating value might increase or decrease with time depending on new reviews. The database that was collected in the first stage was stored locally. The size of the database is 1.74 KB, which is considered a small size. Therefore, at this time, it is not necessary to store the database using a cloud-storage service. The existence of the database locally contributes to the reliability and speed of the app. Another concept that plays a role in the app performance is what is called asynchronous programming (async) in Dart programming language. A Future is a foundational class in async programming. Therefore, if you call a function that might take a long time until it returns a value, you can make that function return a future value, and the program will not remain waiting but returns to the main routine without any delay. Once the future value is ready, you can use it (Windmill, 2020).

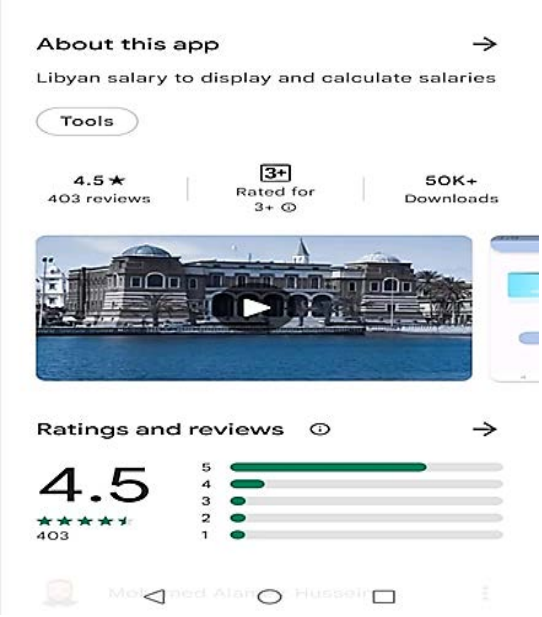


**Figure: (9).** App Rating

This method was used in the salary app in many cases. For instance, when the app checks the internet connection when launching or navigates between screens. Another example is bringing the correct salary table from the database. Therefore, the user will find the app working smoothly without any problems because all the previous procedures, which take a long time, are working in the background in a synchronized way. The app has been installed by users from different countries, as demonstrated in Google Analytics. However, you can notice in Figure 10 that Libya comes in the top list for user distribution per country over the last six months. It has 10k new Libyan users. Due to different users' behaviour, they might spend different times using the Libyan salary app. The average engagement time of app users was 3m 57s, as shown in Figure 11, also obtained from Google Analytics. Although, The Libyan salary app is the first to contain employee salaries from different ministries, which was released on 14 May 2021. However, there is a government app that works on Android published by the social security fund, which is called "الضمان موبايل".

The app is specified for retired people and was released on 16 December 2020. It displays details of their pensions. However, this app differs from the Libyan salary app because it has access to the government database for the retired. Table 2 illustrates a comparison between the two apps. The first column is about the app's download size. This feature affects the required time for the app to be installed. The Libyan salary app has also a bigger size. The same applies to the second column (App size). The app size is the amount of space required by the app to be installed on your device. Cross-platform apps have a bigger size than native apps. However, there some optimizing work should be done to reduce the app size. The minimum requirement for the salary app is Android 4.4. The app in this paper has many positive reviews. It also overcomes the first app in Table 2 in the average rating with a 4.5 rating, despite it being available for less than one year in the Google Play Store.


**Figure: (10).** Users by Country

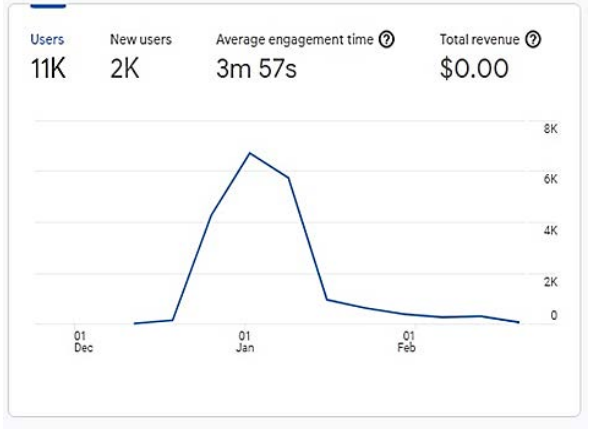


**Figure: (11).** Users Engagement

**Table: (2).** Apps Comparison

| App Name | App Download Size | App Size | Rviews | Rating |
|---|---|---|---|---|
| "الضمان موبايل" | 6 MB | 14.9MB | 840 | 4 |
| "الراتب" | 8.3 MB | 48 MB | 403 | 4.5 |

## CONCLUSION

The paper introduced a solution for many people who want to know all details about their salary, which includes promotions and deductions. By using Cross-Platform Development (Flutter), The Libyan Salary app was programmed to work for Android mobiles. It also works as a web application. The user needs to choose his field of work. The app will then show a screen where you can choose your occupation grade and salary increment, and with one button click, all salary details will appear. A notification service is available in the app to show news related to salaries, and notify app users about any new updates. The app has a reasonable size and great performance. Although the app has many positive comments from its users, there is a significant demand to add more ministries and sectors in the future. Other future work is to add a registration procedure, where each user can have a unique profile. As a result, he might only receive notifications related to his occupation. In addition, users' information will be protected using Firebase security rules.

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# تطبيق الراتب الليبي باستخدام برنامج فلاتر

**أيمن مختار حسين**

*قسم هندسة الحاسوب، جامعة الزاوية، الزاوية، ليبيا*

تاريخ الاستلام: 05 مايو 2022 / تاريخ القبول: 17 أغسطس 2022
Doi:https://doi.org/10.54172/mjsc.v37i3.802

**المستخلص** : انتشرت تطبيقات الهاتف المحمول في كثير من جوانب حياتنا اليومية. يقوم العديد من الأشخاص بمعظم أعمالهم عبر هواتفهم مثل الخدمات المصرفية عبر الإنترنت والتسوق وما إلى ذلك. ونظرًا لأن العديد من الأفراد لا يعلمون تفاصيل رواتبهم، تقدم هذه الورقة تصميمًا وتنفيذاً لتطبيق الراتب الليبي باستخدام برنامج فلاتر. يتيح التطبيق للمستخدمين معرفة تفاصيل رواتبهم بما في ذلك من علاوات وخصومات حسب جدول الرواتب لكل وزارة أو قطاع. يحتوي التطبيق حاليا على جداول رواتب عشرة وزارات. تتألف عملية بناء التطبيق من الخطوات الأربع التالية: جمع القوانين وجداول المرتبات لكل الوزارات لاستخدامها كقاعدة بيانات لتطبيق الراتب، تصميم شاشات التطبيق بحيث تكون سهلة الاستخدام، ربط التطبيق الخاص بنا مع منصة Firebase، وأخيراً إعداد نسخة أندرويد وويب لتطبيق الراتب، ونشرها. ومن الجدير بالذكر أن النتائج التي تم الحصول عليها من المقيمين في متجر قوقل بلاي مرضية نوعا ما. بالإضافة إلى ذلك ، هناك طلب متزايد من أفراد مجتمعنا لإضافة وزارات أخرى إلى التطبيق. ويشجع مجتمعنا تقديم مثل هذه الخدمة التي تعد الأولى من نوعها. وقد وصل التطبيق إلى أكثر من 50 ألف تنزيل في أقل من ستة أشهر، ومتوسط التقييم 4.5 من 5 في متجر قوقل بلاي وقت كتابة هذه الورقة.

**الكلمات المفتاحية** : تطبيقات الهاتف المحمول، تطبيق الراتب.

* أيمن مختار حسين: a.hassan@zu.edu.ly، قسم هندسة الحاسوب، جامعة الزاوية، الزاوية –ليبيا

*Al-Mukhtar Journal of Sciences* 37 (3): 261-273, 2022
*ISSN: online 2617-2186 print 2617-2178*
**Journal Homepage** https://omu.edu.ly/journals/index.php/mjsc/index

# Effects of *streptomyces rochi* Biosurfactants on Pathogenic *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

**Nadia H. Al-Healy[1*], Essra Gh.Al-Sammak[2]**

[1] *Department of Techniques, Faculty of Medical Laboratory, Al-Hadba University, Iraq*
[2] *Department of Biology, Faculty of Science, Mosul University, Iraq*

Received: 22 June 2022/ Accepted: 07 September 2022
Doi: https://doi.org/10.54172/mjsc.v37i3.947

**Abstract:** Fifty soil samples were collected from soils contaminated and uncontaminated with hydrocarbons. Six isolates belonging to *Streptomyces rochei* were diagnosed, 8% from contaminated soil and 6% from uncontaminated soil. Isolates were diagnosed depending on the study of 16s rDNA compared to standard isolates within the National Center for Biotechnology Information site. 28 smears of wounds and 12 smears of burns were collected, including *Staphylococcus aureus* isolates (35.7%) and *Pseudomonas aeruginosa* (16.6%). The isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed multiple resistance to antibiotics, Oxacillin, Erythromycin, Nalidixic acid, and Tetracycline. Isolate *Streptomyces rochei* 19 showed the ability to produce biosurfactants that have antagonistic properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In addition, the biosurfactant production from *Streptomyces rochei* 19 were non-toxic to the potential growth of *Brassica oleracea* seeds at 1.5 and 50 mg/cm$^3$. The biosurfactant was diagnosed as Lipopeptide using thin layer chromatography and GC-Mass technique.

**Keywords:** *Streptomyces rochei*, Antimicrobial, Biosurfactant, GC-Mass.

## INTRODUCTION

The genus of Streptomyces represents the largest group within Actinobacteria (Prescott et al., 2018). In general, the members of the Streptomycetaceae family can be distinguished by means including morphological, physiological traits, the chemical composition of the cell wall, the type of peptidoglycan, and type of fatty acid chains (Madhaiyan et al., 2016). Molecular methods including GC ratio, 16s rRNA sequence techniques, and hybridization methods were also implied (Law et al., 2019). It is characterized by being a gram-positive filamentous bacterium, non-acid fast stain, heterotrophic, able to grow in different environments, catalase positive, nitrate reduces to nitrite and has the ability to hydrolyze adenine, ascoline, casein, and starch (Chen et al., 2018). The filament is divided into one level to form chains of nonmoving 3-5 spores with a smooth, spinal, or hair-like surface (Maleki et al., 2013). The cell wall contains peptidoglycan with large amounts of L-diaminopimelic acid and does not contain mycolic acid (van der Aart et al., 2018). Streptomyces species have the ability to produce a distinctive earthy odor because it produces volatile compounds called geosmin (Juttner & Watson, 2007). It produces many pigments which are responsible for giving color to the substratum and aerial mycelium. There are roughly 550 species identified belonging to this genus and more than 3000 strains which have confused the classifiers in

***Corresponding author:** Nadia H. Al-Healy: *nadiaalhealy70@gmail.com*. Department of Techniques, Medical Laboratory, Al-Hadba University, Iraq

finding their diagnostic tables (Lee & Whang, 2014). The Streptomyces genus is known to be a rich source of antibiotics (Alsammak & Alhyaly, 2019), accounting for 75% of the world's naturally produced antibiotics (Barka et al., 2016). *Streptomyces rochei* was first isolated from soil in Russia. It has the ability to produce many secondary metabolites including borrelidin, the surface, or interfacial tension between two liquid phases such as oil/water or air/liquid interfaces (Gudiña et al., 2016). The chemically synthesized surfactants have a wide range of applications in industries (Kaczorek et al., 2013). However, the chemical synthesized surfactants are expensive and are causing environmental pollution. Biosurfactants are natural products produced by microorganisms. The Biosurfactants have unique properties which differ from chemically synthesized surfactants (Nitschke & Silva, 2018). Biosurfactants are environmentally friendly, biodegradable, and have good anti-microbial activities at extreme temperatures, pH, and salinity (Thavasi et al., 2011). These advantages allow biosurfactants to be a substitute for chemically synthesized surfactants. Biosurfactants can be classified depending on their chemical structures into glycolipids, lipopeptides, polysacchride-protein complexes, phospholipids, fatty acids, and neutral lipids (Geetha et al., 2018). The aim of this study was to study antibacterial activities of S*treptomyces rochei* that has the ability to produce biosurfactants.

## MATERIALS AND METHODS

### Isolation of Streptomyces

Fifty soil samples were collected from different sites in Mosul Nineveh city. These included samples contaminated with hydrocarbons (from the generator's area and the oil refineries of Qayarah). Non-polluted soil samples from domestic and public gardens were also included. The samples were taken at a depth of about (2-5) cm. The dilutions of soil were done by taking 1g soil/9 $cm^3$ sterile distilled water, mixed with a vortex for 100 seconds to obtain cell preparation/$cm^3$, and then performing successive decimals to reach dilution to $10^{-4}$. $1cm^3$ of dilution $10^{-3}$ and $10^{-4}$ were taken and distributed by pouring on Starch Casein Agar. The plates were incubated aerobically for 7-10 days at a temperature of 30ºC (Gebreyohannes et al., 2013).

**Isolation of *Staphylococcus aureus* and *Pseudomonas aeruginosa*:** Forty swabs of burns and skin wounds were taken from random people using transport peptone water. The samples were then cultured on the nutrient agar medium to isolate Pseudomonas and the mannitol salt agar to isolate Staphylococcus. Oxacillin resistance screening agar base (ORSAB) was used to isolate methicillin-resistant Staphylococcus (MRSA). The plates were incubated aerobically at 37ºC for 24 hours (Murray et al., 2003).

**Molecular diagnoses of *Streptomyces rochei* and *Staphylococcus aureus:*** Isolation of bacterial DNA using a DNA extraction kit (Wizard ® genomic DNA purification kit) from Promega Company.

### PCR to investigate the 16s rDNA gene

Primers: lyophilized universal primers were used by Promega as in table (1). Primers were dissolved in sterile distilled water to obtain a concentration of 10 picomol/µl.

**Electrophoresis method:** Genetic DNA was detected using electrophoresis on agarose gel electrophoresis at a concentration of 1% agarose. The power supply was adjusted at 50 volts for 80 minutes, and the agarose gel was stained with Diamond $^{TM}$ Nucleic acid.

**Table: (1)**. The sequence of 16s rDNA primers

| The name of gene used | 5′→3′ | Primer | Product size Bp | Reference |
|---|---|---|---|---|
| 16s rDNA | AGAGTTTGATCCTGGCTCAG | 27 Upstream | 1350 | (Cotarlet et al., 2010) |
| | GACGGGCGGTGTGTAC | 1392 Downstream | | (Lane, 1991) |

**PCR reactions to investigate the 16s rDNA gene:** A total of 25µl for all samples containing

the following ingredients were prepared by mixing 12.5µl of green master mix, 1µl from each forward and reverse primers, 4µl of DNA template extracts at a concentration of 50ng/µl, and 6.5 µl of nuclease-free water in a 0.2 Eppendorf tube. Ingredients were blended well by making a spin for 10 seconds with a cold centrifuge, and then the tubes were inserted into the thermocycler device by using the following program:
Min 5 94ºC 1 Cycle
Sec 30 94ºC
Min 1 55ºC 30 Cycles
Min 1 72ºC
Min 5 72ºC 1 Cycle. (Cotarlet et al., 2010)
The PCR products were detected by electrophoresis in 2% agarose at 50 volts for 80 min. The gel was examined after staining with Diamond $^{TM}$ Nucleic acid for 45 minutes using Ultraviolet light to detect the 16s rDNA genes at the site of 1350 base pairs. The molecular size of the DNA fragment was estimated by comparing the band size with the DNA ladder (Sambrook & Russell, 2001).

**Sequences analysis for 16s rDNA gene:** The PCR amplification products were sent with the forward and reverse primers to the Micro-gene laboratory in Korea, where the genotype sequences of the gene 16s rDNA were determined. The results were analyzed using the Basic Local Alignment search tool (BLAST) program, available at the National Center for Biotechnology Information (NCBI) on the site ( http://www.ncbi.nlm.nih.gor).

**Primary screening of the ability of *Streptomyces rochei* 19 to produce biosurfactants**
**First: hemolytic activity:** Hemolysis was car

ried out on plates of blood agar medium supplemented with (5%) human blood. The blood agar plates were inoculated and incubated at 37ºC for 7 days. Plates were examined for a clear zone around the colonies (Carrillo et al., 1996).

**Second: modified drop collapse metho:** Drops of crude oil were dropped as a thin layer on the surface of a glass slide. A 10µl drop of the supernatant of seven days Streptomyces was cultured in maltose Yeast extract broth by cooling centrifuge at 10000 rpm/min for 15 min on the slide. The droplet shape was then observed one minute later. The negative result is explained by the fact the droplet remains convex and the same size, while the positive result is when the drop collapses and becomes flat with a slightly larger size. The result was compared by adding water as a negative control and SDS as a positive control. The test was performed with three replications per isolation (Plaza et al., 2006).

**Third: lipase production:** For lipase activity, Luria-Bertani agar plates supplemented with (1%) of an olive oil emulsion were made (pH of the medium adjusted 7.0) inoculated with a fresh culture of Streptomyces and incubated at 37ºC for seven days. After incubation, plates were observed for a clear zone of hydrolysis around the colony (Maniyar et al., 2011).

**Forth: oil spreading assay method:** 50µl of distilled water was added to the Petri dish, Followed by adding 20µl of crude oil on the surface of the water. 10µl of supernatant was added onto the center of the oil film. The diameter of the clear zone on the oil surface was measured and compared with control using cultured broth (Cornea et al., 2016).

**Fifth- Emulsification index (E24):** Samples were determined by adding 2ml of paraffin oil and 2ml of the cell-free broth in a test tube. Vortexed at high speed for 2 min and allowed to stand for 24h, the percentage of the Emulsification index was calculated using the following equation (Abouseoud et al., 2008).
E24=Total height of emulsion formed (cm)*100/total height of solution (cm)

**Productivity medium used for biosurfactant from *Streptomyces rochei:*** Two media were used for productivity:
1-Maltose Yeast Extract Broth (MYE) supplemented 1% of olive oil (Kokare et al., 2007). The broth culture was incubated aerobically at

30ºC in a shaker incubator at 120 rpm for seven days. 2-Starch nitrate medium, the broth cultures were incubated aerobically at 30ºC in a shaker incubator at 120 rpm for seven days (Shubhrasekhar et al., 2013).

**Biosurfactant Extraction :** First method: the liquid productive culture was incubated for seven days, centrifuged at 100 rpm/min for 15 minutes, the precipitate was discarded, and the supernatant was taken and mixed with the same volume of solvent chloroform-methanol (2:1 v/v) using separating funnels. The bottom layer containing the active substance was taken and evaporated with rotary evaporation, and a dry material was stored in the refrigerator until use (Kiran et al., 2017).

Second method: the culture samples were centrifuged at 14000 rpm/min for 20 min to remove the bacterial cells. 1M HCl was added to the supernatant to reach pH 2.0. The precipitate was collected by centrifugation at 12000 rpm/min for 30 min at 4ºC to obtain crude biosurfactant. The crude biosurfactant was dissolved in deionized water and adjusted to a 7.0 pH and extracted by a solvent having a chloroform: methanol (2:1 v/v), the organic phase (chloroform layer) was evaporated using a rotary evaporator to remove the solvent chloroform. A dry material was stored in the refrigerator until use (Deepa et al., 2015).

**Antibiotics Sensitivity:** By using the Kirby-Bauer method, the isolates of *Staphylococcus aureu*s and *Pseudomonas aeruginosa* at 18 hours with the concentration of ($1.5*10^8$ cell/$cm^3$) compared to McFarland tubes were inoculated by swab on Muller-Hinton Agar. The sensitivity to the following antibiotics: Tetracycline 10µg, Oxacillin 5µg, Carbencillin 25µg, Erythromycin 10µg, Nalidixic acid 30µg, and Gentamicin 10µg was studied, the plates were incubated at 37ºC for 24 hours and then the diameter of the inhibition zone was measured (Winn et al., 2006).

**Antimicrobial activity of biosurfactant:** A sterile filter paper was taken and immersed in biosurfactant extract solution by sterile forceps and put in Muller Hinton Agar inoculated with *Staphylococcus aureu*s and *Pseudomonas aeruginosa* at the age of 18 hours with the concentration of $1.5*10^8$ cell/$cm^3$(compared to McFarland tubes). The plates were incubated at 37ºC for 24 hours (Gebreyohannes et al., 2013).

**Determination of biosurfactant toxicity:** The phytotoxicity test was used to investigate the action of the biosurfactant on cabbage seeds (*Brassica oleracea* L.). Different concentration of biosurfactant solution (1.5 and 50 mg/ml), and control solution (distilled water), were tested. The biosurfactant samples were placed in Petri dishes, each with ten seeds. The plates were incubated for five days at 27ºC. The number of germinated seeds was then counted and the length of the roots was measured from the point of transition from the hypocotyl to the extremity of the root (Yerushalmi et al., 1998). The germination index (GI), which is one of the most commonly used ways to characterize the phytotoxicity of a compound, was calculated as follows:

%GI = (% of seed germination) x (% of root growth): 100

in which % of seed germination = (% of germination in the extract): (% of germination in the control) x 100; and % of root growth = (mean growth in the extract): (mean growth in the control) x 100. The analyses were performed in triplicate.

**Separation of biosurfactant extract using a thin layer chromatography technique**:The obtained extract was separated using 20 $cm^3$ X 20 $cm^3$ silica chromatography plates, the extract was mixed with the solvent DMSO, and the samples were left at room temperature or dried using a hair dryer. The separation solution consisted of chloroform / methanol/ water (65:25: 4, by vol.) (Symmank et al., 2002). The flow rate was calculated for all separated spots according to the following law:

Flow rate = distance traveled by the extract/distance traveled by the solvent.

The compounds separated by TLC were visualized by spraying with 0.5% ninhydrin (w/v, in water or ethanol) to identify the free amino groups. The plates were heated at 110º C for 5 min until the appearance of a reddish-brown color (Bezza & Chirwa, 2015).

**Characterization of biosurfactant by Gas chromatography-mass spectrometry (GC/MS)**: The chloroform: methanol extract was analyzed by gas chromatography-mass spectrometry (GC/MS). The spectrum of the crude component was compared with the spectrum of the known components in the National Institute Standard and Technology (NIST) library. The name, molecular formula, weight, and chemical structure of the components of the test materials were identified.

**Phylogenetic relationships:** The evolutionary relationship among the six *Streptomyces rochei* was obtained using the Unweighted Pair Group Method with Arithmetic mean (UPGM) within Molecular Evolutionary Genetics Analysis version 7 (MEGA7) using the Maximum Composite Likelihood method based on (Tamura et al., 2004). The strains were submitted to NCBI with the accession number for each strain.

## RESULTS AND ISCUSSIONS

**Isolation:** Twenty-four isolates belonging to Streptomyces species were isolated, as shown in table (2), the percentage of *Streptomyces rochei* from hydrocarbon contaminated soils was 8% and uncontaminated soils was 16%, which are considered to be the main bacteria of the soil (Loganathan Karthik & Rao, 2010). The morphological characteristics of the colonies and cells and their adhesion to the nutritional medium and odor production of isolates suspected to belong to the genus of Streptomyces were studied (G Al-Sammak et al., 2009). Species of the genus Streptomyces are difficult to characterize, as indicated by the researcher Holt and his colleagues in 1994 (Holt et al., 1994). While the isolation rate of Pseudomonas from burns was 16.6%, Staphylococcus from wounds was 35.7%. Six isolates belonging to *Streptomyces rochei* were genetically diagnosed using 16s rDNA.

**Table: (2).** Percentage of bacteria isolated from environmental and clinical samples

| Isolated bacteria | Isolation rate % | Number of isolates | Number of samples | Type of samples |
|---|---|---|---|---|
| *Streptomyces rochei* | 8 | 2 | 25 hydrocarbonate soil | Soil |
| | 16 | 4 | 25 uncontaminated soil | |
| Staphylococcus | 35.7 | 10 | 28 swabs | Wound |
| Pseudomonas | 16.6 | 2 | 12 swabs | Burns |

**Phylogenetic relationship:** By using 16s rDNA for molecular diagnosis of *Streptomyces rochei*, the *Streptomyces rochei* strains have shown similarity between (99.42-99.7) % to reference strains in the NCBI. The isolates where clustered together in the phylogenetic tree at 99.4%, as shown in figure (1).

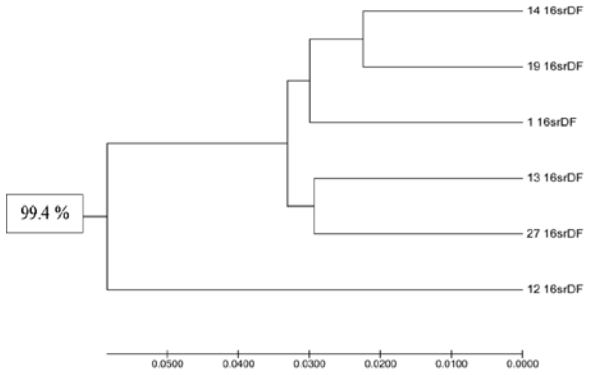


**Figure: (1).** Phylogenetic tree of the 16S rDNA *Streptomyces rochei* based on UPGMA method with aid of MEGA 7. Program.

Depending on some morphological trials and biochemical tests, in addition to the use of 16s rDNA, four isolates were supposed to be *Staphylococcus aureus*, namely 1,6,7,9. These strains showed β-hemolysis, positive for catalase test, and fermentation of mannitol sugar. A special medium (ORSAB) was used to isolate the *Staphylococcus aureus* drug resistance. This medium contains two types of antibiotics,

Oxacillin and Polymixin B, with the presence of sodium chloride salt in the concentration of 5.5%. The methicillin resistance *Staphylococcus aureus,* colonies appeared in blue color on the ORSAB medium and yellow color on the Mannitol salt agar (Simor et al., 2001). While *Pseudomonas aeruginosa* cells showed positive for oxidase and citrate, β-hemolysis, producing pyocyanin stain and foul smell similar to rotting fruit smell as this characteristic was used in the initial diagnosis of the species. And the diagnosis of species was ensured by using an API 20E strip. The *Staphylococcus aureus* and *Pseudomonas aeruginosa* species showed resistance in 100% to Oxacillin, Erythromycin, Carbenicillin, and Nalidixic acid, as shown in table (3). The *Staphylococcus aureus* resistance to β-Lactam antibiotics was due to the ability of the species to produce penicillin-binding protein 2a (PBP2a) encoded by the *mec*-A gene located on the mobile gene element (MGE) that has a low roll of β-Lactam antagonists. In recent years, the species has been resistant to many antibiotics, including Macrolides, Aminoglycoside, Quinolones, and Tetracycline, because resistance genes are carried on plasmids and transposons, which can be transmitted between species and other gram-positive bacteria (Akpaka et al., 2017). While the resistance of the *Pseudomonas aeruginosa* is due to its possession of pumping systems for many antibiotics, in addition to the plasmids responsible for resistance, the mutation also has a role in the resistance (Lister et al., 2009).

**Hemolytic activity**: Initial investigation on the ability of *Streptomyces rochei* isolates to produce biosurfactants using four methods. Six *Streptomyces rochei* isolates were selected, as shown in table (4). The isolates showed complete hemolysis, and the use of this test was based on the fact that biosurfactants can cause the analysis of erythrocytes. The researcher (Chakraborty et al., 2009) pointed out that there is a relationship between the effectiveness of blood hydrolysis and the production of biosurfactants. The use of hydrolysis of blood is a primary indicator to search for the production of biosurfactants.

**Table: (3) .**The resistance of *Staphylococcus aureus* and *Pseudomonas eruginosa* to antibiotics

| No. Isolation | Bacteria | Vancomycin (VA) 30 | Oxacillin (OX) 5 | Erythromycin (E) 10 | Carbencillin (PY) 25 | Gentamicin (CN) 10 | Nalidixic acid (NA) 30 | Tetracyclin e (TE)10 |
|---|---|---|---|---|---|---|---|---|
| 1 | *Staph.aureus* 1 | S | R | R | R | S | R | R |
| 2 | *Staph.aureus* 6 | M | R | R | R | R | R | R |
| 3 | *Staph.aureus* 7 | S | R | R | R | S | R | R |
| 4 | *Staph.aureus* 9 | S | R | R | R | M | R | R |
| 5 | *Staph.aureus* 10 | M | R | R | R | R | R | R |
| 6 | *Staph.aureus* 17 | S | R | R | R | R | R | S |
| 7 | *P.aeruginosa* 1 | R | R | R | R | R | R | R |
| 8 | *P.aeruginosa* 2 | R | R | R | R | R | R | R |

R= resistance S= sensitive M= moderate

**Table: (4).** Initial investigation of *Streptomyces rochei* to produce biosurfactants.

| Accession number in NCBI | Strains | Hydrolysis on blood agar | Lipolysis medium | Modified drop collapse test | Oil dispersion test |
|---|---|---|---|---|---|
| MN589659 | *Streptomyces rochei*1 | + | - | - | + |
| MN594555 | *Streptomyces rochei* 12 | + | + | - | + |
| MN594553 | *Streptomyces rochei* 13 | + | + | - | + |
| MN594540 | *Streptomyces rochei* 14 | + | - | - | + |
| MN589658 | *Streptomyces rochei* 19 | + | + | + | + |
| MN594530 | *Streptomyces rochei* 27 | + | + | - | + |
| | Percentage % | 100 | 66.6 | 16.6 | 100 |

**Lipase production**: Some strains showed a positive result for the lipolysis test. The result showed a clear zone around the colony, evidence of its ownership of the lipase enzyme, which works to breakdown fat to produce fatty acids, as the production of this enzyme is associated with the production of biosurfactant (Deepa et al., 2015). The results were con-

sistent with the study of (Kokare et al., 2007), in which of the 80 strains of the genus Streptomyces, only 56 were positive for the lipase test.

**Modified drop collapse method:** Most of the isolates showed a negative result to the modified drop collapse test, even though it gave a positive result in the hemolysis test. The negative result may be due to the fact that the test is hyposensitivity, in which biosurfactant concentration must be rather high in the filtrate to cause the droplet to collapse (Walter et al., 2010). This method (modified drop collapse) is an easy, fast, and non-specialized for a particular type of biosurfactant and needs a small amount of the sample, easy to perform, and does not need special requirements (Khopade et al., 2012).

**Oil spreading assay method:** All strains that gave good hemolytic activity gave good efficacy to the oil dispersion test. The diameter of the clear zone is in direct correlation with the effectiveness of biosurfactant. When a drop of biosurfactant was added to the oil layer on the water, a clear zone was observed (Figure 2) as in the study of (Al-Safar & E.G.AL-Sammak, 2014)

. The organisms producing biosurfactants alone can disperse oil, as noted by the research (Youssef et al., 2004). The oil dispersion test has been shown to be more sensitive than the method of testing the modified drop collapse in detecting the presence of biosurfactant in the filtrate of the bacterial culture (Ainon et al., 2013). In order to know the productivity of biosurfactants, many researchers use two or more methods to search for biosurfactants. Relying on one method is inappropriate in diagnosing all kinds of biosurfactant, therefore, a set of tests is used to find the product object (Bodour & Maier, 2003).

**Figure (2).** Oil spreading assay: zone of clearance by crude biosurfactant from *Streptomyces rochei*.

**The production of biosurfactants from *Streptomyces rochei* strain 19:** The results showed that the insoluble carbon source in the medium, olive oil, gave the highest increase in productivity over the water-soluble carbon source, glycerol, by observing the diagonal sizes to test the dispersion of oil as indicated by (Abouseoud et al., 2007), probably due to the bacteria's exploitation of to the source of dissolved carbon at high speed in the production of energy and biomass and the lack of the need to produce compounds that analyze the carbon source. In the case of olive oil, the bacterium work to produce biosurfactant for the purpose of analysis of fatty substances and make them more polar, which in turn enter into the synthesis of secondary metabolic products and energy production as well as increasing the area of adhesion of bacteria with olive oil in the medium and this is another benefit (Swadi et al., 2013). The results of the emulsification coefficient test showed that the production medium containing olive oil as a carbon source had a high emulsification coefficient (88%), while the isolation using glycerol as a carbon source showed a low emulsification coefficient (33%). This test is the most reliable in the quantification of active substances dissolved in the medium (Ainon et al., 2013).

**Biosurfactant extraction from *Streptomyces rochei* 19:** The direct method described by (Shubhrasekhar et al., 2013) is to extract biosurfactants from isolation *Streptomyces rochei* 19. The direct method was efficient in extraction, easy and fast, and does not require much effort.

**The antibacterial activity of biosurfactant against *Staphylococcus aureus* and *Pseudomonas aeruginosa***: The biosurfactant extracted from *Streptomyces rochei* 19 showed inhibitory activity against a strain of *Pseudomonas aeruginosa* and *Staphylococcus aureus* as shown in Figure (3).

Since the biosurfactant was effective against gram-negative bacteria and gram-positive bacteria, this is probably because the extracted biosurfactant was from a broad-range type.

**Assessment of toxicity using the germination test:** The germination indexes obtained for seeds of cabbage show higher germination of about 59, 73.5, and 75.5%, with good correlation between average elongation of root and germination index. The biosurfactant produced by *Streptomyces rochei* 19 promoted no toxicity to the seeds of *Brassica oleracea* at concentrations of 1.5 and 50 mg/ml. This result is similar to those of Silva and his colleagues 2010. He shows that the biosurfactant by *Pseudomonas aeruginosa* UCP992 has no toxicity to the seeds of *Lactuca sativa* L. and *Brassica oleracea.*

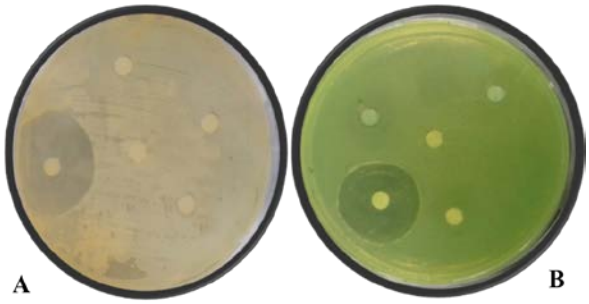


**Figure: (3).** The antimicrobial activity of biosurfactant isolated from *Streptomyces rochei* 19 on
A-*Staphylococcus aureus*
B-*Pseudomonas aruginosa*

**Thin layer chromatography (TLC):** The separation and diagnosis of biosurfactants extracted from strain 19 were detected at a flow rate of 0.9 obtained in a reddish brown spot by using ninhydrin to detect free amino groups and a brown spot by using ioden vapor, thus indicating that the biosurfactant is lipopeptide (Bezza & Chirwa, 2015).

**Gas chromatography and mass spectrometric analysis:** In GC-MS analysis 21 compounds were identified, five of them were bioactive compounds from *Streptomyces rochei* 19 strain, as shown in table (5).

**Table: (5).** Chemical constituents of chloroform-methanol extract of *Streptomyces rochei*19 identified by gas chromatography-mass spectrometer (GC/MS) analysis.

| Compounds | RT | Compound formula | Molecular weight | Activity | References |
|---|---|---|---|---|---|
| Octanoic acid | 11.147 | $C_8H_{16}O_2$ | 144 | Antimicrobial activity | Hismiogullari et ) (al., 2008 |
| Hexanoic acid | 8.048 | $C_6H_{12}O_2$ | 116 | Antimicrobial activity | Hismiogullari et ) (al., 2008 |
| Phenol, 2,4-bis(1,1-dimethylethyl)- | 15.863 | $C_{14}H_{22}O$ | 206 | Antibacterial activity | (Lata, 2015) |
| Undecanoic acid | 12.557 | $C_{11}H_{22}O_2$ | 186 | Antifungal activity | (Peres et al., 2011) |
| Dodecane, 2,6,10- trimethyl- | 12.824 | $C_{15}H_{32}$ | 212 | Antibacterial activity | Nahid et al., ) (2012 |

## CONCLUSIONS

The biosurfactant extracted from *Streptomyces rochei* 19 showed inhibitory activity against Multi-drug resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In addition, the biosurfactant production from *Streptomyces rochei* 19 was non-toxic to the potential growth of *Brassica oleracea* seeds at 1.5 and 50 mg/cm$^3$. Biosurfactants were diagnosed as Lipopeptides by using thin layer chromatography and GC-Mass technique.

## ACKNOLEHGEMENT

Thanks are due to the Al-Hadba University College, Medical Lab. Technology Department, Iraq, and Mosul University, College of Science, Biology Department, Iraq.

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# تأثير منشطات السطح الحيوية للنوع *Streptomyces rochei* على الممرضات *Staphylococcus aureus* و *Pseudomonas aeruginosa*

**نادية حسين الحيالي[1] و اسراء غانم السماك[2]**

[1] *قسم تقنيات المختبرات الطبية، كلية الحدباء الجامعة، العراق*

[2] *قسم علوم الحياة، كلية العلوم، جامعة الموصل، العراق*

تاريخ الاستلام: 22 يونيو 2022 / تاريخ القبول: 07 سبتمبر 2022
Doi:https://doi.org/10.54172/mjsc.v37i3.947

**المستخلص** : جمعت *(50)* عينة من تربة ملوثة، وغير ملوثة بالهيدروكاربونات. شخصت ست عزلات تابعة للنوع *Streptomyces rochei*، *8%* من التربة الملوثة بالهيدروكاربونات، و *6%* من التربة غير الملوثة بالاعتماد على دراسة *16s* rDNA مقارنة مع المركز الوطني لمعلومات التقنية الحيوية. جمعت (*28*) عينة ممرضة لجروح و(12) لحروق عزلت منها عزلات تعود إلى النوع *Staphylococcus aureus* وبنسبة *(35.7%)*، والنوع *Pseudomonas aeruginosa* وبنسبة *16.6%*. أظهرت العزلات التابعة للنوع *Staphylococcus aureus* و *Pseudomonas aeruginosa* مقاومة متعددة للمضادات الحيوية Oxacillin، Erythromycin، Nalidixic acid و Tetracycline . أظهرت العزلة *Streptomyces rochei 19* قابلية على إنتاج المواد الحيوية الفعالة سطحياً، والتي لها فعالية مضادة ضد النوعين الممرضين *Staphylococcus aureus* و *Pseudomonas aeruginosa*. بالإضافة إلى ذلك، كان إنتاج منشطات السطح الحيوية من Streptomyces *rochei 19* غير سام للنمو المحتمل لبذور Brassica oleracea عند 1،5 و50 مجم/سم$^3$. شخصت هذه المواد كدهون ببتيدية بالاعتماد على تقنية كروماتوكرافيا الطبقة الرقيقة TLC وتقنية GC-Mass.

**الكلمات المفتاحية** : ستربتوميس; مضاد للجراثيم ; التوتر السطحي الحيوي; تقنية قياس الكتلة–الغاز.

* نادية حسين الحيالي: *nadiaalhealy70@gmail.com*. قسم تقنيات المختبرات الطبية، كلية الحدباء الجامعة، العراق.

*Al-Mukhtar Journal of Sciences* **37 (3): 274-283, 2022**
***ISSN: online 2617-2186 print 2617-2178***
**Journal Homepage** https://omu.edu.ly/journals/index.php/mjsc/index

# Surgical Management of Traumatic Teat Fistulas in Crossbred Goats with Polyester Sutures

**Khaled. M. A. Hussin[1*], Hamdi. Faraj Naji[1], Asma Saleh W[2] and Marwan Saleh[3]**

*[1]Department of Clinical Veterinary Medical Sciences, Faculty of Veterinary Medicine, Omar Al- Mukhtar University, Al-Bayda, Libya*
*[2]Department of Botany, Faculty of Science, Darna University, Darna, Libya*
*Agricultural Research Center, Al-Bayda, Libya Agricultural Research Center, Al-Bayda, Libya*

Received:13 July 2022 / Accepted: 06 September 2022
Doi: https://doi.org/10.54172/mjsc.v37i3.943

**Abstract:** For the current investigation, a total of six goats with traumatic teat fistulas that were brought to Omar Al-Mukhtar University's University Veterinary Hospital (UVH) were chosen. Using a basic continuous suture pattern and polyglactin 910, the mucosal and muscle layers were sutured individually after preoperative examination. Polyester sutures that were braided and coated with polybutilate were used to oppose the skin edges. The morphological evaluation and ultrasonographical evaluation were used to assess the restored teat postoperatively. The success of the procedure in restoring the functional capacity of the teat postoperatively was demonstrated by the quality of the milk and milkability. Due to the issues experienced, which may be related to the management procedures, postoperative care, and the suture material, the surgical technique used for the management of teat wounds with fistula was shown to be less effective.

**Keywords:** Goats, Traumatic teat fistula, Polyglactin 910, Braided Polybutilate coated Polyester sutures.

## INTRODUCTION

For dairy farmers, the health of their udders and teats is becoming more and more important because diseases that damage these organs reduce productivity and cause big financial losses. (Abd-El-Hady, 2015). The mucosa, submucosa, highly vascularized connective tissue, muscularis, and skin make up the goat's teat's five layers. (Hendrickson, 2007) Stenosis (31.5%), laceration without perforation (22.2%), skin wound (21%), rupture or splitting (20%), and perforating injury (4.9%) are the several types of teat injuries that can occur in goats. Teat lacerations are frequently flap wounds with varied forms and penetration depths rather than neat, straight, and incised wounds. (Molaei & Ebrahimi, 2014). The two types of teat injury are external or "uncovered" lesions, and internal or "covered" lesions. (Nichols, 2009). They can also be divided into superficial and deep categories based on the various layers involved. (Ashraf. abdel-hamed. Hegazi & Khaled. M. A. Hussin, 2008; Nouh et al., 2014; Rafi. Mustafa. Elkaseh. Abdulla et al., 2008). Injuries to the papillary duct and teat cistern are examples of internal injuries. Teat wall lacerations of any kind are considered external injuries, while injuries to the papillary duct and teat cistern are considered interior injuries. (Sreenu et al., 2014). When cattle are housed (78%) as opposed to maintained in pastures (21%), the majority of teat injuries occur. (Molaei et al., 2002). They can happen in tie stalls or free stall barns and are frequently self-

***Corresponding author:** Khale M. A. Hussin khaled.alhmri@omu.edu.ly, Department of Clinical Veterinary Medical Sciences, Faculty of Veterinary Medicine, Omar Al- Mukhtar University, Al-Bayda, Libya.

inflicted when the goat rises. (Nichols et al., 2016). Due to the goat's larger udder and awkward mobility following parturition, teat wounds are frequent. They are most common in animals between the ages of five and six or in the early stages of lactation. (Molaei & Ebrahimi, 2014). According to the authors, the majority of the teat injuries take place within a month of calving, when goats are at their peak of production. On a goat's teat, better results can be obtained by utilizing the proper suture material and stitch design. (Couture & Mulon, 2005).

The strongest non-absorbable synthetic suture material at the time was polyester. They perform better than stainless steel sutures and are only outperformed by nylon, polypropylene, and polybuster sutures. (Banks. Alan S et al., 2001). These synthetic braided multifilaments, which are constructed of polyethylene terephthalate, are non-absorbable and have traits like low tissue reactivity, high tensile strength, simplicity in handling, and long life. They were offered either uncoated or coated in polybutylene, Teflon, or silicone (Kudur et al., 2009).

## MATERIALS AND METHODS

Six goats presented to University Veterinary Hospital (UVH) at Omar Al-Mukhtar University and were diagnosed to have deep lacerated wounds with exposed teat cistern (Figure. 1) were selected for the present study.

**Figure: (1).** Showing deep lacerated wounds with exposed teat cistern

The animal particulars viz; breed, age, body weight, feeding pattern- grazing/ stall feeding, stage of lactation, and calving history were documented. A complete history was obtained from the farmer regarding the time of occurrence of the wound and its duration and its etiology. A clinical examination of the affected teat was performed and the parameters included were udder morphology, teat which was affected, shape of the affected teat, and length of the teat (Table 1).

**Table: (1).** Animal Particulars, Anamnesis and Clinical examination (n = 6)

| Animal No | Breed | Age (Years) | B.W (KGs) | Feeding Pattern | Stage of Lactation | Calving History (Days) | Duration (Days) | Etiology | Treatment adapted | Udder morphology | Affected Teat | Shape of affected teat | Length (cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Crossbred | 3 | 36 | Grazing | 1 | 17 | 2 | Barbed wire | Yes | Globular | LFT | Cylindrical | 4 |
| 2 | Crossbred | 4 | 42 | Grazing | 1 | 23 | 5 | Barbed wire | Yes | Globular | RHT | Funnel | 6 |
| 3 | Crossbred | 5 | 45 | Stall fed | 1 | 11 | 3 | Barbed wire | Yes | Pendulous | LHT | Cylindrical | 3.7 |
| 4 | Crossbred | 4.3 | 39 | Grazing | 1 | 30 | 12 | Barbed wire | Yes | Pendulous | RFT | Funnel | 2.9 |
| 5 | Crossbred | 3.2 | 33 | Stall fed | 1 | 26 | 6 | Barbed wire | Yes | Globular | RHT | Cylindrical | 7 |
| 6 | Crossbred | 4 | 36 | Grazing | 1 | 32 | 8 | Barbed wire | Yes | Globular | LHT | Cylindrical | 5 |

LFT- Left Fore Teat; RFT – Right Fore Teat; RHT – Right Hind Teat; LHT – Left HindTeat.

On the day of presentation and following suture removal, the afflicted teat's ability to produce milk was evaluated using the following parameters: milk yield per quarter (kg), the

kind of milk flow, and affected quarter's milk flow rate (kg/min) by hand milking. The color, consistency, pH, California mastitis test, and somatic cell count (1x105cells/ml) of the milk on the day of presentation and suture removal were used to evaluate its quality. Following preoperative examination, all animals were denied food and drink for 12 to 18 hours prior to surgery. They were then all given intramuscular injections of Xylazine at a dose rate of 0.05 mg/kg, and the ring block technique was used with 0.5% bupivacaine hydrochloride to put them to sleep. The wound was extensively debrided with a No. 11 scalpel blade and irrigated with a 0.5% Povidone Iodine solution. Polyglactin 910 No. 3/0 was used in all of the animals to suture the first layer of the teat, which included the inner mucosal layer and the muscle and connective tissue. For the opposing margins of the skin wound, simple interrupted braided polybutilate-coated polyester sutures of size 2/0 (Ethibond Excel - Ethicon, Johnson and Johnson private limited, India) were used. (Figure. 2).

**Figure: (2).** Showing Size 2/0 polyester sutures with braided polybutilate coating were placed to the skin in an uncomplicated interrupted pattern.

To keep the teat open, a sterile modified polyvinyl tube was inserted (Infant feeding tube No. 10, Romsons Scientific and Surgicals India) and fastened with stay sutures. The altered polyvinyl tube was attached to a sterile, single-use 2 ml syringe, which was then fastened. Injections of meloxicam (0.5 mg/kg) and streptomycin-procaine penicillin (Dicrysticin- S-Zydus AHL) (ten mg/kg body weight) were given intramuscularly for seven days following surgery. After surgery, the surgically corrected teat was assessed on the seventh and tenth postoperative days using the parameters of suture integrity, number of sutures present, suture line type (dry/moist), suture site type (soiled/contaminated), and discharge presence. any wound dehiscence.

## RESULTS AND DISCUSSION

Four goats were allowed to graze among all the animals. Animals who were stall fed were housed in a closed enclosure and were more likely to sustain teat injuries from stamping. The same results were reported. (Khaled et al., 2016; Matzke et al., 1992; Molaei et al., 2002; Nichols et al., 2016; Nichols. S, 2008; Nouh SR et al., 2014).

As they were all in the first stage of nursing, the calves' gestational histories ranged from 11 to 32 days. The majority of teat injuries happened within one month after calving, when goats were in high production, which is consistent with the current findings and (Bristol, 1989). Due to the goat's large udder and movements during parturition, teat wounds were frequent. (Molaei & Ebrahimi, 2014).
The wound's healing time ranged from 2 to 12 days, and barbed wire was always the cause of the damage. Overcrowding of animals with swollen udders after calving in tie-stall or free-stall barns increases the danger of injury to the teat from the other animals or self-inflicted injury. (Mulon, 2016; Nichols et al., 2016). Poor udder conformation and low-hanging teats make the goat more likely to injure itself by kicking the teat. (Sreenu et al., 2014).

The animal's udder morphology was examined on the day of the presentation, and two of the animals had pendulous udders, while four had globular udders. These results were postponed (Tiwary et al., 2005) since the majority of ani-

mals with long teats and pendulous udders develop teat lacerations. The right hind teat in two animals, one fore in right and left teat, and left hind teat in two animals were the affected teats. However, the fore and rear teats are both more frequently impacted than the hind teats. (Molaei & Ebrahimi, 2014). Injury rates on the right and left sides are roughly equal. (Kudur et al., 2009). In two of the goats and four of the goats, the affected teats had a funnel-like form. The injured teats were between 2.9 and 7 cm long, indicating that longer teats were more likely to sustain injury. (Grommers et al., 1971).

The milk was leaking from the damaged teat and was difficult to assess on the day of presentation, making it difficult to determine milkability. While on the 10th postoperative day, the milk yield per quarter (kg), the kind of milk flow, and the afflicted quarter's milk flow rate (kg/min) by manual milking were all observed as being the Mean S.E., which was 0.09 0.01. In every instance, a severe cut connecting the skin and mucosa around the cistern led to milk dripping from the wound. So, on the day of the presentation, it was challenging to evaluate the milkability. A similar claim was made by (Mulon, 2016; Nichols. S, 2008).

The milk appeared to be normal on the day of presentation and the tenth postoperative day, showing no changes in color, consistency, or pH. (Table 2). Since all of the animals received antibiotic therapy and the wound was covered with Dynafix without any contamination, the California mastitis test was negative, and the somatic cell count (1x105 cells/ml) on the day of presentation was (Mean S.E.) 27812≠12724.1 and on the 10th postoperative day it was 37452±19754.6, which were found to be within normal range. Similar results were noted by. (Arul jothi N & Balagopalan. T.P, 2012; Khaled. M. A. Hussin & Ashraf. abdelhamed. Hegazi, 2008; Khaled. M. A et al., 2014; Khan & Khan, 2006; Tiwary et al., 2006).

**Table: 2.** Qualitative examination of milk on the day of presentation and on 10th Postoperative day (n=6)

| Animal NO | Milkability | | Colour of milk | | Consistency of milk | | pH | | California mastitis test | | Somatic cell count (1.00.000cell/mi) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day of Presentation | $10^h$ Postoperative Day | Day of presentation | $10^h$ Postoperative Day | Day of presentation | $10^h$ Postoperative Day | Day of presentation | $10^h$ Postoperative Day | Day of presentation | $10^h$ Postoperative Day | Day of presentation | $10^h$ Postoperative Day |
| 1 | 0 | 0.134 | White | White | Normal | Normal | 6.5 | 7 | Negative | Negative | 249893 | 294876 |
| 2 | 0 | 0.122 | White | White | Normal | Normal | 7 | 7.5 | Negative | Negative | 273524 | 334972 |
| 3 | 0 | 0.080 | White | White | Normal | Normal | 7 | 7.5 | Negative | Negative | 308953 | 390328 |
| 4 | 0 | 0.093 | White | White | Normal | Normal | 6.5 | 7 | Negative | Negative | 258986 | 294765 |
| 5 | 0 | 0.071 | White | White | Normal | Normal | 6.5 | 7 | Negative | Negative | 230472 | 289745 |
| 6 | 0 | 0.102 | White | White | Normal | Normal | 7 | 7.5 | Negative | Negative | 300467 | 359870 |
| Mean ± SE | | 0.096±0.013 | | | | | | | | | 27812≠12724.1 | 37452±19754.6 |

It was discovered that thoroughly debriding the wound edges and irrigation with 0.5% Povidone Iodine solution were very efficient ways to revive the surgical site. (Arul jothi N & Balagopalan. T.P, 2012; Khaled. M. A. Hussin et al., 2021; Khaled. M. A.Hussin et al., 2018; Mulon, 2016; Nichols. S, 2008). With Polyglactin 910 No. 3/0, the mucosal and muscle layers were sutured separately in a straightforward continuous pattern. In the current investigation, a three-layer suture technique was used, and it proved to be quite successful in com-

pletely closing the teat cistern. (Arul jothi N & Balagopalan. T.P, 2012; Arul Jothi et al., 2006; Balagopalan & Aruljothi, 2016; Ghamsari et al., 1995).The number of braided and polybutilate-coated polyester sutures used to close the skin varied from 5 to 12, depending on the length, direction, and size of the incision. Because these sutures are synthetic, they stay eternally in tissues. In humans, they are usually utilized for tendon lacerations where the sutures last longer inside the tissues. (Singer et al., 2010). The suture pattern used was an interrupted, straightforward design. (Nichols et al., 2016).Morphologically intact in three animals (33%) but not in four (67%)

**Table: (3).** Group II animal morphological examination (n = 6)

| Animal no | Intactness of sutures | No. of sutures present | | Nature of suture line | Nature of the suture site | Discharge | Wound dehiscence |
|---|---|---|---|---|---|---|---|
| | $7^{th}$ day | $0^{th}$ day | $7^{th}$ day | $7^{th}$ day | $7^{th}$ day | $7^{th}$ day | $7^{th}$ day |
| 1 | Not Intact | 8 | 3 | Moist | Contaminated | Yes | Yes |
| 2 | Not Intact | 5 | 3 | Moist | Contaminated | Yes | Yes |
| 3 | Intact | 6 | 6 | Dry | Not Soiled | No | No |
| 4 | Intact | 8 | 8 | Moist | Not Soiled | No | No |
| 5 | Not Intact | 7 | 4 | Moist | Contaminated | Yes | Yes |
| 6 | Not Intact | 10 | 6 | Dry | Contaminated | Yes | Yes |
| Mean ± S.E. | | 7.4±1.4 | 5±0.5 | | | | |

**Figure (3):** showing fistula growth, wound dehiscence, and gaping wound borders on day 7 after surgery in gout.

Due to its multifilament structure, poor knot security, increased tissue reactivity, and inflammatory response, the average number of sutures present was only 5.3. It's possible that using polyester sutures led to a localized infection that persisted and exacerbated the tissue reaction. (Boothe, 2003). Only 33% of the animals had dry suture lines, while 67% had moist suture lines, which may have been caused by an inflammatory reaction at the suture line and the multifilament structure of the suture material, which favors infection. The same results were reported. (Boothe, 2003; Premsairam et al., 2020). Because of its multifilament structure, postoperative wound infection was more likely to occur. (Modi, 2009). Suture site discharge was present in 67% of the animals, which led to a polluted suture site and the moist nature of the suture line. Four animals (or 67%) had discharge at the suture site, while only three animals (or 33%) did not, indicating that the suture site's local infection persisted and that an exaggerated tissue reaction to the polyester suture encouraged the development of a postoperative wound infection that led to the suture site's discharge. (Banks. Alan S et al., 2001; Boothe, 2003). The polyester sutures' braided design made them more susceptible to infection and contaminated suture sites. (Al-Mubarak & Al-Haddab, 2013). In 33% of the cases, there was no evidence of wound dehiscence, but in 67% of the animals, the suture site exhibited dehiscence due to contamination, which led to an enhanced inflammatory response since the outer coated layer had been torn. (Boothe, 2003).

All the animals had their skin sutures removed on the tenth postoperative day. (Nichols et al., 2016), It showed signs of wound dehiscence,

fistula development, and edge gaping. Complete wound healing was not achieved in four animals because of wound dehiscence, which may have been brought on by the moist nature of the suture line, contaminated suture site with discharge, postoperative surgical complications like tissue flap necrosis, wound dehiscence, and fistula formation with the gaping of wounds exposing the inner lying structures. (Azizi S et al., 2007; Mulon, 2016). According to an explanation, 67% of animals with polyester sutures did not have them intact, which resulted in increased tissue reactivity, inflammatory response, contamination of the suture site, chronic local infection, and exaggerated tissue reaction. (Boothe, 2003; Chellamani et al., 2013), while in 33% of the cases, the wounds healed without dehiscence and without any complications (Figure. 4).

**Figure (4):** showing a straightforward wound healing process without any dehiscence on day 10 after sutures were removed.

To maintain the teat patency and drain the milk from the injured quarter, it was very helpful to install a sterile prosthetic tube made of modified polyvinyl chloride number 10 in place. The surgical site was found to be effectively protected by the application of an adhesive bandage (Dynafix). A 2 ml syringe that was attached to the tube helped complete the circuit.

## CONCLUSION

The quality of milk and milkability reflected the effectiveness of the technique in regaining the functional capacity of the teat postoperatively. The surgical technique employed for the management of teat wounds with fistula was found to be less effective due to the encountered complications which could be due to the management practices, postoperative care, and the suture material.

## ACKNOWLEDGEMENT

The Dean of the Faculty of Veterinary Medicine and the Head of the Department of Clinical Veterinary Medical Sciences are to be thanked for the resources made available and the assistance given during the study.

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# المعالجة الجراحية لنواسير الحلمة الرضحية في الماعز الهجين بخيوط البوليستر

**خالد. مسعود عبدالخالق حسين [*1] حمدي. فرج ناجي [1]، أسماء صالح ونيس[2]، مروان صالح [3]**

*[1] قسم العلوم الطبية البيطرية الإكلينيكية، كلية الطب البيطري، جامعة عمر المختار، البيضاء، ليبيا*

*[2] قسم النبات، كلية العلوم، جامعة دارنا ، درنة ، ليبيا*

*[3] مركز البحوث الزراعية، البيضاء، ليبيا للبحوث الزراعية. المركز، البيضاء، ليبيا*

تاريخ الاستلام:13 يوليو 2022 / تاريخ القبول: 06 سبتمبر 2022

Doi:https://doi.org/10.54172/mjsc.v37i3.943

**المستخلص:** تم اختيار ما مجموعه 6 ماعز تم تقديمها إلى المستشفى البيطري الجامعي (UVH) بجامعة عمر المختار، مصابة بناسور الحلمة الرضحية في هذه الدراسة. بعد التقييم قبل الجراحة، تم خياطة الطبقات المخاطية والعضلية بشكل منفصل عن طريق نمط خياطة بسيط مستمر باستخدام بولي جلاكتين 910. تمت مواجهة حواف الجلد باستخدام خيوط بوليستر مضفرة ومغلفة بالبوليبوتيلات. تم إجراء تقييم ما بعد الجراحة للحلمة التي أعيد بناؤها من خلال التقييم المورفولوجي والتقييم فوق الصوتي. عكست جودة الحليب وقابلية الحليب فعالية التقنية في استعادة القدرة الوظيفية للحلمة بعد الجراحة. وجد أن التقنية الجراحية المستخدمة لإدارة جروح الحلمة مع الناسور تكون أقل فعالية بسبب المضاعفات المصادفة والتي يمكن أن تكون بسبب ممارسات الإدارة والرعاية بعد الجراحة ومواد الخياطة.

**الكلمات المفتاحية:** ماعز ، ناسور حلمة رضحي ، بولي جلاكتين 910 ، خيوط بوليستر مضفرة مغلفة بالبوليبوتيلات.

[*1] خالد مسعود عبد الخالق Khaled.elhmri@omu.edu.ly قسم العلوم الطبية البيطرية الإكلينيكية ، كلية الطب البيطري ، جامعة عمر المختار ، البيضاء ، ليبيا

مجلة المختار للعلوم 37 (3): 284-290، 2022
ISSN: online 2617-2186 print 2617-2178
Journal Homepage https://omu.edu.ly/journals/index.php/mjsc/index

# تأثير فتحات التشكيل وحرارة ماء الترطيب في بعض صفات النوعية الفيزيائية للحبيبات العلفية لتغذية الدواجن

**باسم عبود عباس**

*قسم الانتاج الحيواني، كلية الزراعة، جامعة ديالى، العراق.*

تاريخ الاستلام: 07 ديسمبر 2021/ تاريخ القبول: 01 سبتمبر 2022
Doi:https://doi.org/10.54172/mjsc.v37i3.531

**المستخلص** : يهدف البحث إلى معرفة تأثير ظروف تصنيع الأعلاف في بعض صفات النوعية الفيزيائية للحبيبات العلفية لتغذية الدواجن. نفذت تجربة عاملية بعاملين تم فيها دراسة تأثير قطر فتحات التشكيل وبمستويين 2.5 و 4.5 ملم، و حرارة ماء ترطيب العليقة، وبمستويين 25 و 40 مْ. وقياس تأثيرهما في الحبيبات العلفية غير المتكسرة، والحبيبات المتكسرة، وتمدد الحبيبات، ونسبة الغبار. أظهرت النتائج أن زيادة قطر فتحات التشكيل من 2.5 إلى 4.5 ملم أدت إلى انخفاض معنوي في نسبة الحبيبات غير المتكسرة، وزيادة نسبة الحبيبات المتكسرة، ونسبة تمدد الحبيبات، ونسبة الغبار. ومع زيادة حرارة ماء العليقة من 25 إلى 40 مْ زادت معنوياً نسبة الحبيبات غير المتكسرة، وانخفضت معنوياً نسبة الحبيبات المتكسرة، ونسبة تمدد الحبيبات، فيما لم يظهر وجود تأثير معنوي في نسبة الغبار. وكانت أعلى مقاومة لتكسر الحبيبات 96.66% وأقل تكسر بالحبيبات 3.38% وأقل نسبة غبار 0.48% مع الفتحات 2.5 ملم والحرارة 40 مْ، أما أقل نسبة تمدد كانت 6.13% مع الفتحات 2.5 ملم والحرارة 25 مْ. استنتج أن زيادة قطر فتحات التشكيل أدت إلى زيادة نسبة الحبيبات المتكسرة، ونسبة تمدد الحبيبات ونسبة الغبار. زيادة حرارة ماء العليقة أدت إلى زيادة نسبة الحبيبات المقاومة للتكسر، فيما لم يظهر وجود تأثير معنوي في نسبة الغبار. نوصي بزيادة قطر فتحات التشكيل حسب الحاجة العمرية للحيوانات، واستعمال البخار للترطيب بدل الماء الحار.

**الكلمات المفتاحية** : الحبيبات المتكسرة؛ تشكيل الحبيبات؛ تصنيع الأعلاف؛ حرارة الحبيبات؛ رطوبة الحبيبات.

## المقدمة

توفر التكنولوجيا الحديثة طرقاً جديدة لتغذية الطيور الداجنة تتناسب بشكل وثيق مع الاحتياجات الغذائية لها، بهدف تقليل تكلفة العلف، والتي تشكل حوالي 65–70 % لتكلفة الإنتاج (Aljebory & Naji, 2021). تقدم الأعلاف للدواجن بأشكال عدة منها العلف المجروش Mash والعلف المفتت Crumble والحبيبات العلفية Pellet ويعد استخدام الحبيبات العلفية في تغذية الطيور الداجنة من الوسائل المهمة للحد من الفقد الحاصل في الأعلاف المقدمة للطيور التي أثبتت فائدتها (Behnke & Beyer, 2002; Lowe, 2005)، إن التحسن في وسائل قياس نوعية الحبيبات العلفية يمكن أن يساهم بشكل كبير في نمو هذه الصناعة لما له من نتائج إيجابية نتيجة التداول التجاري لهذه الحبيبات خلال إنتاجها ونقلها وأثناء تقديمها للحيوانات حيث أن قياس النوعية الفيزيائية لحبيبات الأعلاف يمكنها المساعدة بشكل كبير في إنتاج حبيبات قوية بعد تنظيم عمليات الإنتاج حيث يوجد العديد من أجهزة الاختبار لقياس النوعية الفيزيائية للحبيبات العلفية المتداولة تجاريا (Salas-Bringas et al., 2007). بين

*[1] باسم عبود عباس: basimabbas@uodiyala.edu.iq قسم الانتاج الحيواني، كلية الزراعة، جامعة ديالى، العراق. .

(Hemmingsen et al., 2008; Wood et al., 2019) أن مقاومة الحبيبات للتكسر تعرف بأنها القدرة على تحمل التداول والنقل دون توليد كميات زائدة من أجزاء الحبيبات الناعمة المتكسرة. أوضح (Maier & Briggs, 2000) أن الدقائق الناعمة المتكسرة بعد تصنيع الحبيبات العلفية تشكل علاقة عكسية سلبية مع نوعية الحبيبات المصنعة، وزيادتها يعني حبيبات علفية رديئة النوعية حيث إن معدل الدقائق المتكسرة تمثل مؤشرا يرتبط مع مقاومة الحبيبات للتفتت. ذكر Hancock (2000) إذا كانت عملية تصنيع الحبيبات غير صحيحة فإنها تنتج دقائق متكسرة بكميات كبيرة، وبذلك تقل فوائد الحبيبات الغذائية بشكل كبير. عرف (Dozier, 2001) مقاومة الحبيبات العلفية للتفتت بأنها التماسك الفيزيائي للعلائق المصنعة بشكل حبيبات علفية مع أقل دقائق ناعمة، أو أجزاء متكسرة من تلك الحبيبات أثناء المعاملة، أو النقل، والتي يتم تقديرها اعتمادا على النسبة المئوية للحبيبات الكاملة، أو الدقائق الناعمة المتولدة منها. بينت FAO (1980) أن تنظيم الحرارة والرطوبة قبل تصنيع الحبيبات العلفية يحسن مقاومة الحبيبات للتكسر، ويقلل كمية المواد الناعمة المتولدة من المنتوج النهائي. أوضح New (New, 1987) أن قطر فتحات قرص التشكيل لآلة إنتاج الحبيبات تكون متنوعة، وبمدى يعتمد على قطر الحبيبات المطلوب إنتاجها. أكد (Tanveer et al., 2018) أن من الضروري قياس الخواص الفيزيائية للحبيبات العلفية. إذ ستكون المعرفة كونها مفيدة جداً لمعرفة طريقة الحفاظ على هذه الحبيبات من التكسر بعد عملية التصنيع، وأثناء النقل، والتعبئة، والتخزين، والتغذية. يهدف البحث إلى دراسة تأثير فتحات التشكيل، وحرارة ماء العليقة في بعض صفات النوعية الفيزيائية للحبيبات العلفية لتغذية الدجاج.

## المواد وطرق البحث

استعمل لإجراء التجربة عليقة خاصة بتغذية الدجاج مكونة من عدد من المواد العلفية وفق النسب المحددة ذرة صفراء (16 %)، حنطة (53 %)، كبسة فول الصويا (23 %)، بروتين حيواني(6 %)، زيت نباتي (1%)، مسحوق حجر كلس (0.5 %)، ملح طعام (0.25 %) و فيتامينات ومعادن ( 0.25 %).

استعملت آلة بريمية النوع لغرض تصنيع الحبيبات العلفية، مواصفاتها صينية المنشأ نوع (Gosonic) موديل -682 (Gmg) أبعادها (طول× عرض× ارتفاع) 257*251*308 ملم. تم التحكم في ظروف التصنيع من حيث :

1- قطر فتحات التشكيل وبمستويين 2.5 و 4.5 ملم
2- حرارة ماء ترطيب العليقة وبمستويين 25 و 40 مْ

نظمت معاملات الاختبار وفق التصميم العشوائي الكامل (CRD) وبثلاث مكررات ليكون عدد الوحدات التجريبية 12 وحدة تجريبية. واختبرت الفروق بين المعاملات باستعمال اختبار أقل فرق معنوي (LSD) عند مستوى احتمالية (0.05). واستعمل البرنامج (Spss) الإصدار العاشر وفقا لـ (بشير، 2003) لإجراء التحليل الإحصائي، تم احتساب رطوبة العليقة وكانت 35.8 % باستعمال الفرن الكهربائي وعلى أساس الوزن الرطب وفق ما ذكره (Pfost, 1976) أما درجة نعومة الجرش للعليقة فقد حددت باستعمال غربال بقطر 1.5 ملم.

تم حساب صفات الحبيبات العلفية كالآتي:

**الحبيبات غير المتكسرة (%) Un broken pellet**: حسبت بعد وزن عينة من الحبيبات ووضعها في جهاز اختبار ليتم بعدها غربلتها وفق ما جاء في (Cubes, 2007) ومن ثم عزل ما يتبقى من حبيبات أعلى الغربال لتوزن مرة أخرى الحبيبات المتبقية فوق الغربال فقط، وتحسب على أساسها نسبة الحبيبات غير المتكسرة وفقا إلى ( Salas-Bringas et al., 2007) بتطبيق المعادلة الآتية:

الحبيبات غير المتكسرة (%) = وزن المتبقي فوق الغربال (غم) / وزن العينة الأولي (غم) * 100

**الحبيبات المتكسرة (%) broken pellets**: وهي ما يتكسر ويتفتت إلى قطع صغيرة وأجزاء من الحبيبات تمر عبر الغربال إلى أسفل تجمع في وعاء بعد عملية اختبارها بجهاز الاختبار، وإجراء عملية الغربلة لها، و التي تم احتسابها وفقا لـ (Salas-Bringas et al., 2007) بتطبيق المعادلة الآتية:

الحبيبات المتكسرة (%) = وزن المتبقي أسفل الغربال (غم) / وزن العينة الأولي (غم) * 100

**تمدد الحبيبات العلفية (%)**: وهي تمثل التغير الحاصل في قطر الحبيبات العلفية المنتجة بالنسبة لقطر ثقوب تشكيلها في ماكنة الإنتاج وقد تم احتسابها باستعمال جهاز Micrometer لقياس أقطار المواد وحسب الطريقة المتبعة من (Misra et al., 2002) باستخدام المعادلة الآتية:

تمدد الحبيبات (%) = [(قطر الحبيبة العلفية (ملم) )$^2$ / قطر ثقب التشكيل (ملم))$^2$ – 1] * 100

**نسبة الغبار (%)**: وهي نسبة ما يتبقى من مادة بعد إجراء عملية غربلة للمتفتت من الحبيبات بعد عملية الإسقاط بجهاز الاختبار وإجراء عملية الغربلة حيث يستعمل غربال بقطر مناسب وما ينزل أسفل الغربال يحسب غبارا، وحسب طريقة (Salas-Bringas et al., 2007) وفق المعادلة الآتية:

نسبة الغبار (%) = وزن الغبار (غم)/ وزن العينة الأولي (غم) * 100

## النتائج والمناقشة

**الحبيبات غير المتكسرة (%)** : يبين الجدول (1) تأثير فتحات التشكيل وحرارة ماء الترطيب والتداخل بينهما في الحبيبات العلفية غير المتكسرة. حيث إن زيادة قطر فتحات التشكيل من 2.5 إلى 4.5 ملم رافقها انخفاض معنوي في الحبيبات غير المتكسرة من 95.60 % إلى 72.41 %، ويعزى السبب إلى تعرض العليقة للضغط لمدة أقل من قبل بريمة الآلة داخل غلافها مع زيادة قطر الفتحات الذي يؤدي إلى تسريع خروجها ما ينتج عنه قلة إحكام تماسك مكوناتها ليؤدي بالنتيجة إلى انخفاض نسبة الحبيبات غير المتكسرة المصنعة منها، وهذا يتفق مع ما وجده (Khater et al., 2014). كما أدت زيادة حرارة ماء الترطيب من 25 إلى 40 مْ إلى زيادة معنوية في الحبيبات غير المتكسرة من 81.94 إلى 86.07 % ويعود السبب إلى أن ارتفاع الحرارة تسبب تحرر بعض الزيوت من المواد، وإحداث جلتنة جزئية لبعض حبيبات النشأ في مكونات العليقة مما يساعد على تماسك المكونات مع بعضها وإعطائها الصلابة، وهذا يتفق مع ما وجده (Čolović et al., 2010). وقد أعطى التداخل بين فتحات التشكيل، و حرارة ماء الترطيب تأثيرا معنويا مسجلا أعلى حبيبات غير متكسرة 96.66 % مع الفتحات 2.5 ملم، و الحرارة 40 مْ، أما أقل نسبة حبيبات غير متكسرة فكانت 69.34 % مع الفتحات 4.5 ملم، وحرارة الترطيب 25 مْ.

**جدول:(1)**. تأثير فتحات التشكيل، وحرارة ماء الترطيب في الحبيبات غير المتكسرة (%)

| فتحات التشكيل(ملم) | حرارة ماء الترطيب (مْ) 25 | 40 | متوسط تأثير الفتحات |
|---|---|---|---|
| 2.5 | **a** 94.54 | **a** 96.66 | a 95.60 |
| 4.5 | **c** 69.34 | b 75.48 | b 72.41 |
| متوسط تأثير الحرارة | b 81.94 | a 86.07 | |
| قيم LSD على مستوى **5%** | | | |
| الفتحات: 1.54 | الحرارة: 1.54 | التداخل: 2.18 | |

**الحبيبات المتكسرة (%)**: يوضح الجدول (2) تأثير فتحات التشكيل، وحرارة ماء الترطيب، والتداخل بينهما في الحبيبات العلفية المتكسرة. فمع زيادة قطر فتحات التشكيل من 2.5 إلى 4.5 ملم زادت نسبة تكسر الحبيبات بشكل كبير جدا من 4.42 إلى 27.58 % ويعزى ذلك إلى قلة إحكام تماسك مكونات الحبيبات مع زيادة قطر فتحات التشكيل ليؤدي بالنتيجة إلى زيادة نسبة الحبيبات المتكسرة وهذا يتفق مع ما ذكره (Behnke, 2001). ومع زيادة حرارة ماء العليقة من 25 إلى 40 مْ حصل انخفاض معنوي في الحبيبات المتكسرة من 18.05 إلى 13.95%. ويعود السبب إلى زيادة خاصية

الالتصاق والتماسك بين مكونات العليقة نتيجة إحداث جلتنة جزئية لحبيبات النشا وبالتالي تقل نسبة المتكسر منها وهذه النتيجة تتفق مع (Winowiski, 1995). أعطى التداخل بين فتحات التشكيل 2.5 ملم، وحرارة ماء الترطيب 40 مْ أقل حبيبات متكسرة وبنسبة 3.38 %، أما أعلى نسبة حبيبات متكسرة فكانت 30.66 % مع فتحات التشكيل 2.5 ملم، وحرارة الماء 25 مْ.

**جدول:(2)**. تأثير فتحات التشكيل، وحرارة ماء الترطيب في الحبيبات المتكسرة (%)

| فتحات التشكيل(ملم) | حرارة ماء الترطيب (مْ) | | متوسط تأثير الفتحات |
|---|---|---|---|
| | 25 | 40 | |
| 2.5 | c 5.45 | d 3.38 | b 4.42 |
| 4.5 | a 30.66 | b 24.51 | a 27.58 |
| متوسط تأثير الحرارة | a 18.05 | b 13.95 | |
| قيم LSD على مستوى 5% | | | |
| الفتحات: 1.54 | الحرارة: 1.54 | التداخل: 2.18 | |

**تمدد الحبيبات العلفية (%):** يبين الجدول (3) تأثير فتحات التشكيل، و حرارة ماء الترطيب، والتداخل بينهما في تمدد الحبيبات العلفية. حيث أدت زيادة قطر فتحات التشكيل من 2.5 إلى 4.5 ملم إلى زيادة نسبة تمدد الحبيبات، وتحولها من حالة الانتفاخ إلى الانكماش بنسبة من 10.01 إلى -12.58 %. ويعزى السبب إلى تعرض العليقة للضغط لمدة أقل من قبل بريمة الآلة داخل غلافها مع زيادة قطر الفتحات الذي يؤدي إلى تسريع خروجها ما ينتج عنه قلة إحكام تماسك مكوناتها ليؤدي بالنتيجة إلى زيادة مرونة الحبيبات أثناء التشكيل ما يجعلها تتمدد أكثر عند خروجها من ماكنة التصنيع، وهذه النتيجة تتفق مع ما أوضحه (محمد علي ودميان ،.1988). كما يتضح أن زيادة حرارة ماء الترطيب من 25 إلى 40 مْ أدت إلى انخفاض معنوي في تمدد الحبيات العلفية، وتحولها من حالة الانكماش إلى الانتفاخ أي من 4.36- إلى 1.80 % ويعود السبب إلى تماسك أكبر لمكونات العليقة وزيادة الترابط بين جزيئاتها نتيجة تأثرها بزيادة درجة الحرارة مما يجعلها أقل تمدداً. هذا وقد أثر التداخل معنوياً إذ أعطى أقل نسبة تمدد (انتفاخ) وبمقدار 6.13 % مع قطر الفتحات 2.5 ملم، و حرارة الماء 25 مْ، أما أعلى نسبة تمدد (انكماش) فكانت 14.86- % مع فتحات التشكيل 4.5، و حرارة الماء 25 مْ.

**جدول:(3)**. تأثير فتحات التشكيل وحرارة ماء الترطيب في تمدد الحبيبات العلفية (%)

| فتحات التشكيل (ملم) | حرارة ماء الترطيب (مْ) | | متوسط تأثير الفتحات |
|---|---|---|---|
| | 25 | 40 | |
| 2.5 | c 6.13 | a 13.90 | b 10.01 |
| 4.5 | a -14.86 | b -10.30 | a -12.58 |
| متوسط تأثير الحرارة | a - 4.36 | b 1.80 | |
| قيم LSD على مستوى 5% | | | |
| الفتحات: 0.92 | الحرارة: 0.92 | التداخل: 1.30 | |

**نسبة الغبار (%):** تشير بيانات الجدول (4) إلى تأثير فتحات التشكيل وحرارة ماء الترطيب، والتداخل بينهما في نسبة الغبار. فمع زيادة قطر فتحات التشكيل من 2.5 إلى 4.5 ملم زادت نسبة الغبار للحبيبات المصنعة من 0.54 إلى 1.67 %، ويعزى السبب إلى زيادة تمدد جسم الحبيبات، وارتفاع نسبة المتكسر منها لترتفع نسبة الغبار بناء على ذلك. ومن الجدول 4 يتضح أن زيادة حرارة ماء الترطيب من 25 إلى 40 لم تؤثر معنويا في نسبة الغبار. كذلك لم يعطِ التداخل بين فتحات التشكيل وحرارة ماء الترطيب تأثيراً معنويا في نسبة الغبار.

**جدول:(4)**. تأثير فتحات التشكيل، وحرارة ماء الترطيب في نسبة الغبار (%)

| فتحات التشكيل (ملم) | حرارة ماء الترطيب (مْ) | | متوسط تأثير الفتحات |
|---|---|---|---|
| | 25 | 40 | |
| 2.5 | a 0.61 | a 0.48 | b 0.54 |
| 4.5 | a 1.73 | a 1.60 | a 1.67 |
| متوسط تأثير الحرارة | a 1.17 | a 1.04 | |
| قيم LSD على مستوى 5% | | | |
| الفتحات: 0.94 | الحرارة: n.s | التداخل: n.s | |

## استنتاج

1- أظهرت النتائج أن زيادة قطر فتحات التشكيل أدت إلى انخفاض نسبة الحبيبات غير المتكسرة، وزيادة نسبة الحبيبات المتكسرة، ونسبة تمدد الحبيبات، ونسبة الغبار.

2- أدت زيادة حرارة ماء العليقة إلى زيادة نسبة الحبيبات غير المتكسرة، وانخفاض نسبة الحبيبات المتكسرة، ونسبة تمدد الحبيبات، فيما لم يظهر وجود تأثير معنوي في نسبة الغبار.

## الشكر والتقدير

تم تمويل البحث من النفقة الخاصة، ولا يوجد أي تضارب في المصالح جراء إنجاز المخطوطة المقدمة. مع الشكر الجزيل لكل من ساهم بتقديم يد العون لي لإنجاز البحث.

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# Effect of Forming Holes and Moistening Water Temperature on Some physical Quality Characteristics of Poultry Feed Pellets

**Basim Aboud Aabbas**
*Department of Animal Production, College of Agriculture, University of Diyala, Iraq.*

Received:07 December 2021./ Accepted: 01 September 2022
Doi: https://doi.org/10.54172/mjsc.v37i3.531

**Abstract:** The research aims to investigate the effect of feed manufacturing conditions on some significant physical traits of feed pellets for poultry feeding. The study was conducted using a factorial experiment of two factors that included the effect of the studied forming holes' diameters at 2.5 and 4.5 mm, and the feed pellet moistening water temperature at 25 and 40 C°. Hence to find out their effect on the broken pellets, unbroken pellets, pellet expansion, and dust ratio. The results showed that increasing the diameter of the forming holes from 2.5 to 4.5 mm significantly affected the decrease in unbroken pellets, the increase in the percentage of broken pellets, pellet expansion ratio, and dust ratio. Increasing the temperature of the water from 25 to 40 C°, led the percentage of unbroken pellets to increase significantly and the percentage of broken pellets, and pellet expansion ratio decreased significantly. Whereas no significant effect on dust was recorded. The highest resistance to pellet breakage was 96.66%, the least percentage of unbroken pellets was of 3.38%, and the lowest dust content was 0.48%, with 2.5 mm holes and 40 C°. The lowest expansion ratio was 6.13% with 2.5 mm holes and 25 C°. It was concluded that the increase in the diameter of the forming holes led to an increase in the percentage of broken pellets, the percentage of expansion ratio, and the percentage of dust. Increasing temperature of the water led to an increase in the percentage of unbroken pellets, while there was no significant effect on the percentage of dust. We recommend increasing the diameter of the forming holes according to the age of the animals and using steam for moistening rather than hot water.

**Keywords:** Broken pellet; Pellet formation; Feed manufacturing; Pellet temperature; Pellet moisture.

***Corresponding author:** Basim Aboud Aabbas: *basimabbas@uodiyala.edu.iq* Department of Animal Production, College of Agriculture, University of Diyala, Iraq

مجلة المختار للعلوم 37 (3): 291-296، 2022
ISSN: online 2617-2186 print 2617-2178
Journal Homepage https://omu.edu.ly/journals/index.php/mjsc/index

# تأثير كلوريد الكالسيوم على الصفات الكيميائية لثمار التفاح المبردة من صنف Golden Delicious

**انتصار محمد الشريف بن ريمة**

*قسم النبات، كلية الآداب والعلوم، المرج، جامعة بنغازي – ليبيا*

تاريخ الاستلام: 09 مارس 2022 / تاريخ القبول: 01 أغسطس 2022
Doi:https://doi.org/10.54172/mjsc.v37i3.598

**المستخلص** : تهدف هذه الدراسة للتعرف على إمكانية استخدام محاليل كلوريد الكالسيوم للحفاظ على أفضل جودة لثمار صنف التفاح جولدن دليشيس المخزنة تخزيناً مبرداً والنامية تحت ظروف منطقة المرج بليبيا، وقد أجريت خلال موسمي الزارعة 2017 و2018 واشتملت المعاملات الرش بتركيزين من كلوريد الكالسيوم وهما: (0 % ، 0.06 %) حيث كانت الرشة الأولى بعد شهر من أوج التزهير، وكرر الرش ثلاث مرات بواقع رشة كل 30 يوم، وبعد الجني تم غمر الثمار قبل التخزين في محاليل كلوريد الكالسيوم بتركيزات (0 %، 2 %، 4 %، 6 %) وخزنت لمدة ( 120 يوماً) عند درجة حرارة صفر مئوي، وأظهرت النتائج المتحصل عليها أن استعمال كلوريد الكالسيوم رشاً على الثمار حافظ بشكل أفضل على جودتها مقارنة بمثيلتها غير المرشوشة، كما أن الغمر بمحاليل كلوريد الكالسيوم حافظ أيضاً على جودة الثمار بزيادة تركيزه وذلك في حدود التركيزات الداخلة في هذه الدراسة مما يجعلنا نستنتج الدور الهام لكلوريد الكالسيوم في المحافظة على جودة ثمار هذا الصنف.

**الكلمات المفتاحية** : كلوريد الكالسيوم؛ التفاح؛ التخزين؛ المرج؛ Golden Delicious.

## المقدمة

يعود الموطن الأصلي للأصل البري للتفاح (*Malus sylvestris* ) إلى آسيا الوسطى، وينتسب التفاح( *Malus domestica* Borkh) إلى العائلة الوردية (Rosaceae) (حداد وآخرون،2017)، وهي من الثمار ذات الإقبال الكبير على استهلاكها بصور مختلفة نظراً لاعتدال أسعارها وقيمتها الغذائية العالية بمكوناتها الأساسية في تغذية وصحة الإنسان (بلقيس وآخرون،2007) ويعد الصنف (جولدن دليشيس) صنف أمريكي، ثماره صفراء اللون، يحتاج إلى فترة متوسطة من البرودة شتاء، ثماره متوسطة إلى كبيرة الحجم متطاولة، الطعم حلو والنكهة محببة، صنف مرغوب لقابلية زراعته في الشتاء الدافئ، يحمل بغزارة، يتحمل النقل والتخزين، صنف تجاري ومنتشر في أكثر البلدان (باشا، 2015) ونظراً للأهمية الكبيرة لثمار التفاح فقد أجريت الكثير من الدراسات للوصول لأفضل المعاملات للمحافظة على جودة الثمار سواء قبل أو بعد القطاف أو حتى في أثناء التخزين، ولإطالة مدة تخزينها وتوافرها بالسوق على مدار العام، وهذا ما يتحقق من خلال تقليل انخفاض الصفات النوعية والإصابة بالأمراض التخزينية المختلفة الناتجة عن تراجع في هذه الصفات مما يؤدي لانخفاض قيمتها التسويقية. وقد أظهرت عدة دراسات أن استخدام مركبات الكالسيوم سواء رشاً على الأشجار أو بتغطيس الثمار قبل التخزين لها تأثير إيجابي في الحفاظ على نوعية الثمار (Conway et al., 1994).

ويعد الرش الورقي بالكالسيوم أكثر فاعلية للثمار من حيث حصولها على مستويات مناسبة من عنصر الكالسيوم

*انتصار محمد الشريف بن ريمة entesar.abdalah@uob.edu.ly قسم النبات، كلية الآداب والعلوم، المرج، جامعة بنغازي – ليبيا..

(Conway et al., 2001; Fallahi et al., 2005) حيث إن الثمار المرشوشة به تحتوي على 10 أضعاف على الأقل من تركيز الكالسيوم مقارنة بغير المرشوشة(Drake,2008) .

الغمر بكلوريد الكالسيوم يحافظ على جودة الثمار ( Al Shoffe et al., 2021) فقد وجد ( Barreiro et al., 2003) أنه بزيادة تركيز الكالسيوم في المحاليل التي غمرت فيها ثمار التفاح والمحددة بالتركيزين 2% و4% ، أدى لزيادة الكالسيوم في قشرة لب الثمار، وانخفاض في نسبة المواد الصلبة الذائبة؛ وذلك بعد التخزين لمدة 6 أشهر من التخزين عند درجة 1 $^{o}$م وهذا ما أكده حداد وحداد ،2012 عند الرش بنترات الكالسيوم والغمر في كلوريد الكالسيوم وتأثيرهما على زيادة محتوى الثمار من الكالسيوم ، وما أكده أيض ( Watkins et al., 2005) بأن غمر الثمار بكلوريد الكالسيوم قبل التخزين له تأثيره العكسي على خفض نسبة المواد الصلبة الذائبة الكلية في الثمار، أما (خليفة،2018) فلم يجد أي تأثير معنوي على نسبة المواد الصلبة الذائبة بزيادة تركيز الغمر بكلوريد الكالسيوم بينما أدى ذلك للمحافظة على الأحماض الكلية بزيادة التركيز، لذا يهدف هذا البحث إلى دراسة تأثير المعاملة بكلوريد الكالسيوم خلال نمو الثمار وبعد القطاف على جودة الثمار بعد التخزين المبرد.

## المواد وطرق البحث

تم تنفيذ هذه الدراسة في عامي2017 و 2018 على صنف التفاح Golden Delicious للحصول على أفضل جودة للثمار وإطالة عمرها التخزيني، حيث تم رش الأشجار في الحقل بالماء ومحلول كلوريد الكالسيوم بتركيزي (0%، 0.06%) بعد 30 يوماً من أوج الإزهار ثم كرر الرش كل 30 يوماً بمجموع أربع رشات، وبعد القطف تم اختيار الثمار الخالية من الكدمات والعيوب الفسيولوجية والمتجانسة في الحجم وتم غمرها لمدة 20 دقيقة في محاليل من كلوريد الكالسيوم بتركيزات (0، 2، 4 و6%) وتم تخزينها عند درجة حرارة 0 $^{o}$م ورطوبة نسبية 90 ± 2% لمدة (120 يوماً)، اشتملت الدراسة على 8 معاملات ( معاملتي رش بكلوريد الكالسيوم وأربع معاملات غمر في كلوريد الكالسيوم) كل معاملة ثلاث مكررات وكل مكررة احتوت 5 ثمار بمجموع 15 ثمرة لكل معاملة ، وتم إجراء التحاليل الآتية:

**المواد الصلبة الذائبة :** وتُقاس باستخدام جهاز الرفراكتوميتر المعملي(Schwallier, 2012) .

**نسبة الأحماض الكلية:** تم تقديرها في العصير كنسبة مئوية على أساس حمض الماليك طبقاً لما ذكره ( Graham et al., 2004)، وذلك بمعايرتها بمحلول هيدروكسيد الصوديوم (0.1عياري) ودليل فينول فيثالين، وتستخدم العلاقة التالية لحساب نسبة الحموضة:

$$\% \text{ الكلية للحموضة} = \frac{\text{الوزن المكافئ لحمض} \times \text{العيارية} \times \text{كمية القلوي}}{1000 \times \text{كمية العصير المستخدم}} \quad X100$$

تقدير محتوى الثمار من فيتامين "ج": تم تقديره حسب ما ذكر في (A.O.A.C.,1990).

**تقدير محتوى الثمار من عنصر الكالسيوم (Ca):** جرت القراءة على 15 ثمرة لكل معاملة مقسمة إلى 3 مكررات، أُجري التحليل بعد تجفيف العينات وطحنها وتحضيرها للقياس على أساس الوزن الجاف للعينة.

استخدم تصميم القطاعات العشوائية التامة لتحليل البيانات وعزلت المتوسطات باختبار أقل فرق معنوي LSD عند معنوية 0.05 (Little & Hills, 1978).

## النتائج والمناقشة

يتضح من خلال الجدول (1) أن رش الأشجار بكلوريد الكالسيوم له تأثير إيجابي ومعنوي على جودة الثمار المخزنة تخزيناً مبرداً حيث حافظ على (TSS) ونسبة الحموضة ومحتوى الثمار من فيتامين "ج" ومن الكالسيوم مقارنة بالثمار المرشوشة بالماء فقط، فقد كانت (TSS) بالثمار المرشوشة بكلوريد الكالسيوم متفوقة معنوياً لعامي الدراسة بقيم 15.98 و16.15 مقارنة بالثمار غير المرشوشة بقيم 14.81 و 15.43 ويتعارض هذا مع ما وجده (Watkins et al., 2005)،

وكذلك نسبة الحموضة حيث تفوقت معاملة الرش بكلوريد الكالسيوم على معاملة الرش بالماء معنوياً فقد كانت نسبة الحموضة للثمار المرشوشة بكلوريد الكالسيوم 0.275% و0.301% بينما في الثمار المرشوشة بالماء 0.261% و0.287% لعامي الدراسة وتتفق هذه النتائج مع ما وجده (خليفة، 2018)، كما أن الثمار المرشوشة بالكالسيوم تفوقت معنوياً بقيم 4.51 و4.62 ملجم/100جرام في احتفاظها بمحتواها من فيتامين "ج" مقارنة بمثيلتها المرشوشة بالماء فقط بقيم 3.37 و4.14 ملجم/100جرام وذلك لكلا عامي الدراسة، وقد حافظت الثمار على محتواها من الكالسيوم بشكل أفضل عند رشها بكلوريد الكالسيوم فكان تركيز الكالسيوم بها 9.18% ، 8.74% مقارنة بالثمار المرشوشة بالماء فقط بتركيز 8.56% ، 8.20% لعامي الدراسة على التوالي، وهذه النتائج تتفق مع الدراسات التي أوضحت أن الرش بمركبات الكالسيوم يقلل من إنتاج غاز الإيثلين مما يقلل من التنفس (Lafer, 2003) وبالتالي يقلل من فقد المواد الغذائية وتحللها بفعل التنفس حيث يعزى دور الكالسيوم في منعه لتحلل أغشية السيتوبلازم والإندوبلازم والمايتوكندريا مما يعني قلة إنتاج غاز الأثيلين، وانخفاض معدل التنفس، وتأخير الشيخوخة، كذلك يعمل على خفض نفاذية الأغشية الخلوية، مما يؤدي إلى انخفاض نفاذية غاز الأثيلين (Conway et al., 1994).

يتضح من خلال الجدول (1) أن غمر الثمار بكلوريد الكالسيوم له تأثير إيجابي ومعنوي على جودة الثمار المخزنة تخزيناً مبرداً وذلك بزيادة تركيزه في محلول الغمر بشكل عام في حدود ما اشتملته هذه الدراسة، حيث إنه كلما زاد تركيز كلوريد الكالسيوم بمحاليل الغمر كلما قل فقد (TSS) في الثمار معنوياً وذلك في العام الثاني ، حيث كانت قيم (TSS) ( 13.93، 14.65، 15.60، 16.33) للتراكيز (0% ، 2%، 4% ، 6%) على التوالي وبفروق معنوية، بينما في العام الأول تفوق تركيز 6% معنوياً على جميع التراكيز بقيمة (15.77) إلا أن التركيزين 2% و4% لم يختلف معنوياً فيما بينهما وكانت قيمهما (15.14، 15.12) برغم تفوقهما على التركيز 0% بقيمة (14.43)، كما أن نسبة الحموضة الكلية بالثمار تأثرت بارتفاع تركيز كلوريد الكالسيوم في محاليل الغمر حيث بزيادته قل فقد الحموضة في كلا عامي الدراسة معنوياً، فكانت نسب الحموضة للتراكيز (0% ، 2%، 4% ، 6%) على التوالي للعام الأول كالآتي (0.232%، 0.254%، 0.282%، 0.331%) وللعام التالي كالآتي (0.261%، 0.284%، 0.298%، 0.311%) ويتفق هذا مع ما وجده (بن ريمة،2009) وكذلك (Han et al., 2021).

ويُمكن أن يُعزى تأثير كلوريد الكالسيوم على حُموضة الثِمار لتأثيره على تقليل التنفس وأكسدة الأحماض مما يقلل من فقدها خلال التنفس، كذلك قل فقد الثمار لمحتواها من فيتامين "ج" بزيادة تركيز كلوريد الكالسيوم في محاليل الغمر بشكل طردي وبتأثير معنوي، فقد كان محتوى الثمار من الفيتامين (3.41، 3.89، 4.38، 4.87 ملجم/100جرام) و(3.37، 3.86 ، 4.33، 4.79 ملجم /100 جرام) للعامين على التوالي للتراكيز (0%، 2%، 4%، 6%)، ويفسر ذلك بأن الكالسيوم يمنع تحلل أغشية السيتوبلازم، والإندوبلازم، والمايتوكندريا مما يقلل إنتاج غاز الأثيلين وبالتالي ينخفض معدل التنفس وتقل أكسدة المواد الغذائية (Conway et al., 1994).

كما أظهرت النتائج أنه بزيادة تركيز كلوريد الكالسيوم في محاليل الغمر زاد محتوى الثمار من الكالسيوم طردياً وذلك في كلا عامي الدراسة فقد كانت نسب كلوريد الكالسيوم للعامين على التوالي (7.44%، 8.25%، 8.83%، 9.40%) و(7.30%، 7.84%، 8.39%، 8.93%) للتراكيز (0%، 2%، 4%، 6%) على التوالي أيضاً وتتفق مع ما وجده (حداد وحداد،2012)، هذا ويرجع تفسير زيادة الكالسيوم بالثمار نتيجة المُعاملة بكلوريد الكالسيوم إلى أنَّ الكالسيوم يخترق الثمرة ويتحد مع البروتوبكتين في الصفيحة الوسطى ويُدعَّم السيليلوز الموجود في جدار الخلية فيزيد محتواه بالثمرة (الشيخ،2011).

ويتضح من خلال هذه الدراسة أن أفضل تركيز من كلوريد الكالسيوم حافظ على جودة الثمار بشكل أفضل هو الرش بتركيز 0.06% والغمر في تركيز 6% وهما أعلى تركيزين

في المعاملتين.

**الجدول (1).** تأثير فترات التخزين والمعاملة بكلوريد الكالسيوم على الصفات الكيميائية لثمار التفاح صنف Golden Delicious:

| المعاملات | | الصفات الكيميائية | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | TSS(%) | | للحموضة % | | فيتامين"ج" 100(ملجم/جرام) | | تركيز الكالسيوم% | |
| | | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| الرش بكلوريد الكالسيوم | 0% | 14.81b | 15.43b | 0.261b | 0.287b | 3.70b | 4.14b | 8.56b | 8.20b |
| | 0.06% | 15.98a | 16.15a | 0.275a | 0.301a | 4.51a | 4.62a | 9.18a | 8.74a |
| الغمر في كلوريد الكالسيوم | 0% | 14.43c | 13.93d | 0.232d | 0.261d | 3.41d | 3.37d | 7.44d | 7.30d |
| | 2% | 15.12b | 14.65c | 0.254c | 0.284c | 3.89c | 3.86c | 8.25c | 7.84c |
| | 4% | 15.14b | 15.60b | 0.282b | 0.298b | 4.38b | 4.33b | 8.83b | 8.39b |
| | 6% | 15.77a | 16.33a | 0.331a | 0.311a | 4.87a | 4.79a | 9.40a | 8.93a |
| قيم LSD عند P 0.05 | | 0.61 | 0.70 | 0.012 | 0.011 | 0.47 | 0.45 | 0.563 | 0.53 |

**الاستنتاجات والتوصيات**

نستنتج من خلال هذه الدراسة أن رش ثمار التفاح صنف جولدن دليشيص بكلوريد الكالسيوم حافظ على جودتها كما أن غمرها في محاليل كلوريد الكالسيوم حافظ على جودتها بزيادة تركيزه في هذه المحاليل، ونوصي باستخدام تراكيز أعلى من كلوريد الكالسيوم سواء عند الرش أو الغمر في تجارب أخرى وكذلك مناطق أخرى وذلك لأهمية هذه المعاملات في المحافظة وتحسين جودة الثمار مما ينعكس على المردود الاقتصادي لإنتاج التفاح في ليبيا.

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# Effect of Calcium Chloride on the Chemical Properties of the Refrigerated Fruits of the Apple Variety Golden Delicious

**Entesar M. A.ben Rema**

*Department of Botany, Faculty of Arts and Sciences, Al-Marj, University of Benghazi, Libya*

Received:09 March 2022./ Accepted: 01 August 2022
Doi: https://doi.org/10.54172/mjsc.v37i3.598

**Abstract:** This study aims to identify the possibility of using calcium chloride solutions to maintain the best quality of the fruits of the Golden Delicious apple variety stored in cold storage and grown under the conditions of the AL-Marj region in Libya. It was conducted during the 2017 and 2018 planting seasons, and the treatments included spraying with two concentrations of calcium chloride, namely (0% and 0.06%) where the first spray was a month after the peak of flowering, and the spraying was repeated three times, one spray every 30 days, after harvesting, the fruits were immersed before storage in calcium chloride solutions at concentrations of (0%, 2%, 4%, 6%) and stored for (120 days) at a temperature of zero Celsius. The obtained results showed that the use of calcium chloride sprayed on the fruits better preserved their quality compared to the non-sprayed ones. The immersion in calcium chloride solutions also preserved the quality of the fruits by increasing its concentration within the limits of the concentrations included in this study, which makes us conclude the important role of calcium chloride in maintaining the quality of the fruits of this variety.

**Keywords:** Calcium Chloride; Apple; Storage; Al-Marj; Golden Delicious.

***Corresponding author:** Entesar M. A.ben Rema: *entesar.abdalah@uob.edu.ly* , Department of Botany, Faculty of Arts and Sciences, Al-Marj, University of Benghazi, Libya

مجلة المختار للعلوم 37 (3): 297-308، 2022
ISSN: online 2617-2186 print 2617-2178
Journal Homepage https://omu.edu.ly/journals/index.php/mjsc/index

# تأثير المستخلصات المائية لأوراق نبات القرع وفيتامين B12 على بعض صفات النمو والصفات الفسلجية لأنواع من البقوليات

**بان عبد العزيز عيدان ووسن صالح حسين** *

*قسم علوم الحياة، كلية العلوم، جامعة الموصل، العراق*

تاريخ الاستلام: 13 أبريل 2022/ تاريخ القبول: 25 يوليو 2022
Doi:https://doi.org/10.54172/mjsc.v37i3.741

**المستخلص** : أجريت هذه الدراسة في مختبرات قسم علوم الحياة /كلية العلوم /جامعة الموصل لمعرفة تأثير المعاملة بالمستخلصات المائية لأوراق القرع، B12 والفيتامين، والخليط على بعض الصفات الفسلجية لثلاثة أنواع من البقوليات (الحمص، اللوبيا، والفاصوليا )، حيث تناول البحث قياس نسبة التشرب، فعالية انزيم α–اميليز، نسبة الإنبات، طول الرويشة والجذير وأوزانهما الجافة في البذور المعاملة بمستخلص أوراق القرع، فيتامين B12 والخليط، أعطت النتائج زيادة في نسبة التشرب في جميع المعاملات، وفعالية الانزيم في أغلب المعاملات، أما نسبة الإنبات ومعدل طول الرويشة والجذير وأوزانهما الجافة فقد تباينت ما بين الزيادة والنقصان وكانت أعلى نسبة تحفيز في بذور الفاصوليا المعاملة بالخليط عند التركيز 2% حيث بلغت 48.34% في حين كانت أعلى نسبة مئوية للانخفاض في إنبات البذور 21.05% 6% وبلغت في بذور الفاصوليا، كما أظهرت النتائج أن المعاملة بالفيتامين سببت زيادة في معدل طول الرويشة والجذير وأوزانهما الجافة لبادرات الأنواع النباتية الثلاث(حمص، لوبيا، فاصوليا) ، ولوحظ أيضا عند معاملة الأنواع المختبرة بالخليط بأن الفيتامين قلل من التأثير الضار لمستخلص القرع، وأعطى التأثير التحفيزي لجميع الصفات المختبرة.

**الكلمات المفتاحية** : مكون التنسيق. قلم المدقة؛ التصميم. إدراج (كلمات رئيسية)

## المقدمة

عرف الأليلوباثي قديماً ولأول مرة من قبل العالم ( Molisch, 1937) على أنه التداخلات البايوكيميائية التي تحدث بين جميع أنواع النباتات ويشمل أيضا الأحياء المجهرية ويضم هذا المصطلح كلا من التداخلات المحفزة(الإيجابية) والتداخلات المثبطة (السلبية). تتضمن هذه التداخلات إنتاج مركبات كيميائية بواسطة النباتات أو الأحياء المجهرية التي تؤثر على نمو وتطور، وانتشار النباتات، والكائنات المجهرية الأخرى الموجودة في المجتمعات الطبيعية أو الأنظمة الزراعية(Einhellig, 1995)، تسمى هذه المركبات Allelochemical وهي مواد غير مغذية يتم إنتاجها بشكل أساسي كنواتج ايض ثانوية أو نواتج تحلل الميكروبات (Rice, 1984)، وتتحرر هذه المركبات إلى البيئة بأربع طرق رئيسية هي: إفرازات الجذور (Root exudate)،الغسيل (Leaching) تغسل المركبات من الأوراق، السيقان أو الأفرع بماء المطر أو قطرات الندى، التطاير (Volatilization) تتحرر المركبات الكيميائية إلى البيئة بشكل غازات أو أبخرة (Kamal, 2020)، وكذلك عن طريق تحلل المتبقيات النباتية (Decomposition) والتي تتضمن تحلل الأنسجة الميتة للنباتات في التربة بفعل عوامل حية وغير حية ( Hussain, 2020).

* وسن صالح حسين : wassbio54@uomosul.edu.iq قسم علوم الحياة، كلية العلوم، جامعة الموصل، العراق.

تتركز الأهداف الرئيسية للبحوث الأليلوباثي بتطبيق التأثيرات الأليوباثية في المجال الزراعي لتحسين الإنتاج والحاصل (بمثابة سماد طبيعي) وتقليل استخدام المبيدات وما يترتب عليها من تلوث في البيئة (Jabran et al., 2015) إذ أن (Allelochemicals) لها تأثيرات متعددة على النباتات سواء كانت مباشرة أو غير مباشرة فنجد أن العديد من المركبات الكيميائية (Allelochemicals) تؤثر في الصفات الفسلجية للنبات كنفاذية الخلية ووظيفة الغشاء للبذور المتشربة بها وعند تراكيز معينة (Li et al., 2010)، يمكن دراسة تأثير المركبات الكيميائية المتحررة من المستخلصات المائية للعديد من النباتات الراقية ومن الأجزاء النباتية المختلفة على حيوية البذور في الظروف المختبرية، حيث ركزت التجارب المختبرية الأولية على دراسة تأثير هذه المركبات على إنبات البذور ونمو البادرات (Vyvyan, 2002)، إن الاختبار الحيوي للبذور لدراسة طريقة عمل المركبات الأليوباثية هو أحد الاعتبارات المهمة، فوجد بأن المركبات الأليوباثية تؤثر في انبات البذور ويمكن إن يعزى هذا التأثير الى ان تشرب البذور وفعالية انزيم α–اميليز ترتبط ارتباطاً قوياً بعملية الانبات ولهذا يمكن قياس نسبة تشرب البذور ونشاط انزيم α–اميليز لتقييم التغييرات التي تطرأ على البذور ودراسة كفاءة إنباتها عند معالجتها بمختلف المركبات الألليوباثية ( Baskin et al., 2006). تعد البقوليات من المحاصيل المهمة التي تزيد من خصوبة التربة وتقوم بعملية تثبيت النتروجين الجوي عن طريق علاقتها التكافلية مع بكتيريا الرايزوبيا ( Pervin et al., 2017) كما تعد مصدراً للبروتين وكذلك تحتوي على نسب عالية من الكربوهيدرات ما يقارب 58.2%، كما تحتوي على نسب من الكالسيوم والحديد والفسفور والبوتاسيوم بالإضافة إلى احتوائها على فيتامين B1 و B2 (علوان وحميد ،2013).

أما العائلة القرعية فمعروفة بتأثيراتها الأليوباثية والتي سجلت بعدة بحوث ودراسات، فقد سجلت إحدى الدراسات انخفاضاً كبيراً في نسبة الإنبات وطول النبات بالإضافة إلى الوزن الرطب لدغلي السعد والرزين النامية في ترب حاوية على مخلفات نبات القرع والخيار (طباش وبوزقلي 2012)، كما ولوحظ في نفس الدراسة انخفاض في معدل ارتفاع النبات ومعدل المساحة الورقية ومحتوى الكلوروفيل لنبات الخس عند معاملته بالمستخلصات المائية لبذور الخيار بالتراكيز 10 و20%، كما سبب انخفاض في امتصاص الأيونات الضرورية (S,K, Mg. Fe, Ca) وزيادة تراكم الأيونات المعدنية الثقيلة ( Zn, Na, Cu ).

فيتامين B12 مهم في تغذية الإنسان والحيوان ، ينتج فيتامين B12 بواسطة البكتيريا وبعض الكائنات وحيدة الخلية التي تعيش في الأمعاء الدقيقة في الحيوانات ولا يتواجد في النباتات الوعائية يعد عنصر الكوبلت عنصراً مهماً في تركيب فيتامين B12 كما يعد من العناصر المهمة للنباتات والبقوليات على وجه الخصوص إذ إنه يحفز تكوين العقد وتثبيت النيتروجين، يوجدB12 في الطبيعة بصورة cobalamine cyanocobalamine, والتي تعد الأشكال الفعالة للفيتامين، إن مجموعة فيتامين B تعمل كمضادات أكسدة قوية تعادل فيتامينات C و E (Elfowiris & Banigesh, 2022). واعتبرت الفيتامينات كمنظمات نمو لها دور مهم أثناء وبعد إنبات البذور إذ استخدمت الفيتامينات كمنظمات للنمو (مثل فيتامين B و E) والذي أعطى النبات مقاومة و قدرة للتغلب على أي عوامل محددة في بيئته وذلك بتحسين معدل النمو وتحمل الملوحة (Khatun et al., 2016)، لذا تهدف هذه الدراسة لتقييم تأثير المستخلصات المائية لأوراق نبات القرع وتأثير فيتامينB12 وكذلك التأثير التازري لكل من (B12 مع مستخلص أوراق القرع) على انبات البادرات وفعالية انزيم α–اميليز وكذلك عملية تشرب البذور

## المواد وطرق البحث

تضمنت الدراسة إجراء تجارب مختبرية في المختبرات التابعة لقسم علوم الحياة \ كلية العلوم ،جامعة الموصل.

**مصدر أوراق القرع*Cucurbita pepo*** : جمعت أوراق نبات القرع بعد الحصاد من إحدى المزارع الخاصة في محافظة نينوى، جففت الأوراق هوائياً وحفظت في علب بلاستيكية لحين

الاستعمال.

**مصدر البذور:** تم الحصول على البذور (الحمص *Cicer aretinum*، اللوبيا *Vgina unguiculata*، الفاصوليا *Phaseolus vulgaris*.) من الأسواق المحلية التابعة لمدينة الموصل .

**اختبار حيوية البذور ونسبة الإنبات:** تم زراعة البذور في أطباق بتري بين ورقتي ترشيح وبواقع أربع مكررات، وتم إضافة 8 مل من (المستخلصات المائية للقرع، B12، الخليط (المستخلصات المائية للقرع مع B12 ) بالتركيزين 2 و6% مع إضافة الماء المقطر لمعاملة السيطرة، حضنت الأطباق في الحاضنة بدرجة حرارة 25 م°، وتم قياس نسبة الإنبات بعد سبعة أيام من الزراعة وبعدها خفض عدد البادرات إلى 5 بادرات لكل طبق وبعد 14 يوم تم قياس أطوال الرويشة والجذير وأخذ الاوزان الجافة لها بعد تجفيفها في الفرن بدرجة 60 مئوية ولمدة 72 ساعة (الجحيشي ،2017) وتم حساب نسبة الانبات حسب معادلة (Saied, 1984):

النسبة المئوية للإنبات = عدد البادرات الطبيعية\عدد البذور المزروعة × 100

**قياس نسبة التشرب:** اتبعت طريقة (Baskin et al., 2006) لحساب نسبة التشرب

نسبة التشرب= [وزن البذور بعد التشرب – وزن البذور قبل التشرب \ وزن البذور قبل التشرب] × 100

**قياس فعالية انزيم** α-amylase: تتم استخلاص الانزيم حسب طريقة (Białecka & Kępczyński, 2010)، وتم قياس فعالية الأنزيم بالرجوع للمنحني القياسي للمالتوز.

## النتائج

توضح بيانات الجدول (1) ارتفاع نسبة التشرب للبذور في جميع المعاملات وكانت أعلى نسبة تحفيز في بذور الفاصوليا المعاملة بالخليط عند التركيز 2% حيث بلغت 48.34%. وقد يعود السبب لتأثير المركبات الكيميائية التي أثرت على الغشاء وسببت زيادة في نفاذيته (Li et al., 2010).

**الجدول(1).** يوضح تأثير معاملة البذور بالمستخلص المائي (لأوراق القرع، بالفيتامين والخليط) على نسبة التشرب %.

| نوع المستخلص | التركيز | النوع النباتي | | | نوع المعاملة * التركيز | تأثير المعاملة | تأثير التركيز |
|---|---|---|---|---|---|---|---|
| | | حمص | لوبيا | فاصوليا | | | |
| مستخلص القرع | مقارنة | 110.16 j | 96.87 o | 71.81 u | 92.94 g | | |
| | 2 | 121.4 i | 120.26 h | 89.68 q | 110.45 c | | |
| | 6 | 125.3 e | 100.2 n | 79.63 t | 101.71 f | | |
| فيتامين B12 | مقارنة | 110.16 j | 96.87 o | 71.81 u | 92.94 g | | |
| | 2 | 130.26 c | 122.54 f | 84.35 r | 112.38 b | | |
| | 6 | 112.26 i | 104.24 l | 96.43 p | 104.31 d | | |
| خليط | مقارنة | 110.16 j | 96.87 o | 71.81 u | 92.49 g | | |
| | 2 | 133.14 b | 134.4 a | 106.53 k | 124.69 a | | |
| | 6 | 126.4 d | 103.29m | 82.53 s | 104.08 e | | |
| نوع المعاملة * النوع النباتي | مستخلص القرع | 118.95 b | 105.77 f | 80.37 i | | 101.7 c | |
| | فيتامين B12 | 117.56 c | 107.88 e | 84.19 h | | 103.21 b | |
| | خليط | 123.24 a | 111.52 d | 86.95 g | | 107.24 a | |
| التركيز * النوع النباتي | مقارنة | 110.16 d | 96.87 f | 71.81 i | | | 92.94 c |
| | 2 | 128.27 a | 125.73 b | 93.52 g | | | 115.84 a |
| | 6 | 121.33 c | 102.57 e | 86.2 h | | | 103.37 b |
| تأثير النوع النباتي | | 119.92 a | 108.39 b | 83.84 | | | |

**فعاليـة أنـزيم** α-amylase:حصلت زيادة في فعالية الأنزيم عند جميع المعاملات وكان أعلى معدل للزيادة كانت في بذور الحمص بتأثير المعاملة بالخليط 6% وبلغت أكثر من ثلاثة أضعاف ما موجود في المقارنة (معاملة السيطرة) في حين نجد إن بذور الفاصوليا المعاملة بالمستخلص المائي لأوراق القرع بتركيز 6%اظهرت انخفاضاً في فعالية الأنزيم إذ انخفضت من 93.31 في معاملة السيطرة إلى 74.46 .

**جدول(2)**. يوضح تأثير معاملة البذور بالمستخلص المائي لأوراق القرع، بالفيتامين والخليط على فعالية إنزيم الفاamylase-

| نوع المستخلص | التركيز | النوع النباتي | | | نوع المعاملة * التركيز | تأثير المعاملة | تأثير التركيز |
|---|---|---|---|---|---|---|---|
| | | حمص | لوبيا | فاصوليا | | | |
| مستخلص القرع | مقارنة | 81.65 p | 77.77 q | 93.31 n | 84.24 g | | |
| | 2 | 311.14 c | 166.64 k | 228.26 h | 235.35 c | | |
| | 6 | 189.33 j | 107.26m | 74.46 r | 123.68 e | | |
| فيتامين B12 | مقارنة | 81.65 p | 77.77 q | 93.31 n | 84.24 g | | |
| | 2 | 166.64 k | 255.36 g | 108.31 l | 176.77 d | | |
| | 6 | 82.31 o | 81.56 p | 107.14 m | 90.34 f | | |
| خليط | مقارنة | 81.65 p | 77.77 q | 93.31 n | 84.24 g | | |
| | 2 | 401.4 a | 308.2 d | 288.16 e | 332.59 a | | |
| | 6 | 355.34 b | 265.16 f | 224.26 i | 281.59 b | | |
| نـوع المعاملـة * النوع النباتي | مستخلص القرع | 194.04 d | 117.22 g | 132.01 f | | 147.76 b | |
| | فيتامين B12 | 110.2 h | 138.23 e | 102.92 i | | 117.12 c | |
| | خليط | 279.46 a | 217.04 b | 201.91 c | | 232.8 a | |
| التركيـز * النـوع النباتي | مقارنة | 81.65 h | 77.77 i | 93.31 g | | | 84.24 c |
| | 2 | 293.06 a | 243.4 b | 208.25 d | | | 248.23 a |
| | 6 | 208.99 c | 151.33 e | 135.29 f | | | 165.2 b |
| تأثير النوع النباتي | | 194.57 a | 157.5 b | 145.61 c | | | |

تبين نتائج الجدول(3) حصول انخفاض في نسبة الإنبات بذور الأنواع النباتية المختبرة (حمص ،لوبيا ، فاصوليا) المعاملة بالمستخلص المائي لأوراق القرع بالتركيز 6% وبلغت أعلى نسبة مئوية للانخفاض 21.05% في بذور الفاصوليا، أما الفيتامين فقد سبب زيادة في نسبة الإنبات وأعطى أعلى نسب إنبات، أما أعلى نسبة تثبيط فقد بلغت 14% في نبات اللوبيا عند المعاملة بالخليط بالتركيز 6% ومن تأثير نوع المعاملة في النوع النباتي تتفوق المعاملة بالفيتامين لبذور اللوبيا بإعطائها اعلى نسبة للإنبات مقارنة بباقي الأنواع والمعاملات.

من نتائج الجدول (4) يتبين حدوث زيادة في معدل طول الرويشة في بادرات الحمص، اللوبيا والفاصوليا عند معاملتها بالفيتامين بالتركيزين 2 و6% ، وإن أعلى نسبة زيادة في طول الرويشة سجلت في بادرات اللوبيا عند معاملتها بالفيتامين بالتركيز 6% ، بينما انخفض في البادرات المعاملة بالمستخلص المائي لأوراق القرع وكذلك الخليط. كما نلاحظ من نتائج التحليل الإحصائي تفوق المعاملة بفتامين B12 أعطت أعلى معدل للزيادة عند جميع الأنواع النباتية كما نجد إن التركيز 2% كان له الدور الأكبر في زيادة طول الرويشة.

**جدول(3).** يوضح تأثير معاملة البذور بالمستخلص المائي لأوراق القرع، بالفيتامين والخليط على نسبة الإنبات.

| نوع المستخلص | التركيز | النوع النباتي | | | نوع المعاملة * التركيز | تأثير المعاملة | تأثير التركيز |
|---|---|---|---|---|---|---|---|
| | | حمص | لوبيا | فاصوليا | | | |
| مستخلص القرع | مقارنة | 96.66 ab | 100 a | 76.66 f | 91.1 ab | | |
| | 2 | 96.66 ab | 93.33 abc | 76.66 f | 88.88 ab | | |
| | 6 | 90 bcd | 90 bcd | 60 g | 80 c | | |
| فيتامين B12 | مقارنة | 96.66 ab | 100 a | 76.66 f | 91.11 ab | | |
| | 2 | 100 a | 100 a | 80 f | 93.33 a | | |
| | 6 | 96.66 ab | 100 a | 76.66 f | 91.11 | | |
| خليط | مقارنة | 96.66 ab | 100 a | 76.66 f | 91.11 | | |
| | 2 | 100 a | 90 bcd | 83.33 def | 91.11 ab | | |
| | 6 | 96.66 a | 86.66 cde | 76.66 f | 86.66 b | | |
| نـــوع المعاملـــة * النوع النباتي | مستخلص القرع | 94.44 bc | 94.44 bc | 71.11 e | | 86.66 b | |
| | فيتامين B12 | 97.77 ab | 100 a | 77.77 d | | 91.85 a | |
| | خليط | 97.77 ab | 92.22 c | 78.88 d | | 89.63 b | |
| التركيـــز * النـــوع النباتي | مقارنة | 96.66 abc | 100 a | 76.66 d | | | 91.11 a |
| | 2 | 98.88 ab | 94.44 bc | 80 d | | | 91.11 a |
| | 6 | 94.44 bc | 92.22 c | 71.11 e | | | 85.92 b |
| تأثير النوع النباتي | | 96.66 a | 95.55 a | 75.92 | | | |

**جدول(4).** يوضح تأثير معاملة البذور بالمستخلص المائي لأوراق القرع، بالفيتامين والخليط على معدل طول الرويشة(سم)

| نوع المستخلص | التركيز | النوع النباتي | | | نوع المعاملة * التركيز | تأثير المعاملة | تأثير التركيز |
|---|---|---|---|---|---|---|---|
| | | حمص | لوبيا | فاصوليا | | | |
| مستخلص القرع | مقارنة | 9.25 m | 11.62 e | 9.96 j | 10.27 c | | |
| | 2 | 8.28 r | 12.28 d | 9.21 o | 9.92 e | | |
| | 6 | 7.21 u | 11.13 g | 7.94 t | 8.76 g | | |
| فيتامين B12 | مقارنة | 9.25 m | 11.62 e | 9.96 j | 10.27 c | | |
| | 2 | 9.89 k | 13.73 a | 10.32 h | 11.31 a | | |
| | 6 | 9.63 l | 12.64 b | 11.24 f | 11.17 b | | |
| خليط | مقارنة | 9.25 m | 11.62 e | 9.96 j | 10.27 c | | |
| | 2 | 9.12 q | 12.38 d | 9.22 n | 10.24 d | | |
| | 6 | 9.14 p | 10.21 i | 8.13 s | 9.16 f | | |
| نـــوع المعاملـــة * النـــوع النباتي | مستخلص القرع | 8.24 I | 11.67 b | 9.03 h | | 9.65 c | |
| | فيتامين B12 | 9.59 e | 12.66 a | 10.5 d | | 10.92 a | |
| | خليط | 9.17 f | 11.4 c | 9.1 g | | 9.89 b | |
| التركيز * النوع النباتي | مقارنة | 9.25 f | 11.62 b | 9.96 d | | | 10.27 b |
| | 2 | 9.09 h | 12.79 a | 9.58 e | | | 10.49 a |
| | 6 | 8.66 i | 11.32 c | 9.1 g | | | 9.69 c |
| تأثير النوع النباتي | | 9.00 c | 11.91 a | 9.54 | | | |

أظهرت نتائج الجدول (5) عدم وجد فروقات معنوية في طول الجذير للبادرات المعاملة بالمستخلص المائي لأوراق القرع بالتركيز 2%، أما التركيز 6% فقد سبب اختزالاً في طول الجذير للأنواع النباتية الثلاث، وأزداد معدل طول الجذير في البادرات (حمص، لوبيا، فاصوليا) المعاملة بالفيتامين وفي كلا التركيزين وأعلى نسبة تحفيز هي 19.78% في بادرات الفاصوليا عند التركيز 6%. المعاملة بالخليط سببت زيادة في معدل طول الجذير لبادرات الحمص وبالتركيزين(2,6%) بينما انخفض في اللوبيا والفاصوليا. ونجد أيضاً تفوق المعاملة بالفيتامين والتركيز 2% في إعطاء أعلى نسبة زيادة في طول الجذير.

حصل انخفاض في الوزن الجاف للرويشة لجميع البادرات المعاملة بالمستخلص المائي لقرع لكلا التركيزين(2 و6%) اقصى انخفاض في الوزن كان في بادرات الحمص المعامل بالتركيز 6% وكما موضح بالجدول(6). بينما ارتفع الوزن في جميع البادرات عند معاملتها بالفيتامين ولكلا التركيزين. أما المعاملة بالخليط فسببت زيادة في الوزن الجاف للرويشة بالتركيز 2% لبادرات اللوبيا والفاصوليا، وزيادة في بادرات الحمص في كلا التركيزين. وأعطت اللوبيا أعلى معدل للزيادة في الوزن الجاف للرويشة.

الجدول (7) يوضح زيادة في الوزن الجاف للجذير لبادرات الأنواع النباتية الثلاثة المعاملة بمستخلص القرع بالتركيز 2%، بينما انخفض الوزن بالتركيز 6%.وكذلك أزداد الوزن في البادرات المعاملة بالفيتامين وفي كلا التركيزين وأعطت بادرات الحمص أعلى نسبة زيادة بلغت 45.26% عند التركيز 2%.أما معاملة الخليط فقد أدت إلى زيادة الوزن الجاف في جميع الأنواع النباتية المختبرة وكلا التركيزين ماعدا بادرات الفاصوليا المعاملة بالتركيز 6% حيث انخفض الوزن إلى 0.215 مقارنة بمعاملة السيطرة 0.235.

**جدول(5)**. يوضح تأثير معاملة البذور بالمستخلص المائي لأوراق القرع، بالفيتامين والخليط على معدل طول الجذير(سم)

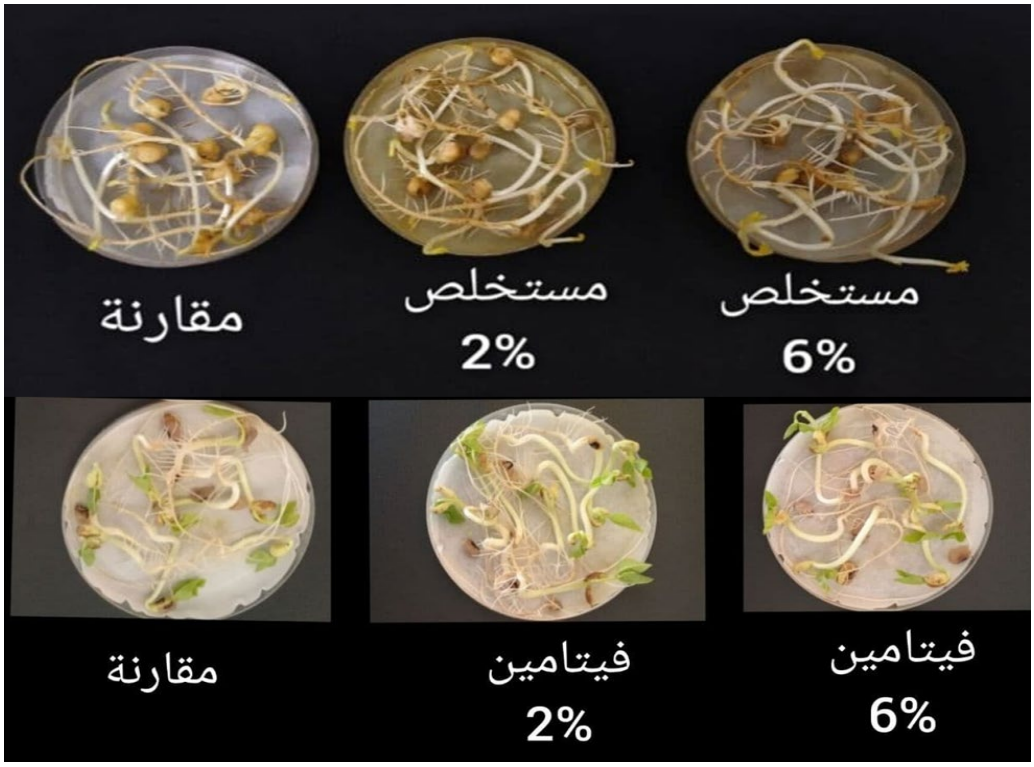
| نوع المستخلص | التركيز | النوع النباتي | | | نوع المعاملة * التركيز | تأثير المعاملة | تأثير التركيز |
|---|---|---|---|---|---|---|---|
| | | حمص | لوبيا | فاصوليا | | | |
| مستخلص القرع | مقارنة | 11.03 o | 13.36 d | 11.27 l | 11.88 d | | |
| | 2 | 11.1 n | 13.29 e | 11.32 k | 11.90 d | | |
| | 6 | 9.26 q | 11.61 i | 9.32 p | 10.06 f | | |
| فيتامين B12 | مقارنة | 11.03 o | 13.36 d | 11.27 l | 11.88 d | | |
| | 2 | 11.26 lm | 14.18 a | 11.59 i | 12.34 c | | |
| | 6 | 12.42 g | 13.62 b | 13.51 c | 13.18 a | | |
| خليط | مقارنة | 11.03 0 | 13.36 d | 11.27 l | 11.88 d | | |
| | 2 | 13.31 e | 12.91 e | 11.53 j | 12.58 b | | |
| | 6 | 11.23 m | 11.74 h | 8.81 r | 10.59 e | | |
| نـــوع المعاملـــة * النوع النباتي | مستخلص القرع | 10.46 i | 12.75 b | 10.63 g | | 11.28 c | |
| | فيتامين B12 | 11.57 f | 13.72 a | 12.12 d | | 12.46 a | |
| | خليط | 11.85 e | 12.67 c | 10.53 h | | 11.68 c | |
| التركيـــز * النـــوع النباتي | مقارنة | 11.03 g | 13.36 b | 11.27 f | | | 11.88 b |
| | 2 | 11.89 d | 13.46 a | 11.48 e | | | 12.22 a |
| | 6 | 10.97 h | 12.32 c | 10.54 i | | | 11.28 c |
| تأثير النوع النباتي | | 11.29 b | 13.04 a | c 11.1 | | | |

**جدول(6)**. يوضح تأثير معاملة البذور بالمستخلص المائي لأوراق القرع، بالفيتامين والخليط على الوزن الجاف للرويشة(غم)

| نوع المستخلص | التركيز | النوع النباتي | | | نوع المعاملة * التركيز | تأثير المعاملة | تأثير التركيز |
|---|---|---|---|---|---|---|---|
| | | حمص | لوبيا | فاصوليا | | | |
| مستخلص القرع | مقارنة | 0.454 r | 1.016 d | 0.702 k | 0.724 d | | |
| | 2 | 0.442 s | 0.973 e | 0.677 l | 0.697 e | | |
| | 6 | 0.413 u | 0.912 g | 0.653 m | 0.659 g | | |
| فيتامين B12 | مقارنة | 0.454 r | 1.016 d | 0.702 k | 0.724 d | | |
| | 2 | 0.521 o | 1.240 a | 0.738 i | 0.833 a | | |
| | 6 | 0.463 q | 1.078 b | 0.872 h | 0.804 b | | |
| خليط | مقارنة | 0.454 r | 1.016 d | 0.702 k | 0.724 d | | |
| | 2 | 0.438 t | 1.041 c | 0.722 j | 0.734 c | | |
| | 6 | 0.478 p | 0.955 f | 0.632 n | 0.688 f | | |
| نـــوع المعاملـــة * النوع النباتي | مستخلص القرع | 0.436 i | 0.967 c | 0.677 f | | 0.693 c | |
| | فيتامين B12 | 0.479 g | 1.11 a | 0.770 d | | 0.787 a | |
| | خليط | 0.456 h | 1.004 b | 0.685 e | | 0.715 b | |
| التركيـــز * النـــوع النباتي | مقارنة | 0.454 h | 1.016 b | 0.702 f | | | 0.724 b |
| | 2 | 0.467 g | 1.084 a | 0.712 e | | | 0.754 a |
| | 6 | 0.451 i | 0.981 c | 0.719 d | | | 0.717 c |
| تأثير النوع النباتي | | 0.457 c | 1.027 a | B 0.711 | | | |

**جدول(7)**. يوضح تأثير معاملة البذور بالمستخلص المائي لأوراق القرع، بالفيتامين والخليط على الوزن الجاف للجذير (غم)

| نوع المستخلص | التركيز | النوع النباتي | | | نوع المعاملة * التركيز | تأثير المعاملة | تأثير التركيز |
|---|---|---|---|---|---|---|---|
| | | حمص | لوبيا | فاصوليا | | | |
| مستخلص القرع | مقارنة | j30.24 | 0.217 qr | 0.235 k | 0.231 g | | |
| | 2 | i2450. | 0.228 n | 7 h40.2 | 0.272 c | | |
| | 6 | 7 k30.2 | 0.213 s | m300.2 | 0.252 e | | |
| فيتامين B12 | مقارنة | 0.243 j | 0.217 qr | 0.235 l | 0.231 g | | |
| | 2 | 0.353 a | 0.250 g | 0.275 f | 0.293 a | | |
| | 6 | 0.342 b | 0.220 p | 0.304 d | 0.288 b | | |
| خليط | مقارنة | 0.243 j | 0.217 qr | 0.235 k | 0.231 g | | |
| | 2 | 0.322 c | 0.224 o | 0.243 j | 0.263 d | | |
| | 6 | 0.285 e | 0.218 q | 0.215 r | 0.239 f | | |
| نـــوع المعاملـــة * النوع النباتي | مستخلص القرع | 0.241 d | 0.219 h | 0.237 e | | 0.232 c | |
| | فيتامين B12 | 0.312 a | 0.229 g | 0.271 c | | 0.270 a | |
| | خليط | 0.283 b | 0.219 h | 0.231 f | | 0.244 b | |
| التركيـــز * النـــوع النباتي | مقارنة | 0.243 e | 0.217 g | 0.235 f | | | 0.231 c |
| | 2 | 0.306 a | 0.234 f | 0.255 c | | | 0.265 a |
| | 6 | 0.288 b | 0.217 g | 0.249 d | | | 0.251 b |
| تأثير النوع النباتي | | 0.279 a | 0.222 c | 0.246 b | | | |




**الشكل (1)** تأثير المستخلصات المائية للقرع وفيتامين B12 على انبات ونمو اللوبيا.

**المناقشة**

نلاحظ تفوق المعاملة بالفيتامين على بقية المعاملات كما هو موضح في النتائج ،فدلت النتائج إلى زيادة نسبة التشرب في جميع المعاملات وقد يعود السبب لتأثير المركبات الكيميائية على الأغشية الخلوية والتي سببت زيادة في نفاذيتها (Li et al., 2010). وإن الزيادة في فعالية أنزيم الاميليز للبذور قد يعزى إلى تحفيز المركبات الكيميائية لحامض الجبريلين (Beck & Ziegler, 1989)، الذي يحفز تكوين أنزيم الاميليز من خلال تأثيره في طبقة الاليرون الموجودة في غلاف البذرة والذي يؤدي إلى تنشيط الجينات المؤثرة في تكوين الانزيمات، وتختلف هذه الجينات من نبات إلى آخر (الجحيشي،2017)، وفي المقابل نلاحظ انخفاض نسبة الإنبات للبذور(حمص، لوبيا، فاصوليا) المعاملة بالمستخلص المائي لأوراق القرع وأيضا بذور اللوبيا المعاملة بالخليط قد يرجع السبب الى اضطراب توازن مستوى هورموني Absessic acid و Ethylene (Bogatek & Gniazdowska, 2007) بتأثير المركبات الكيميائية المتحررة من أوراق القرع والتي أدت إلى ارتفاع نسبة هورمون Absessic acid الذي سبب كمون البذرة ومنع نمو الجنين بتثبيط النشاط الأيضي والذي جعل البذور في حالة كمون اصطناعية (Oracz et al., 2007) أو يمكن إن يعود السبب إلى الانخفاض في فعالية أنزيم الاميليز حسب نتائج هذه الدراسة بتأثير المركبات الأليوباثية المتحررة من أوراق القرع إذ بينت الدراسات إن هذه المركبات لها تأثير في الصفات الفسيولوجية والبايوكيمائية الضرورية لحدوث عملية الإنبات كتشرب البذور بالماء وتنبه الجنين وفعالية الإنزيمات كذلك تأثيرها في الجهد الأزموزي ونشاط المايتوكوندريا (Weir et al., 2004).

أما معدل طول الرويشة والجذير والوزن الجاف للرويشة والجذير فقد تباين ما بين الارتفاع والانخفاض عند المعاملة بمستخلصات أوراق القرع وهذه النتيجة تتفق مع ما ذكرته الحافظ (2020)، وإن زيادة معدل طول الرويشة والجذير

وأوزانهما الجافة قد يكون بسبب المركبات الكيميائية القابلة للذوبان في الماء والمتحررة من المستخلصات المائية لأوراق القرع ومنها Ferulic acid والذي تم تشخيصه بدراسات سابقة وهو معروف بدوره التحفيزي في النمو حيث يزيد نشاط الأنزيمات و ATP داخل النبات وكذلك يزيد انقسامات الخلايا النباتية وبالتالي يزيد من معدل النمو والكتلة الجافة(الجحيشي.2017).أما التثبيط الحاصل في طول الرويشة والجذير وأوزانهما الجافة فقد يعزى إلى المركبات الكيميائية المتحررة مثل galic acid و palmtic acid حيث اثبت (Kalinova et al., 2007) إن لها تأثيرات مثبطة لنمو أنواع من النباتات.

اظهرت النتائج إن المعاملة بالفيتامين سببت زيادة في معدل طول الرويشة والجذير وأوزانهما الجافة لبادرات الأنواع النباتية الثلاث(حمص، لوبيا، فاصوليا)،ونلاحظ أيضا من معاملة الخليط بأن الفيتامين استطاع إن يغطي التأثير الضار لمستخلص القرع وأعطى التأثير التحفيزي لجميع الصفات المختبرة، وهذا يعود للتأثير الإيجابي للفيتامين حيث يعمل كمحفز لنمو الجذور في عدة أنواع نباتية ( Ansari et al., 1990) وإن سبب التأثير التحفيزي للفيتامين قد يعود لعنصر الكوبلت الذي يمثل الذرة المركزية في تركيب الفيتامين، حيث وجد (Jaleel et al., 2009) بأن عنصر الكوبلت سبب زيادة في ارتفاع النبات وطول الجذر لنبات الذرة عند معاملتها بالتركيز 50 mg\Kg، كما سبب زيادة في الوزن الجاف للمجموع الخضري والجذري لنبات الحنطة مقارنة بمعاملة السيطرة (Hu et al., 2022).

## الاستنتاجات والتوصيات

نستنتج من خلال هذه الدراسة إن اظهرت النتائج أن المعاملة بالفيتامين سببت زيادة في معدل طول الرويشة والجذير وأوزانهما الجافة لبادرات الأنواع النباتية الثلاث(حمص، لوبيا، فاصوليا)، ولوحظ أيضاً عند معاملة الأنواع المختبرة بالخليط بأن الفيتامين قلل من التأثير الضار لمستخلص القرع وأعطى التأثير التحفيزي لجميع الصفات المختبرة.

ومن خلال النتائج نوصي باستخدام B12 مع مستخلصات نباتية اخرى وبتراكيز معينة وتطبق على مستوى التجارب الحقلية لمطابقة النتائج مع نتائج الدراسة الحالية.

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# Effect of Aqueous Extracts of Pumpkin Leaves and Vitamin B12 on some Growth and Physiological Characteristics of Types of Legumes

**Ban A.Idan and Wasan S. Hussain***

*Department of Biology, Faculty of Science, University of Mosul, Mosul, Iraq*

Received: 13 April 2022/ Accepted: 25 July 2022
Doi: https://doi.org/10.54172/mjsc.v37i3.741

**Abstract:** The current study was conducted in the laboratories of the Department of Life Sciences / College of Science / University of Mosul to know the effect of treatment with aqueous extracts of pumpkin leaves, vitamin B12, and the mixture on some physiological characteristics of three types of legumes (chickpeas, cowpeas, and beans), where the research dealt with measuring the percentage of impregnation, the effectiveness of α-amylase enzyme, germination percentage, stem and root length, and their dry weight in seeds treated with pumpkin leaf extract, vitamin B12, and the mixture, the results gave an increase in the percentage of impregnation in all treatments and enzyme activity in most of the treatments. As for the percentage of germination, the average length of the plumule and the radicle, and their dry weights, they varied between increasing and decreasing, and the highest percentage of stimulation was in the bean seeds treated with the mixture at the concentration of 2%, which amounted to 48.34%, while the highest percentage decrease in the germination of the seeds was 21.05% and reached 6% in the bean seeds.The results also showed that the vitamin treatment caused an increase in the average length and dry weight of the plumule and seedlings of the three plant species (chickpeas, cowpea, and beans), and it was also noted when treating the tested species with the mixture that the vitamin was able to cover the harmful effect of the pumpkin extract and gave the stimulating effect to all tested traits.

**Keywords:** Pumpkin; B12, Allelopathic Compounds; Impregnation; Amylase Enzyme.

***Corresponding author:** Wasan Saleh Hussain: *wassbio54@uomosul.edu.iq* , Department of Biology, Faculty of Science, University of Mosul, Mosul, Iraq

مجلة المختار للعلوم 37 (3): 309-315، 2022
ISSN: online 2617-2186 print 2617-2178
Journal Homepage https://omu.edu.ly/journals/index.php/mjsc/index

# تأثير درجة حموضة الماء على كفاءة قشور البيض في إزالة أيون معدن الكروم سداسي التكافؤ من المياه

**سامية أحمد الباهي**

*قسم الوبائيات ومكافحة العدوى ، كلية العلوم الصحية ، جامعة مصراته ، ليبيا*

تاريخ الاستلام: 26 مايو 2022 / تاريخ القبول: 21 يوليو 2022
Doi:https://doi.org/10.54172/mjsc.v37i3.838

**المستخلص :** في الآونة الأخيرة، تم استكشاف أنواع مختلفة من الممتزات الحيوية ذات مصدر حيواني أو نباتي ذات الحد الأدنى من حيث التكلفة والجهد، والتي يتم الحصول عليها من النفايات المنزلية ونفايات الزراعة. في العمل الحالي، تمت دراسة إمكانية استخدام مسحوق قشور بيض الدجاج كمادة مدمصة لإزالة معدن الكروم السداسي من المحلول المائي. تعد كربونات الكالسيوم، وكربونات المغنيسيوم، وفوسفات الكالسيوم من المكونات الأساسية لمسحوق قشور البيض، والتي بدورها لها دور كبير وفعال في عملية الامتزاز. في هذه الدراسة تم مزج 6 جم من مادة الامتزاز (قشور البيض) مع 3 ملجم من الكروم السداسي لكل لتر من محلول الماء النقي عند درجة حرارة (40) درجة مئوية، وضمن مجال الرقم الهيدروجيني (3،6،9). وقد تبين أن قشور البيض المتكلسة تمتلك القدرة على امتزاز أيونات الكروم السداسي، حيث بلغت نسبة الإزالة 88.767 % عند الرقم الهيدروجيني 6 خلال 60 دقيقة.

**الكلمات المفتاحية :** قشور البيض، الامتزاز، الرقم الهيدروجيني، الكروم السداسي.

## المقدمة

قد تكون العولمة قد أدت إلى التقدم السريع في الأنشطة الصناعية ولكنها في الوقت نفسه، تلحق أضرارًا جسيمة بالبيئة (Mia et al., 2020). فالصناعات هي المساهم الرئيسي في إطلاق النفايات السامة في أشكالها الصلبة والسائلة والغازية بشكل مباشر في نظامنا البيئي، حيث تتضمن النفايات السامة على المعادن الثقيلة، فلزات وملوثات عضوية يمكن أن تسبب أضرارًا جسيمة للتربة وأنظمة المياه (Elahi et al., 2020). وكان أكثر الملوثات المعدنية المدرجة سمية من قبل وكالة حماية البيئة الأمريكية، معدن الكروم سداسي التكافؤ فهو يمثل عنصر انتقالي يوجد بشكله الطبيعي في الصخور والتربة (Mane et al., 2016; Mia et al., 2020).

يعد معدن الكروم من أكثر المعادن التي تسبب تلوث المياه، رغم أنه أحد العناصر الأساسية لبناء جسم الإنسان، حيث يشارك بعمق في عملية التمثيل الغذائي للدهون، والأنسولين، والجلوكوز، وقد يؤدي نقصه إلى الإصابة بمرض السكري (Martone et al., 2013). ومن ناحية أخرى، يصنف معدن الكروم واحدًا من أكثر الأنواع سمية، ومسرطنة خصوصا الكروم سداسي التكافؤ عند مقارنته بالكروم ثلاثي التكافؤ (Ahmed & Mokhtar, 2020; Korshoj et al., 2015). من بين العديد من أنواع الكروم، يستخدم معدن الكروم Cr على نطاق واسع في العديد من الصناعات بما في ذلك دباغة الجلود والطلاء الكهربائي والطلاء، وصناعات المعادن، والأصباغ، والفولاذ المقاوم للصدأ، والأسلاك المسخنة

*[1] سامية أحمد الباهي، samiaahmed7499@gmail.com ، قسم الوبائيات ومكافحة العدوى، كلية العلوم الصحية، جامعة مصراته، ليبيا.

كهربائياً. وعلى الرغم من أهميته في الصناعات المختلفة والأنشطة الصناعية، اهتم به الباحثون فيما يخص دراسات أثره البيئي (Al-Obaidi et al., 2020; Gu & Zhu, 2011). عليه تم وضع معيار لجودة المياه، ومراقبة محتوى الكروم السداسي لمياه الصنبور، والمياه البيئية، لذلك وضعت منظمة الصحة العالمية (WHO) عام 2004، حدًا أقصى مسموحًا به صحيا وهو 50 ميكروغرام /لتر لتركيز الكروم السداسي في مياه الشرب (Rajoriya et al., 2021).

تم إجراء جهود بحثية مكثفة لاكتشاف أكثر الطرق فعالية لإزالة الكروم من الماء مثل طرق التبادل الأيوني، طريقة الترسيب الكيميائي والترشيح (Liu et al., 2017; Peng & Guo, 2020). إضافة إلى وجود طرق أخرى مثل المعالجة البيولوجية ذات التكلفة المنخفضة والكفاءة الممتازة في إزالة المعادن الثقيلة، حيث يمكن تعريف عملية الامتزاز على أنها ارتباط مجموعة متنوعة من الغاز، أو المادة السائلة في الجزيئات، أو الذرات، أو الأيونات على سطح مادة صلبة (المادة المعالجة) وهذا الارتباط يمكن أن يكون ارتباطًا فيزيائيًا، أو كيميائيًا لجزيئات المواد في المواقع النشطة على سطح المواد المازة من خلال قوة Van der Waals الموجودة بين الجزيئات.

يمكن أن تتأثر عملية الامتزاز بعوامل مختلفة مثل درجة الحرارة، ودرجة الحموضة، ووقت التلامس، والتركيز الأولي، ومساحة السطح (Komur et al., 2017). وبشكل عام فإن المعالجة الحيوية للمحاليل المائية، هي أحد الطرق الخضراء لها ثلاث مهام رئيسية: إعادة تدوير النفايات بشكل آمن على الإنسان والبيئة، إزالة المواد الضارة، التقليل من سميتها (Lee et al., 2022; Zhang et al., 2017).

تم إجراء الدراسات والأبحاث في السنوات الأخيرة حول إمكانية استخدام قشور البيض كمواد ماصة في عمليات معالجة المياه (Sankaran et al., 2020; Tsai et al., 2008). تعتمد فعالية تنقية محاليل المياه على عدد من العوامل، من بينها الرقم الهيدروجيني، والتركيز، وحجم جزيئات المواد الماصة، والجرعة، وفترة التلامس (Ghasemi et al., 2020; Govindarajan et al., 2011).

قشور البيض هي مصدر بديل لكربونات الكالسيوم التي تزيل بكفاءة أيونات المعادن الثقيلة عند ارتباطها بها في المحلول المائي، حيث تعتمد قدرة الامتصاص الخاصة بقشور البيض على خصائصها الفيزيائية، والكيميائية، وتأثير الظروف التجريبية، حيث تتكون قشر البيض من مواد خزفية مكونة من هيكل ثلاثي الطبقات، طبقة البشرة على السطح الخارجي، والطبقة الإسفنجية الوسطى (الجيرية) والطبقة الداخلية الصفائحية أو الثديية (Rajoriya et al., 2021; Stadelman, 2000). وهي ألياف بروتينية مرتبة لتكوين غشاء مزدوج الطبقات شبه منفذ، وألياف غير قابلة للذوبان في الماء، ولها مساحة سطح عالية ذات خاصية الامتصاص (Sankaran et al., 2020; Tsai et al., 2006).

## المواد وطرق البحث

**تحضير المحلول القياسي من الكروم سداسي التكافؤ:** تم تحضير 1000 جزء في المليون من الكروم سداسي بأخذ 0.283 جم من مركب $K_2Cr_2O_7$ ثنائي كرومات البوتاسيوم (يجفف عند 100 درجة مئوية لمدة ساعة) مع 100 مل ماء في دورق حجمي (Klatt & Kunze, 2009).

**تحضير مادة الامتزاز المعالجة (قشور البيض):** تم الحصول على قشور البيض الطبيعي الخام من مطعم الإفطار الخاص بالحرم الجامعي في كلية علوم البيئة التطبيقية صربيا. بعد تنظيف قشر البيض. تم تجفيف هذه العينات عند 55 درجة مئوية في فرن ثم طحنها إلى مسحوق بواسطة مطحنة خاصة. بعد ذلك، تمت غربلة هذه العينات بواسطة منخل رقم 200 للحصول على العينات المعالجة النهائية للاستخدام التجريبي.

**تحضير عينات المياه الملوثة بالكروم السداسي**: تم وضع 6 جم/ لتر من المواد الماصة الجافة (قشور البيض) في سلسلة من القوارير المعملية عبوة 100 مل والتي تحتوي على عينات المياه المحضرة مسبقا، وذات تركيز 3 ملجم من معدن الكروم

سداسي التكافؤ Cr (VI)/ لتر تم استخدام HNO3 أو NH3 للحصول على معايير مختلفة من الأس الهيدروجيني المستخدمة في البحث (3، 6، 9 ).

رجت محتويات القوارير باستخدام جهاز الطرد المركزي 300 دورة في الدقيقة عند 40 درجة مئوية. تمت معايرة محتويات القوارير في أوقات زمنية مختلفة (20، 40، 60، 80، 100، 120) دقيقة من بداية التعرض. تم ترشيح النفايات السائلة المعالجة بورق ترشيح Whatman 1 وحساب الكمية الممتصة للكروم بواسطة جهاز مقياس الطيف الضوئي Atomic adsorption spectrometer (AAS).

تم حساب النسبة المئوية لكفاءة امتصاص الكروم على النحو التالي:

النسبة المئوية لكفاءة الامتزاز (%) = $\frac{C0-Ct}{C0} \times 100$

$C_0$= التركيز الأولي لأيون الكروم قبل المعالجة

$C_t$= التركيز المتبقي لأيون الكروم بعد المعالجة

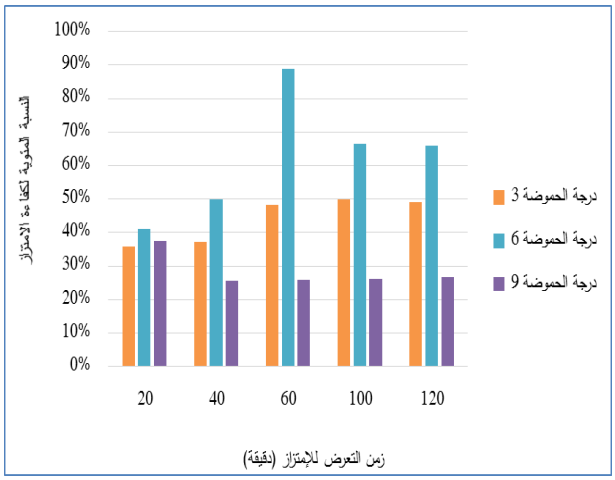
**التحليل الإحصائي**: تم تنفيذ كل مجموعة تجريبية على شكل مكررات. تم تحليل التجارب من خلال تحليل التباين (ANOVA) في برنامج التحليل الإحصائي (SPSS) وتعتبر P <0.05 ذات دلالة إحصائية.

## النتائج والمناقشة

**تأثير درجة حموضة الماء والزمن على كفاءة امتصاص الكروم السداسي بواسطة قشور البيض:** يبين الشكل (1) تأثير اختلاف الرقم الهيدروجيني على قدرة وكفاءة قشور البيض في عملية امتزاز أيونات معدن الكروم السداسي من المحلول المائي، كما تظهر البيانات في الجدول (1) أفضل نسبة كفاءة للامتزاز عندما كان PH=6 للمحلول المائي، حيث بلغت 88.767 % خلال 60 دقيقة من وقت التفاعل، يمكن أن تعزى الزيادة السريعة في معدل الامتزاز خلال الدقائق الأولى إلى وجود عدد كبير من المواقع الشاغرة على أسطح قشور البيض، كما لوحظ انخفاض نسبة كفاءة قشور البيض بعد 60 دقيقة من زمن المعالجة نتيجة انخفاض كمية المواقع النشطة، وامتلاء مسام أسطح قشور البيض بأيونات الكروم السداسي مما يحد من حركة عملية الامتزاز (Adebisi et al., 2017; Naghipour et al., 2020).

كما لوحظ الانخفاض والارتفاع في قيمة الرقم الهيدروجيني يؤدي إلى انخفاض أداء قشور البيض في عملية الامتزاز.

يمكن أن تُعزى عملية تأثير امتصاص أيونات المعدن على عامل الرقم الهيدروجيني إلى حد كبير إلى النوع، والحالة الأيونية للمجموعة الوظيفية الموجودة في المادة الماصة وأيضًا إلى كيمياء المعدن في المحلول (Gupta, 2009; Jain et al., 2010; Maleki et al., 2015; Naghipour et al., 2020).




**شكل(1)**. تأثير درجة حموضة المحلول المائي على كفاءة قشور البيض في امتزاز الكروم السداسي خلال فترات زمنية مختلفة.

**جدول**:**(1)**. تأثير درجة حموضة المحلول المائي على كفاءة قشور البيض في امتزاز الكروم السداسي خلال فترات زمنية مختلفة

| درجة حموضة الماء | زمن التعرض للامتزاز (دقيقة) | | | | |
|---|---|---|---|---|---|
| | 20 | 40 | 60 | 100 | 120 |
| | % النسبة المئوية لكفاءة الامتزاز | | | | |
| 3 | 35.967 | 37.200 | 48.267 | 49.978 | 48.967 |
| 6 | 40.956 | 49.933 | 88.767 | 66.589 | 65.911 |
| 9 | 25.567 | 26.033 | 26.167 | 26.633 | 37.544 |

## استنتاج

تم التوصل إلى عدد من الاستنتاجات من خلال هذه الدراسة، حيث يمكن استخدام قشور البيض المجففة كمواد مازة "خضراء" منخفضة التكلفة في معالجة وإزالة الكروم السداسي من المحاليل المائية تحت ظروف معينة كالرقم الهيدروجيني للماء المراد معالجته بالإضافة إلى وقت التلامس، يتناقص امتزاز أيونات الكروم على قشور البيض مع انخفاض أو زيادة درجة الحموضة عن درجة 6. علاوة على ذلك، نستنتج أن كفاءة امتزاز الكروم تزداد مع وقت التلامس. يمكن تبني هذه الفكرة لإزالة الكروم على نطاق واسع من نفاياته مثل نفايات المدابغ ومصانع الأصباغ السائلة ومياه الصرف السمية الملوثة بأيونات الكروم السداسي.

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# Effect of Water pH on the Efficiency of Eggshells in Removing Cr (VI) Ion from Water

**Samia A. Elbahi**

*Department of Epidemiology and Infection Control, Faculty of Health Sciences, Misurata University, Libya.*

Received:26 May 2022 / Accepted: 21 July 2022
Doi: https://doi.org/10.54172/mjsc.v37i3.838

**Abstract:** Recently, various types of bio-adsorbents of animal or vegetable sources with minimal cost and less effort, which are obtained from domestic and agricultural waste, have been explored. In the present work, the possibility of using chicken eggshell powder as an adsorbent material to remove hexa-chromium metal from an aqueous solution was studied. Calcium carbonate, magnesium carbonate, and calcium phosphate are the primary components of eggshell powder, which in turn have a substantial and effective role in the adsorption process. In this study, 6 g of adsorbent (egg shells) was mixed with 3 mg of hexa-chromium per liter of pure water solution at a temperature of (40) °C and within the pH range (3, 6, 9). It was found that calcified eggshells have the ability to adsorb hexa-chromium ions, with a removal rate of 88.767% at pH 6 within 60 minutes.

**Keywords:** ; Adsorption; pH; Cr (VI).

***Corresponding author:** Samia A. Elbahi: samiaahmed7499@gmail.com, Department of Epidemiology and Infection Control, Faculty of Health Sciences, Misurata University, Libya.