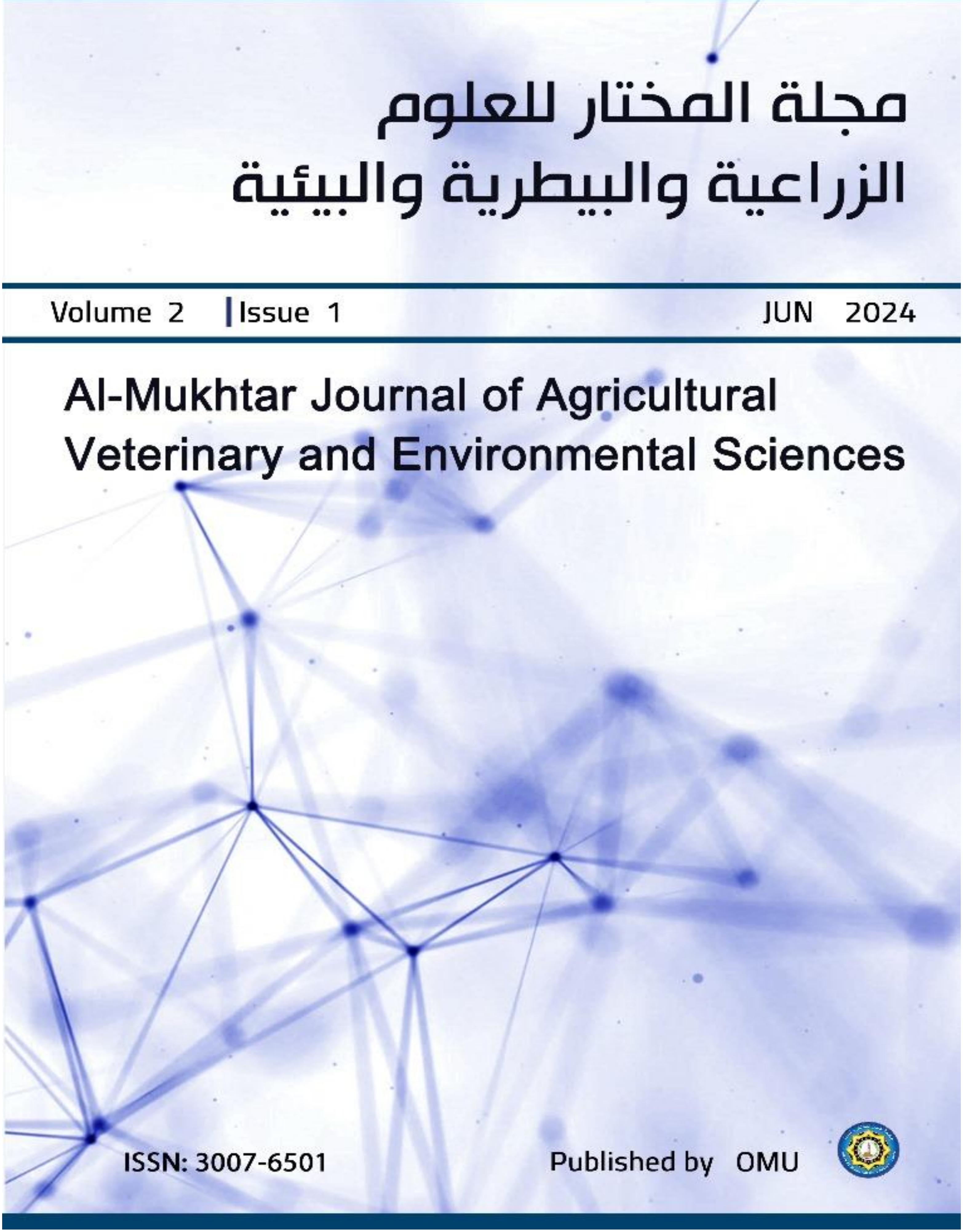

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Research Article **Open Access**

Mycoflora Detection in Some Dry Plants Used in Libyan Folk Medicine

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Abstract: laurel Bay, Guava and Senna were purchased from different herbalist shops in Al-Beyda city. Moisture content was determined, and the levels of fungal contamination were detected in all samples by incubating them on 2% water agar petri dishes and purifying on potato sucrose agar at 25 ± 1^{o}C. The higher moisture contents of 50% and 25% were recorded in Guava and Greek laurel Bay respectively. Seven different genera and 12 species were isolated and identified as *Alternaria alternata*, *Aspergillus flavus*, *A. carbonarius*, *A. niger*, *A. parasiticus*, *Cheatomium globosm*, *C. funicola*, *Cheatomium* sp., *Cladosporium cladosporides*, *Phoma* sp., *Pealosporium herbarum* and *Ulocladium charturm*. The higher diversity of 14 isolates and the higher frequency of 38.9% were found in guava following by 11 isolates and 30.6% in Greek laurel Bay.The relative density of contamination for fungi isolated revealed that *A. alternata* and *A. niger* are maximum (15.7%) followed by *C. funicola* (13.7%) and the minimum number of fungi recorded was 2% for *P. herbarium***.** Screening tests for mycotoxins producibility in isolates of fungi by using vapor ammonia liquid on PSA medium, among which *A. flavus*, *A. carbonarius*, *A. niger*, showed positive results for mycotoxins production.

Keywords: Medicinal Plant; Moisture content; Fungi; Mycotoxins, Libya.

كشف الميكوفلورا في بعض النباتات الجافة المُستعملة في الطب الشعبي الليبي

المستخلص: أُجريت الدراسة الحالية لبحث الفطريات المرافقة لبعض النباتات الجافة المُستعملة في الطب الشعبي. جُمعت أوراق خمسة عشر عينة تعود لخمسة نباتات هي: السدر، الزريقة، الغار، الجوافة، والسنامكي تم شراؤها من محلات عطارة مختلفة في مدينة البيضاء. قُدر المُحتوى الرطوبي ومستوى التلوث في العينات باستخدام الاجار المائي فيما تمت التنقية على الوسط *PSA*، وبينت النتائج تسجيل أعلى مُحتوى رطوبي 50% و25% في أوراق الجوافة والغار على التوالي. تم تعريف سبعة أجناس فطرية ضمت 12 نوعاً مختلفاً: *Alternaria alternata*، *Aspergillus flavus*، *A. carbonarius*، *A. niger*، *A. parasiticus*، *Cheatomium globosm*، *C. funicola*، *Cheatomium sp.*، *Cladosporium cladosporides*، *Phoma sp*، *Pealosporium herbarum* و *Ulocladium charturm* . سجل نبات الجوافة وجود 14 عزلة فطرية بتكرار 38.9% متبوعاً بـ11عزلة في نبات الغار وتكرار 30.6%. كانت أعلى كثافة 15.7% للفطريات *A. alternata* و *A. niger* متبوعاً بـ13.7% لـ*C. funicola* وأقل كثافة 2% لـ *P. herbarium*. اختبار قُدرة العزلات على إنتاج السموم في الوسط *PSA* بالتعريض لأبخرة الأمونيا السائلة بين أن الأنواع *A. flavus*، *A. carbonarius* و *A. niger* مُنتجة للسموم.

الكلمات المفتاحية: نباتات طبية، المحتوى الرطوبي، الفطريات، سموم فطرية، ليبيا.

INTRODUCTION

In Libya, the use of folk medicine is one of the oldest, richest, and most diverse traditions, associated with the use of medicinal plants. Different parts of medicinal plants, like (bark, stem, leaves, root, fruit etc.) are used in crude, powered form, as well as boiled or soaked in water to treat cough and common cold, asthma, diarrhea and constipation, accelerated heart rate, and getting rid of fat, colic, and kidney stones. (Al-Kady & Ebshaina, 1988; Al-Kady, 2004). The fungi accompany medicinal plants during their growth in the field, and others contaminate plants during drying, harvesting and storage processes (Roy & Chourasia, 1989; Rashidi & Deokule, 2012).

Many reports about medicinal plants contamination with fungi were published. In a study conducted by (Shakhenib et al. 2011), were able to isolate 43 genera of fungi from *Thymus* sp., *Mentha longifolia* and *Trigonlla foenun- graecun* plants. The dominant fungi were *Aspergillus* (100%), *Alternaria* (90%), followed by *Cladosporium* spp., *Emericella*, *Ulocladium*, *Mucor* and *Cheatomium* at rates 76.66%, 73.33%, 53.33%, 56.66% and 40% respectively. In another study, (Siakrwar et al. 2014) demonstrated that *Saraca indica*, *Terminalia arjuna*, *Hemidesmus indicus* were contamination with molds such as *Alternaria*, *Aspergillus*, *Penicillium*, *Rhizopus* and *Syncepalastrum*. Also, *A. flavus*, *A. niger*, *Mucor* spp. and *Rhizopus* spp. were dominant in some stored medicinal plants (Rawat et al., 2014).

Samples of 12 medicinal plants were collected from markets and analyzed for fungi contamination. The results showed that most of the species found belong to the genus *Cladosporium*, *Fusarium*, *Aspergillus* and *Penicillium* (Pereira et al., 2015). Fungi *A. alternata*, *A. terreus*, *C. cladospoiodes*, *Curvularia* spp., *C. globosum*, *Hansfordia sinuosae*, *Phoma multirostrata*, *Pleospoales* sp. and *Trametes versicolor* were associated with the aerial parts of *Calotropis procera*, *T. graecum*, *Vernonia amygdalina*, *Catharanthus roseus* and *Euphorbia prostrata* Sudanese medicinal plants (Khiralla et al., 2016).

In the Kingdom of Saudi Arabia, Al-Hindi *et al.*, (2017) noted that the most commonly isolated fungal genera were in the following descending order: *Aspergillus*, *Penicillium*, *Fusarium* and *Rhizopus* in agarwwod (Oudh). In China, a total of 126 fungi were identified from the surface of samples of medicinal herbs by morphology and ITS sequencing, with *Aspergillus* and *Penicillium* genera as the predominant contaminants (Zheng et al., 2017).

(Mbilu et al. 2018) reported that the medicinal plant *Warburgia ugandensis* was a carrier for stored fungi such as *Alternaria* sp., *A. flavus*, *Bionectria ochroleuca*, *Cladosporium* sp., *Cochliobolus sativus*, *C. acutatum*, *Diaporthe amygdali*, *Guignardia mangiferae*, *Fusarium oxysporum*, *Nigrospora oryzae*, *Phomopsis* sp., *Phyllostica gardeniicola*, *Trichoderma harzianum* and *Tricharina gilva*.

(Yodsing et al. 2018) recorded the presence of seven species of fungi: *A. candidus*, *Neosartorya fischeri*, *N. tatenoi*, *Paecilomyces herbarum*, *Phomopsis* sp., *Xylaria berteri* and *X. feejeensis* was a companion to the *casearia growiifolia* plant.

In another study, (Gaddawi et al., 2022) found many fungi, such as *A. alternata*, *Aspergillus* spp., *Fusarium oxysporum* and *Penicillium* sp. at different frequencies in alabaster, mint and anise herbal plants. Also, (Wei et al., 2023) evaluated the levels of contamination by toxigenic fungi in herbal medicines. They recorded *Aspergillus* (43.77%), *Fusarium* (5.17%) and

Cladosporium (4.46%) as the three predominant genera.

All plants and their products were exposed to contamination with fungi and their toxins during field growth, storage or selling. Different taxonomic groups of fungi were detected in medicinal plant samples collected from different regions, suggesting the *Aspergillus* and *Penicillium* groups as the most predominant genera. Many species of *Aspergillus* and *Penicillium* genera are known mycotoxin-producers (Al-Hindi et al., 2017; Zheng et al., 2017; Gaddawi, et al., 2022; and Wei et al., 2023). Many reports showed the role of relative humidity and moisture content in stored plant materials to justify contamination with mold fungi (Moreno-Romo, 1985; Roy and Chourasia, 1989; El-Gali, 1996).

The fungi caused discoloration, quality deterioration, a reduction in commercial value, and a risk to human health, especially after medication with them because some fungi produce mycotoxins in these plants that cause several ailments to the liver, kidney, nervous system, muscles, skin, etc. in low dosages (Rodricks, 1976; Rai and Mehrotra, 2005; Truckesses and Scott, 2008).

Because of the increase in the use of storage medicinal plants and obtaining them easily without any adequate conditions for their use, the present work investigated the presence of fungi in five types of medicinal plants that may be contaminated with storage mold and the rapid detection of toxins from fungal isolates.

MATERIALS AND METHODS

Source of samples

A number of 15 samples of dry medicinal plants exposed for sale were collected from different herbalist shops in Al-Beyda city, Libya. The plants listed in Table (1) were packed in polythene bags and brought to the microbiology laboratory at Agriculture College, Omer Al-Mukhtar University, for further studies.

Table (1): List of used medicinal plants

S. No	English name	Scientific name	Family
1	Greek laurel Bay	*Laurus nobilis*	Lauraceae
2	Guava	*Psidium guajava*	Myrtaceae
3	Lotos	*Zizyphus lotus*	Rhamnace
4	Senna	*Senna acutifolia*	Caesalpiniaceae
5	Zoreka	*Globularia alypum*	Globulariacea

Moisture content

To calculate the moisture content of the sample, 12 grams were weighted and placed in the oven at 100°C for 24 hours. The sample was taken out of the oven and placed in a glass desiccator until it cooled completely, then weighted again and returned to the oven for an hour. After drying, samples were weighted for three times and the moisture content was estimated according to Essono *et al*., (2007): $MC = [(W_i - W_f)/ W_i\ ] \times 100$, where MC = moisture content, W_i = initial weight ,and W_f = final weight. Therefore, the difference between the Initial and final weights equals the amount of moisture.

Isolation and identification of mycoflora:

Samples were washed with tap water to remove soil and dust particles. They were cut into segments (0.5 cm), surface sterilized with 1% sodium hypochlorite solution for 2 minutes, then rinsed three times in sterile distilled water, placed on a filter paper and left to dry. The pieces were distributed on 2%WA Petri dishes at a rate of 5 pieces per plate, and incubated at 25 ± 1^{o}C for 5-7 days under 12 hours photoperiod. The fungal growth visible on the infected parts was purified dishes by hyphal tip transfer (Burgess, et al. 2008). A small agar block taken from the margin of the colony was transfered to plate on clean PSA using a flat needle. All fungi were maintained on PSA slants for further studies.

The fungi were primarily identified with the naked eye based on apparent characteristics such as colony color, existence or lack of aerial mycelia, and by lactophenol cotton blue stain for checking color and density, presence or lack of acervulus, pycnidium, and other asexual reproductive organs in cultural media, consisting of shape and size of conidia, conidiophores, pycnidia, and formation of hair in pycnidium under the compound microscopic (Malone & Muskett, 1997 ; Barnett & Hunter, 1998 ; Kidd et al., 2016).

Recorded of results

Frequency (Fr) and relative density (RD) of different fungal species can be expressed by means and calculated according to the following formulas (Girridher & Ready, 1997): Fr(%) = (ns/N) **x** 100 ns , number of samples in which the genus or species is detected; N – the total number of samples, RD(%) = (ni/Ni) x 100 ni, the number of isolates of the genus or species, Ni the total number of detected isolates.

Determination of toxigenic fungi:

In this experiment, a rapid detection of toxins was used. All isolates were grown on PSA medium in Petri dish at 25c° for 7 days. The dish was inverted, and 2ml of ammonium hydroxide solution 25% was placed in the plate cover, and closed by para film and returned to the incubator for 4 days to expose for ammonia vapor. Each treatment was repeated 3 times, and the dishes were monitored during this period to note and record any change in color for indicating the production of toxins. The change in culture pigments from underside to pink color indicated toxin secretion (Saito & Machida, 1999).

RESULTS

Moisture content

The moisture content observed in all medicinal plants ranged from 8% to 50%. However, the highest mean moisture content was recorded in Guava (50%) followed by Greek laurel Bay (25%) and Zoreka (16.7), while the lowest mean moisture content was observed in Senna (8%), as shown in Table (2).

Table (2): % Moisture content in different medicinal plant samples

Name of Plant	Weight (gm)		% Moisture content
	Initial weight (W_i)	Final weight (W_f)	
Greek laurel Bay	12	9	25
Guava	12	6	50
Lotos	12	11	8
Senna	12	11	8
Zoreka	12	10	16.7

Isolation results from samples showed that presence twelve species of fungi were recorded, including: *A. alternata*, *A. flavus*, *A. carbonarius*, *A. niger*, *A. parasiticus*, *C. globosm*, *C. funicola*, *Cheatomium* sp., *C. cladosporides*, *Phoma* sp., *P. herbarum* and *U. charturm* (Fig.1).

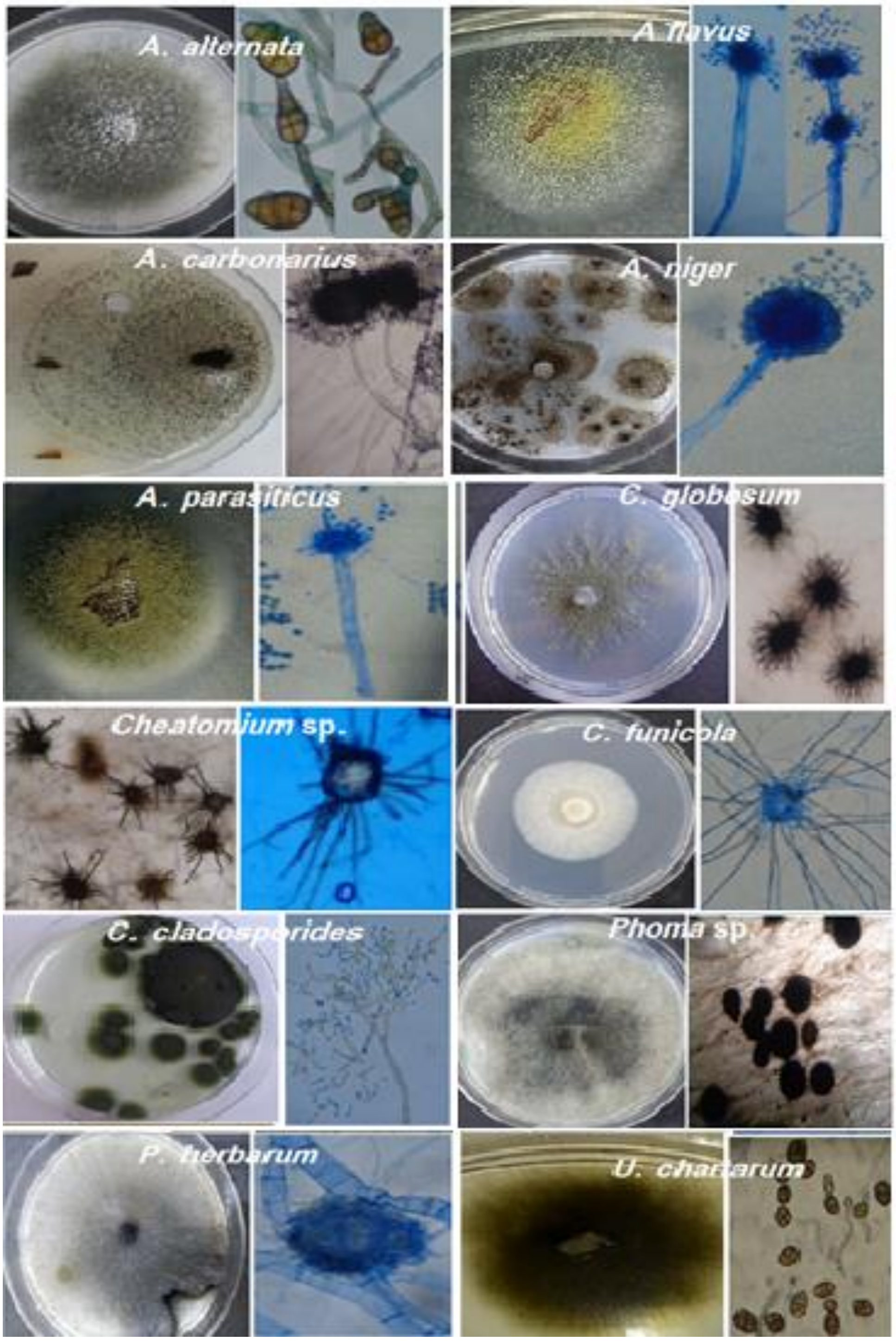


Figure: (1). Colonies of isolated fungi on PSA medium

Results in Table (3) shows the list of fungi in plant samples. Among these fungi, three species belong to *Cheatomuim*, four species belong to *Aspergillus*, whereas *Alternaria*, *Cladosporium*, *Phoma*, *Pealosporium* and *Ulocladium* were appeared as one species. The highest diversity of 14 species of fungi was found in guava followed by Greek laurel Bay (11), Senna (10) and 9 in

Zoreka, and the lowest 7 species in Lotos. *Aspergillus* spp (19) and *Cheatomium* spp (14) were more commonly detected than other genera of fungi. *Alternaria*, *Aspergillus* spp., *Cheatomium* spp. were found in all examined medicinal plants, while *Cladosporium* was found in Greek larel Bay and Lotos. *Phoma* in Zoreka and Lotos, *Ulocadium* in Greek laurel Bay and Guava. *Pealosporium* found only in Zoreka.

Table (3): Samples of medicinal plants contaminated with one or more fungal species

Name of plants	Sample No.	Name of fungi												Fungal contamination per sample	Total
		A. alternata	*A. flavus*	*A. carbonarius*	*A. niger*	*A. parasiticus*	*C. globosum*	*C. funicola*	*Cheatomium* sp.	*C. cladosporides*	*Phoma* sp.	*P. herbarium*	*U. charturm*		
	1	+	-	-	+	-	-	+	+	+	-	-	+	6	
Greek laurel Bay	2	-	-	-	-	-	+	+	+	-	-	-	+	4	11
	3	-	-	-	-	-	-	-	-	-	-	-	+	1	
	4	+	-	-	+	-	-	-	+	-	-	-	-	3	
Zoreka	5	+	-	-	+	-	+	-	+	-	+	+		6	9
	6	-	-	-	-	-	-	-	-	-	-	-	-	0	
	7	-	+	+	-	+	-	-	-	-	-	-	+	4	
Guava	8	+	+	+	-	+	-	+	-	-	-	-	+	6	14
	9	-	+	+	-	+		+	-	-	-	-	-	4	
	10	+	+	-	+	-	-	-	+	-	-	-	-	4	
Senna	11	+	+	-	+	-	-	-	-	-	-	-	-	3	10
	12	+	-	-	+	-	-	+	-	-	-	-	-	3	
	13	+	-	-	-	-	-	-	-	+	-	-	-	2	
Lotos	14	-	-	-	+	-	-	+	-	-	-	-	-	2	7
	15	-	-	-	+	-	-	+	-	-	+	-	-	3	
Total		8	19				14			2	2	1	5	51	

Regarding the frequency of fungi, the results recorded in Table (4) indicated a difference in the frequency of fungi isolated from plants. The minimum percentage of contamination was detected in Lotos (19.4%), while the maximum contamination was in Guava (38.9%) followed by Greek laurel Bay (30.6%).

Considering the density of fungi represented in Table (5), the results showed that *A. alternata* and *A. niger* had the highest density (15.7%) followed by *C. funicola* (13.7%) while *P. herbarium* had the least density at 2%.

Determination of toxigenic fungi:

Results of the ability of fungal isolates to secrete toxins in PSA medium after being exposed to liquid ammonia vapor were recorded, showing a change in culture pigments from underside with different intensities. Among these isolates *A. flavus*, *A. carbonarius*, *A. niger,* showed positive results for mycotoxins production compared to the color before treatments. (Table 6).

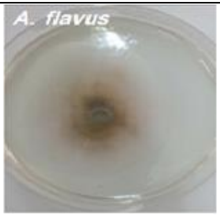
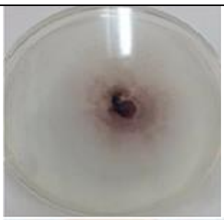
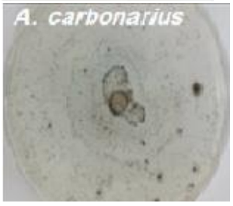
Table (4): % Frequency of fungal species isolated from different medicinal plants

fungi	Greek laurel Bay	Zoreka	Guava	Senna	Lotos
A. alternata	33.3	66.7	33.3	100	33.3
A. flavus	0	0	100	66.7	0
A. carbonarius	0	0	100	0	0
A. niger	33.3	66.7	0	100	66.7
A. parasiticus	0	0	100	0	0
C. globosum	33.3	33.3	0	0	0
C. funicola	66.7	0	66.7	33.3	66.7
Cheatomium sp.	66.7	66.7	0	33.3	0
C. cladosporides	33.3	0	0	0	33.3
Phoma sp.	0	33.3	0	0	33.3
P. herbarium	0	33.3	0	0	0
U. charturm	100	0	66.7	0	0
Mean	30.6	25	38.9	27.8	19.4

Table (5): % Relative Density of fungal species isolated from different medicinal plants

Fungal species	Number of isolates	% contamination
A. alternata	8	15.7
A. flavus	5	9.8
A. carbonarius	3	5.9
A. niger	8	15.7
A. parasiticus	3	5.9
C. globosum	2	3.9
C. funicola	7	13.7
Cheatomium sp.	5	9.8
C. cladosporides	3	5.9
Phoma sp.	2	3.9
P. herbarium	1	2
U. charturm	5	9.8
Total	51	

Table (6): Test of detection of toxins secretion in PSA solid medium

Fungi	Ammonia vapor test		
	Before	After	Response
A. flavus			+
A. carbonarius			+
A. niger	-	-	+

+: found
-: not found

DISCUSSION

During this study, we collected 15 samples of medicinal plants, and isolated 12 species belonging to 7 genera including, *A. alternata*, *A. flavus*, *A. carbonarius*, *A. niger*, *A. parasiticus*, *C. globosm*, *C. funicola*, *Cheatomium* sp., *C. cladosporides*, *Phoma* sp., *P. herbarum* and *U. charturm*. Other similar studies have recorded the presence of such fungi on dried medicinal plants or spices (Zdenk & Stjepan, 1999; Bugno et al., 2006; Rashidi & Deokule, 2013; Gaddawi, et al., 2022; Wei et al., 2023).

Also, the results demonstrated a correlation between the highest moisture and the fungal frequency in medicinal plants. Guava plant has the highest moisture content (50%) and the highest fungal frequency (38.9%). Hale (1998) reported that the presence of moisture content in medicinal plant samples justifies the favorable impact of fungal growth on store medicinal plants. The high level of moisture content and fungal frequency may be due to the fact that most of these samples are imported, and in addition, the preventive measures were not applied during storage and exposure for the sale of these medicinal plants.

From this study,it was noticed *Aspergillus* spp. followed by *Cheatomium* spp. and *A. alternata* were isolated from all samples, they appeared to be the most predominant fungi of medicinal plants. The results agree at large with many of the investigators working on spices and medicinal plants (Sumanth et al, 2010; Shakhenib et al. 2011; Toma & Abdulla, 2013; Gaddawi, et al., 2022; Wei et al., 2023).

Aspergillus spp. recorded the highest incidence. The same result was recorded by (Chen et al. 2020) on medicinal herbs. It is attributed to its ability to produce the large number of conidia spores that widely distributed (Domsch et al., 1980) and exploit the different substances as carbon sources for nutrition and growth (Dickinson & Boardman, 1970), Also, for living at a low level of humidity (Onesirosan, 1982).

From this study, we noticed an increase in the number of fungi (Dematiaceous) that produced melanin pigments, which enhance the survival and competitive abilities of species in certain environments (Bell & Wheeler, 1986).

Cheatomium spp is one of the fungi isolated from all tested samples due to its ability for undergo cellulose analysis by cellulose enzymes and produce fruiting bodies structures to tolerate environment unsuitable conditions (Udagawa et al., 1979).

Considering mycotoxins production, the experiments recorded the ability of *A. flavus A. niger*, and *A. carbonarius* to produce toxins in PSA medium after exposing to ammonium hydroxide vapor. Similar studies have demonstrated the ability of such fungi to secrete toxins (*Wicklow* et al., 1996; Bugno et al, 2006; Rashidi & Deokule, 2013; Pereira et al., 2015; Al-Hindi et al., 2017; Chen et al., 2020).

CONCLUSION

From the present study, it was concluded that all plants samples were contaminated with storage mold fungi and toxigenic fungi. The contamination level was related to the highest moisture content. Because medicinal plants in are used in folk medicine for direct human purposes, so the contamination with fungi must be detected and their use prevented in order to preserve public health.

ETHICS

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

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

Author contributions: The first author supervised the project. He wrote the results, while the second author did the practical experiments.

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Research Article **Open Access**

Effect of Cold Stratitication and Gibberellic Acid Treatment on Seed Germination and Seedlings Growth of Quince (*Cydonia oblonga*)

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Abstract: The study aimed to determine the effect of cold stratification, concentrations of gibberellic acid (GA_3) and the interaction between them on the characteristics of germination and growth of quince seedlings. Quince seeds have embryonic dormancy attributed to inhibitory germination in seed covers; thus, seeds exposed to cold stratification (30, 60, and 90 days) and treated with GA_3 at concentrations (0, 250, 500, and 1000 ppm). A randomized complete block design was used, and each treatment contained 40 seeds. The results showed the cold stratification of 90 days gave the highest percentage and speed of germination, the longest seedling, the largest leaf area and a percentage of dry matter with an average of (87%, 1.88, 37.35cm, 31cm^2 and 55%), and the use of a concentration (500 ppm) gave the best percentage and speed for seed germination. The interaction showed a significant effect on the characteristics of germination and growth. The seed treated with cold stratification for 60 days with a concentration of GA_3 at 500 ppm gave the highest percentage of germination (94%), and the stratification for 90 days with a concentration of 1000 ppm recorded the largest leaf area and the highest percentage of dry matter (34. 40 cm^2 and 57.54%) on respectively. While the seeds stratified for 60 days without GA_3 have the highest value for the diameter (2.89 mm). From this, we concluded that the treatment with GA_3 with cold stratification is of economic importance for its effective role in breaking the dormancy of Quince seeds.

Keywords: Quince, Stratification, dormancy. GA_3

تأثير التنضيد البارد والمعاملة بحمض الجبريليك على إنبات البذور ونمو شتلات السفرجل *Cydonia oblonga*

المستخلص: أجريت الدراسة بهدف تحديد تأثير طول فترت التنضيد البارد، الغمر في حمض الجبريليك والتداخل بينهما على خصائص الإنبات ونمو شتلات السفرجل، تتميز بذور السفرجل بجود سكون الجنين الراجع الي وجود مثبطات الانبات في أغلفة البذرة. حيث عرضت البذور لثلاث فترات تنضيد (30، 60، 90 يوم) وعوملت بحمض الجبريليك بتركيزات (0، 250، 500، 1000 جزء في المليون)، استخدم تصميم القطاعات الكاملة العشوائية في تنفيذ هذه الدراسة واحتوت كل معاملة على 40 بذرة. أظهرت النتائج أن فترات التنضيد أثرت معنوياً على خواص الإنبات والنمو حيث اعطت فترة التنضيد 90 يوم اعلي نسبة وسرعة للإنبات، اطول شتلة أكبر مساحة ورقة ونسبة من المادة الجافة بمتوسط (87%، 1,88، 37.35 سم، 31سم^2 و55%، كما ادى استخدام تركيز (500 جزء في المليون) إلى الحصول على أفضل نسبة وسرعة لانبات البذور، في حين التركيز (1000جزء في المليون) اعطى اطول طول للشتلة وأكبر مساحة للورقة ونسبة من المادة الجافة. أظهر التداخل بين فترات التنضيد وتراكيز حمض الجبريليك تأثيراً معنوياً على خصائص الإنبات والنمو، حيث أعطت البذور المنضدة لفترة 60 يوماً والمعاملة بتركيز (500 جزء في مليون) من حمض الجبريليك أعلى نسبة إنبات (94%) والفترة 90 يوم مع التركيز 1000 جزء بالمليون سجلت أكبر مساحة ورقة وأعلى نسبة من المادة الجافة (34.40سم^2 و57,54%) على التوالي. بينما اعطت البذور غير المعاملة بحمض الجبريليك والمنضدة لمدة 60 يوماً اعلى قيمة لقطر للشتلات بمتوسط (2،89 ملم). ومن ذلك نستنتج ان المعاملة بحمض الجبريليك مع التنضيد البارد ذات اهمية اقتصادية لدورها الفعالة في كسر سكون البذور.

الكلمات المفتاحية: السفرجل، التنضيد البارد، السكون، حمض الجبريليك.

INTRODUCTION

Cydonia oblonga Mill is an important fruit for some pome fruit, particularly pear fruit. Quince as rootstock showed high compatibility and induced a significant dwarfing effect on pear fruit trees. Quince rootstock showed also tolerance to calcareous soil and drought conditions (Tatari at el., 2020; Mohammadi et al., 2015). Moreover, Quince rootstock is quite resistant to moist soil and iron chlorosis (Kafkas at el., 2018). Quince rootstocks have important influences on different fruit production such as canopy architecture, nutritional uptake, flowering, yield and fruit quality (Nimbolkar at el., 2016). Cold stratification is routinely employed to break dormancy in water-permeable seeds (Willis at el., 2014). Several researchers demonstrated that the effect of cold stratification on seed dormancy breaking was a result of a decline in abscisic acid (ABA) and an increase in gibbrrens (GA) (Wang at el., 2020; Chen at el., 2015 & Lewak, 2011). Cold stratification broke embryo dormancy in *Malus domestica* seeds by catabolizing lipids, sugars, and proteins with hydrolytic or proteolytic enzymes (Lewak, 2011). Cold stratification broke the embryonic dormancy of *Mallus domestica* and *Juglanas regia* (Lewak 2011) and *Corylus avellana* (Einali & Sadeghipour, 2007; Li & Ross, 1990) by activating of hydrolytic and proeolytic enzymes that catabolize lipids, sugar and proteins. Cold stratification leads to the activation and transcription of genes involved in the biosynthesis of gibberellins and consequently, an increase in level of endogenous GA_3 (Yamauchi et al., 2004).

GA_3 has been widely used for breaking seed dormancy in many plants (Lee et al., 2022; Su, et a.l., 2016 & Rawat, et al., 2008). Cold stratification has been shown to increase the endogenous GA_3 content of stratified seed and consequently seed germination (Hashemirad et al., 2023; Rawat et al., 2008). Subjecting of exogenous GA seeds to exogenous GA_3 treatments increased their germination rate and decreased their germination time (Ge et al., 2023; Hopkins, & Gravatt 2019; Ali & Elozeiri, 2017). The purpose of this study was to determine the effects of various dormancy-breaking treatments, such as stratification and gibberellic acid (GA_3) on the seed germination of Quince.

MATERIALS AND METHODS

This study was conducted on the farm of the Department of Horticulture, Faculty of Agriculture, Omar Al-Mukhtar University. The seeds were collected from farm No. (4) of the Al-Jabal Al-Akhdar Agricultural Project in the Shahat region in 2018 season. The seeds were divided into 3 groups based on perids of stratification as a main factor (30, 60, and 90 days) then each stratification period was divide into four groups based on concentrations of GA_3 (0,250,500 and 1000 ppm), where each individual treatment contained 40 seeds.

The following treatments were used:

Cold stratification 30 days

Cold stratification 60 days

Cold stratification 90 days

GA_3 0 ppm

GA_3 250 ppm

GA_3 500 ppm

GA_3 1000 ppm

For 24 hours, seeds were soaked with different concentrations of GA_3 based on experiments. After that, these seeds were sterilized with the fungicide (Mancozeb). Seeds were mixed with sand in a ratio of 1:3 (seed and sand, respectively). Then the seeds of each treatment were placed in a polyethylene bag to allow gas exchange and sealed. With weekly flipping and wetting the mix if needed. At the time of planting, the bags were prepared, and each bag contained equal amounts of a mixture of soil, sand and peat moss in a ratio of 1: 2: 1, respectively. At a depth of 2 cm seeds were planted, then irrigated as needed.

Germination percentage (GP) was calculated according to (Peters,2000) $GP=\left(\frac{n}{N}\right)\times\ 100$, where n is the number of germinated seeds, and N is the total number of seeds.

Germination speed (GS): It was estimated according to the equation (Naylor, 1981)

$$GS=\frac{\mathbf{a+(a+b)+(a+b+c)+}\ \ldots\ldots}{\mathbf{N(Total\ number\ of\ seeds/treatment)}}$$

where, a: Number of seeds germinated in the first count , b: Number of seeds germinated in the second count,c: Number of seeds germinated in the third count and N: number of times to count. Seedling length (cm): after 150 days of planting. Seedling diameter (mm): 150 days after planting. Leaf area index of seedling: using Planimeter Kp - 90N. The percentage of dry matter was calculated.

$$\text{as follows: }\frac{\mathbf{dry\ weightdy}}{\mathbf{wet\ weight}}\times 100.$$

Statistical Analysis: The obtained data were subjected to a statistical analysis of variance ANOVA of the combined analysis in a completely randomized block design according to (Ott & Longnecker, 2015) and least significant difference (LSD) of 0.05% was used to compare between the means of the treatments using COSTAT Software (Pacific Grove, CA, USA).

RESULTS

Quince seeds were subjected to cold stratifications and GA_3 concentrations and recorded seed quality parameters.

Table 1shows the results of percentage germination and speed of germination. For percentage germination, results showed that there were significant differences between stratification periods, where stratification for 90 days had the highest germination value (87%).For concentrations of GA_3, also there were significant differences between concentration, where GA_3 500 ppm treatments had the highest ratio of germination (67%). The results of the interaction between stratifications and concentrations show that there were significant differences between treatments in percentage of germination, where stratification 60 days with GA_3 500 ppm treatments had the highest mean (94%).

For speed germination trait (table 1), the results show that there were differences between treatments, with 90 days of stratification having the best results compared with other treatments (1.88). While concentration 500 ppm had the highest mean (1.66). The results of the interaction between the two treatments shown that treated seeds with stratification 90 days with GA_3 500 ppm had the highest value (2.26), whereas the seeds treated with stratification 30 days had the lowest value (0.43).

Table (1): Effect of cold stratification (CS) and GA_3 concentrations on percentage and speed germination of Quince seed.

Germination percentage %

CS	Concentration of GA_3 ppm 0	250	500	1000	x̄ CS
30 days	11^{e}	13^{e}	14^{e}	$16.^{e}$	13.5^{C}
60 days	80^{c}	84^{bc}	94^{a}	90^{ab}	83^{B}
90 days	70^{d}	$83.^{c}$	93^{a}	$86.^{bc}$	87^{A}
x̄ GA3	53.66^{C}	60^{B}	67^{A}	64^{A}	

LSD GA_3= 3.97 Interaction = 6.88 Stratification = 3.44

Germination speed

CS	Concentration of GA3 ppm 0	250	500	1000	x̄ CS
30 days	0.43^{g}	0.46^{g}	0.62^{f}	0.68^{f}	0.54^{C}
60 days	1.14^{e}	1.53^{d}	2.11^{b}	2.08^{b}	1.72^{B}
90 days	1.45^{d}	1.66^{c}	2.26^{a}	2.15^{b}	1.88^{A}
x̄ GA3	1.00^{C}	1.22^{B}	166^{A}	1.64^{A}	

LSD GA_3= 0.051 Interaction= 0.089 Stratification=0.044

Means not sharing the same letter(s) within each column, significantly different at 0.05 level of probability

Table 2 shows the results of seedling length and seedling diameter for Quince seedling. For seedling length (cm) when comparing between stratification period treatments, we found that stratification for 90 days had the highest value (37.35 cm) while stratification for 30 days had the lowest value (31.57 cm). Additionally, both concentrations of GA_3 at 1000 ppm had the highest value (37.53 cm). Moreover, the interaction between two treatments shows there were significant differences in terms of seedling length (cm). The results show that the treatments stratified 90 days with GA_3 at 500 ppm had the highest value of seedling length (39.9cm), but did not differ significantly between these two treatments. However, the stratification for the 30-day treatment without GA_3 had the lowest value of seedling length (28.6 cm). Also, Table 2 shows the Inverse relationship between seedling diameter (mm) with concentrations of GA_3, where seedlings that were not treated with GA_3 and stratified 60 days had the highest diameter (2.89).

Table (2): Effect of cold stratification (CS) and GA_3 concentrations on seedling length (cm) and seedling diameter (mm) of Quince seedling

Seedling length (cm)

(CS)	Concentration of GA_3 ppm 0	250	500	1000	x̄ CS
30 days	28.6^{g}	29.2^{g}	32.7^{f}	35.8^{de}	31.57^{C}
60 days	34.6^{e}	35.4^{de}	37.7^{bc}	38.1^{b}	36.45^{B}
90 days	34.4^{e}	36.4^{cd}	39.9^{a}	38.7^{ab}	37.35^{A}
x̄ GA_3	32.53^{C}	33.66^{B}	36.76^{A}	37.53^{A}	

LSD GA_3= 0.83 Interaction = 1.44 Stratification = 0.72

Seedling diameter (mm)

(CS)	Concentration of GA_3 ppm 0	250	500	1000	x̄ CS
30 days	2.24^{f}	2.13^{g}	2.11^{g}	2.02^{h}	2.13^{C}
60 days	2.89^{a}	2.63^{c}	2.40^{d}	2.37^{de}	2.57^{A}
90 days	2.85^{a}	2.72^{b}	2.31^{ef}	2.03^{h}	2.48^{B}
x̄ GA_3	2.66^{A}	2.49^{B}	2.27^{C}	2.14^{D}	

LSD GA_3= 0.039 Interaction = 0.068 Stratification = 0.034

Means not sharing the same letter(s) within each column, significantly different at 0.05 level of probability

In terms of the influence of treatments on leaf area index (Table 3) there was a significant impact for both main treatments (stratification and GA3 concentrations). Treatment stratification for 90 days with 1000 pm of GA_3 possesses the highest value (34. cm^2). Furthermore, there were differences between treatments in dry matter in seedlings. where stratification 90 days had the highest Values (55.90 %) while GA_3 1000 ppm treatment had the highest percentage of dry matter (56.17%) the treatments stratification 90 days with GA_3 at 1000 ppm had the highest in dry matter (57.54mm).

Table (3): Effect of cold stratification (CS) and GA_3 concentrations on leaf area index(cm2) and percentage of dry matter (%)of Quince seedling.

	Leaf area index(cm^2)				
	Concentration of GA_3 ppm				$\bar{x}$
CS	0	250	500	1000	CS
30 days	23.40^{f}	24.70^{ef}	26.20^{de}	27.10^{d}	25.35^{C}
60 days	25.10^{e}	27.50^{d}	31.10^{c}	32.00^{bc}	28.92^{B}
90 days	26.20^{de}	30.60^{c}	32.80^{ab}	34.40^{a}	31.00^{A}
$\bar{x}$ GA_3	24.90^{D}	27.60^{C}	30.03^{B}	31.16^{A}	
LSD	GA_3= 0.96	Interaction = 1.66	Stratification = 0.83		
Percentage of dry matter (%)					
	Concentration of GA_3 ppm				$\bar{x}$
CS	0	250	500	1000	CS
30 days	50.10^{i}	53.42^{gh}	54.67^{de}	54.58^{def}	53.19^{C}
60 days	52.84^{h}	53.63^{fgh}	54.72^{cd}	56.39^{bc}	54.40^{B}
90 days	53.91^{efg}	55.48^{cd}	56.65^{ab}	57.54^{a}	55.90^{A}
$\bar{x}$ GA_3	52.29^{D}	54.17^{C}	55.35^{B}	56.17^{A}	
LSD	GA_3= 0.57	Interaction = 0.99	Stratification = 0.49		

Means not sharing the same letter(s) within each column, significantly different at 0.05 level of probability

DISCUSSION

The results of the current study demonstrated that stratification for 60 and 90 days or concentrations of 500 and 1000 GA_3 had an obvious effect in terms of improving the speed and germination of seed. These results were explained previously by (Lewak 2011; Han & Yang ,2015; Hopkins & Gravatt 2019; wang et. al 2020 & Ge et. al, 2023). They reported that the effects of cold stratification on seed dormancy breaking occur when the level of abscisic acid (ABA) declines and the level of GAs increases due to cold stratification. Additionally, cold stratification broke embryonic dormancy in seeds by catabolizing lipids, sugars, and proteins with hydrolytic or proteolytic enzymes. Our results are also consistent with the findings of (Sondheimer et. al, 1968; Chen et. al., 2007; Finkelstein et. al, 2008; Leida et.al, 2012 & Ge et. al, 2023). They found that treating with cold stratification and GA_3 markedly increased the speed and germination of seed. The main hormone responsible for maintaining seed dormancy is abscisic acid (ABA). The ABA levels decrease significantly in seeds as the stratification time increases. Because seed dormancy is ultimately controlled by the balance between ABA and gibberellic acid (GA) a decrease in ABA would tip the hormonal balance toward GA and the allowance of germination after sufficient stratification (Guo et al., 2020; El-Yazal 2021 &Ge at el., 2023).

For seedling length (cm), the result shows that the treatments of stratification for 90 days with both GA_3 500 and 1000 ppm have a significant impact on seedling length comparing with other treatments. This confirms the findings by (Yamauchi, at el., 2004), where the cold stratification impacts gene involved in the biosynthesis of gibberellins, thus leading to an increase in the level of bioactive GAs and transcript abundance of GA-inducible genes (Yamauchi, at el., 2004; Chen, at el., 2008). Additionally, our results are consistent with those of (Nedunchezhiyan, at el., 2020) they found that the increase in GA content has an obvious effect in terms of improving the vegetative growth, such as the length of the stem. They reported that the main functions of gibberellins are to increase the intermodal length of the stem, resulting in a reduction in the dwarf stature of the plant.

Stratification 60 and 90 days without GA_3 had the highest value which was markedly and significantly different the rest of the treatments in the seedling diameter (mm). Moreover, the concentrations of GA_3 had an inverse relationship influence on this trait. This may be attributed to the nature of the action and effect of gibberellins, which leads to the division and elongation of cells, giving the highest rate of seedling length, thus having a smaller diameter compared to seedlings produced from untreated seeds. These results agree with what was obtained by (Samaan at el., 2000). Moreover, the treatments with stratification periods and concentrations of gibberellins have a pronounced influence on leaf area and dry matter (%), where stratification of 60 and 90 days with 500 and 1000 ppm GA had a distinct effect. These results agree with (Li at el., 2020; Castro - Camba at el., 2022), they reported that the concentrations of gibberellins play an important role in the enhancement of the efficiency of fruit crops in terms of growth and yield. Therefore, the exogenous applications of GA at different concentrations drastically increase stem elongation and shoot initiation, an addition to modifying several other vital processes in fruit crops.

CONCLUSION

The results of this study revealed that stratification for 60 or 90 days with 500 and 1000 GA_3 ppm had an obvious effect in terms of improving the germination of seed (94% and 93 % respectively). Additionally, it's had a great impact on all other traits, with the exception of the seedling diameter (mm) which revealed that 60 and 90 days of stratification without concentration of GA_3 had the highest value.

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Research Article Open Access

Study of variation patterns for body shape in four endemic mullet species (Mugilidae) from Umm-Hufayn Lagoon, on the Cyrenaica coast of Libya

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Abstract:: The study investigates the morphometric characteristics of four mullet species from the Umm-Hufayn lagoon on the Cyrenaica coast to analyze their morphomeristic variation and possible factors contributing to it. The morphometric analysis showed significant variation in the body shape of mullet species. The results of Discriminant Function Analysis (DFA) show significant variation to separate the four mullet species without any overlap. While Canonical Variate Analysis (CVA) shows the presented four distinct phenotypes differing by mullet species of body shape, Principal Component Analysis (PCA) revealed a wider distribution of body shape variations among the data points, indicating a greater range of body shapes within the sample population. The morphometric differences may be attributed to weight, size, or genetic differences. The study highlights the importance of geometric morphometric analysis to in identifying and differentiating the selected fish species in the same habitat.

Keywords: Mullet species, Umm-Hufayn Lagoon, goemetric morphometric, body shape.

دراسة أنماط التباين في شكل الجسم لأربعة أنواع من أسماك البوري المستوطنة (Mugilidae) في بحيرة أم الحفين في ساحل برقة الليبية

المستخلص : تتناول هذه الدراسة الخصائص المورفومترية لأربعة أنواع من أسماك البوري من بحيرة أم حفين في ساحل برقة، لتحليل تباينها المورفومرتي والعوامل المحتملة التي تساهم في ذلك .حيث أظهر التحليل المورفومتري وجود تباين كبير في شكل الجسم لأنواع البوري .أظهرت نتائج تحليل الوظيفة التمييزية (DFA) تباينًا كبيرًا في فصل أنواع البوري الأربعة دون أي تداخل .بينما يُظهر تحليل التباين القانوني (CVA) الأنماط الظاهرية الأربعة المميزة التي تختلف حسب أنواع البوري في شكل الجسم .تحليل المكونات الرئيسية (PCA)، يُظهر أنه يحتوي على تباين أكثر انتشارًا بين نقاط البيانات، مما يعني أن هناك المزيد من التباين في شكل الجسم .قد تعزى الاختلافات المورفومترية إلى الوزن أو الحجم أو الاختلافات الجينية .تسلط الدراسة الضوء على أهمية التحليل المورفومتري في تحديد وتمييز أنواع الأسماك المختارة في نفس الموطن

الكلمات المفتاحية : اسماك البوري، بحيرة ام حفين، دراسات الشكل الظاهري، ساحل برقة، الاراضي الرطبة.

INTRODUCTION

The diversity of body shapes among fish species often reflects their adaptations to specific habitats. However, the precise causes of these morphological variations remain challenging to elucidate. It is hypothesized that genetic makeup, environmental factors, and their interplay shape the morphological characteristics of fish (Rocamontes-Morales et al., 2021). Understanding body shape is essential for comprehending a species' ecology, life history, and evolutionary trajectory, as well as for comparing populations across different regions (Kitano, 2004). Additionally, patterns of morphometric variations in fishes can reveal observable differences in growth rates and developmental constraints as body form emerges from ontogeny (Azzurro et al., 2014).

Data from morphometric measurements can also be used to distinguish between fish populations. The environmental explanations of morphometric differences could contribute to the development of conservation strategies. Geometric morphometric methods have become increasingly popular in biological research, enabling the examination of diverse structures, from sperm cells to dinosaur skulls (MacLeod et al., 2013). Parallel to various other phenotypic approaches, this methodology allows for the measurement of a vast number of morphometric dimensions, facilitating the investigation of variation patterns. This enables the study of general morphometry, encompassing the overall shape of the organism or the shape of its individual features.

Geometric morphometric methods are useful for measuring thedifferences in phenotypes between closely related sympatric species that live in different ecological niches (Russo et al., 2008). These methods excel in exploratory studies, as a large number of landmarks can be used to capture the overall body shape rather than relying solely on traditional morphometric measurements. This approach provides detailed insights into subtle variations in specific body segments or traits and can serve as an 'early detection' method for investigating hybridization events.

A crucial objective of morphometrics in various biological contexts is to identify shape features that can aid in distinguishing between different groups or species. However, beyond distinguishing between populations or species, we may also seek to gain a comprehensive understanding of the structure of intraspecific variation among individuals in study areas. (Adams et al., 2004; Bookstein, 1991). In essence, morphometrics enables us to delve into the intricate patterns of shape variation within a species, providing valuable insights into population dynamics, evolutionary processes, and the influence of environmental factors on morphological traits. By analyzing shape variations, we can unravel the subtle distinctions that characterize individuals from different populations or geographic regions, shedding light on the intricate tapestry of biological diversity (Zelditch et al., 2012).

Mullet species exhibit uniform morphological characteristics that hinder identification, making it challenging to distinguish between species, as body shape undergoes modifications throughout their lifespan. However, knowledge regarding the genetic and phylogeographic population differentiation of Mullet species in Libyan waters remains somewhat limited.

Several studies have explored the morphological variations and geographic distribution of mullet species in the Mediterranean basin. One study by (El-Zaeem, 2011) examined the morphometric and meristic characteristics of *Mugil cephalus* and *Mugil ramada* from Tunisian lagoons and found significant differences between the two species in terms of body shape, fin ray counts, and meristic characters. Another study by (Cossu et al., 2021) investigated the genetic diversity of mullet species in the Black Sea and revealed distinct genetic groupings among the different species. Despite these advancements, a comprehensive understanding of the genetic and phylogeographic structure of mullet populations in Libyan waters remains elusive. Further research is needed to elucidate the genetic relationships between mullet species in the region and identify potential population boundaries. This information would be crucial for informing conservation and management strategies for these important fisheries resources.

The current study employed geometric morphometrics to investigate the body shape variations of four Mullet species collected along the Cyrenaica coast of Libya.

MATERIALS AND METHODS

Umm Hufayan Lagoon, Libya (32° 33' 13" N, 23° 05' 57" E) is a brackish lagoon located on the eastern coast of Libya (Fig.1). Samples of *Chelon aurata*, *C. labrosus*, *C. ramada*,and *M. cephalus* have been gathered from Umm-Hufayn Lagoon, collection sites in the Cyrenaica -Libya (Fig.1).Approximately 50 specimens of each mullet species were collected from the study area, resulting in a total sample size of 200 individuals. All fish specimens were transported in ice-filled plastic boxes to the laboratories of the College of Natural Resources and Environmental Sciences in Libya.

Geometric Morphometrics

To facilitate geometric morphometric analysis, 180 fish specimens were photographed from their left lateral side against a white backdrop using a digital camera mounted on a tripod at a fixed height and zoom level. Each specimen was placed on a ruler for scale reference. The fish were photographed individually and labeled according to their respective species. *Chelon aurata* (red), *C. ramada* (light blue*), M. cephalus* (dark blue) and *C. labrosu* (green).

The photographs underwent image analysis using the software tpsDIG 2.17 (Rohlf & Evolution, 2009). During this process, 34 landmarks were identified and digitized to represent homologous points on the fish's external shape. These landmarks were scaled to ensure that the variation in shape among the specimens was not too large for the application of statistical methods. The software tpsSMALL (Rohlf & Evolution, 2008; Rohlf, 2003) was employed to verify that the shape variation within the dataset was within the acceptable range for these statistical methods.

Figure (1). Map of Umm-Hufayn Lagoon sampling location

Principal Component Analysis (PCA) was employed to explore the patterns of variation in multiple variables within the sample. The statistical significance of shape differences between groups was evaluated using Discriminant Function Analysis (DFA) applied to the matrix of partial warps, with MorphoJ software serving as the computational tool. To pinpoint the shape features that most accurately differentiate between the groups being studied, we employed Canonical Variate Analysis (CVA) within the MorphoJ software. To ensure the reliability of these classifications, we conducted a leave-one-out cross-validation procedure, involving 1000 permutations.

RESULTS AND DISCUSSION

The Umm-Hufayn lagoon is home to a wide variety of plant and animal life, including many rare and threatened species, for example, *Anguilla anguilla*. It is also an important breeding and nursery ground for fish mullet species and other marine life. It provides a habitat for various birds, including water birds, migratory birds, and shorebirds.

Employing discriminant analysis, we compared the body shapes of the four mullet species. The analysis revealed that the discriminant function was highly effective in distinguishing between the four mullet species. When tested with 10,000 permutations, it successfully separated the species into distinct groups without any overlap. This clear separation is visually depicted in Figure 2. The discriminant scores were calculated in such a way that the threshold for classification into either group was set at a value of zero.

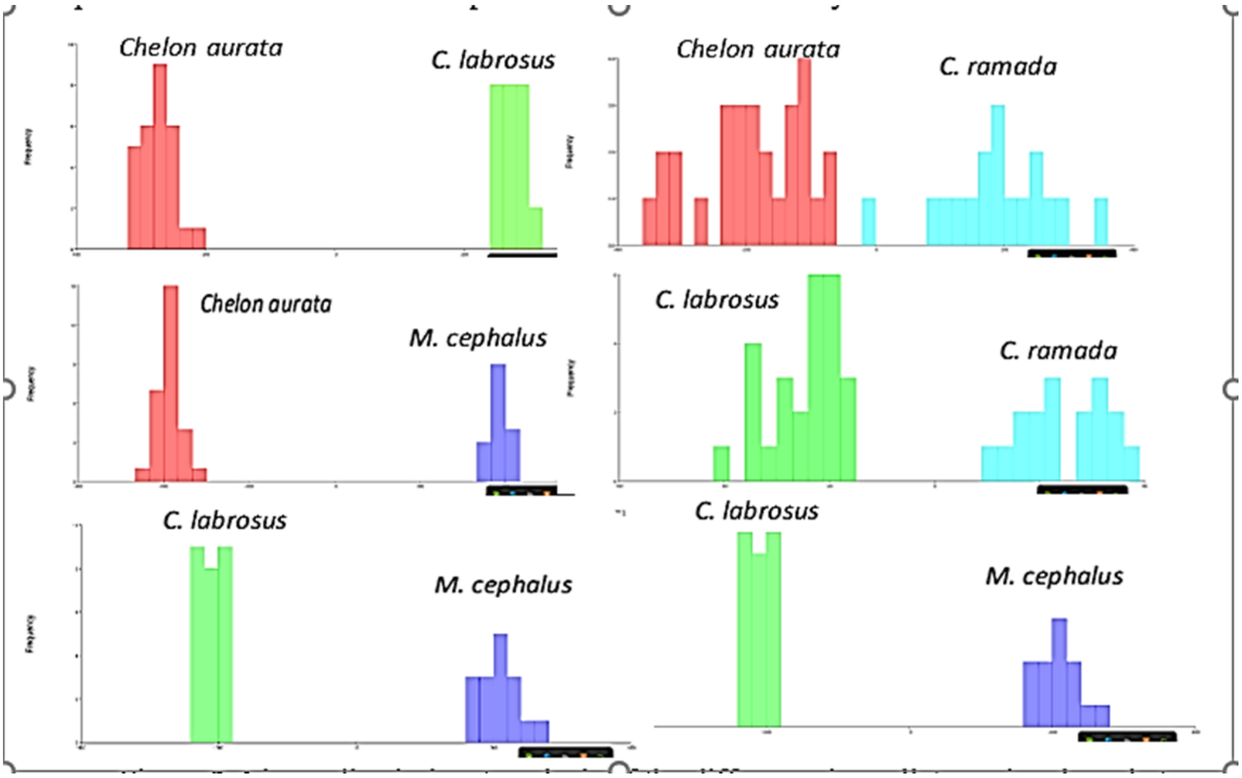

Figure (2). Linear discriminant analysis of the difference in mullet species shape between *Chelon aurata* (Red), *C. ramada* (Light-Blue), *M. cephalus* (Dark-Blue) and *C. labrosus*(Green) combining the study areas.

The Canonical Variate Analysis of *Chelon aurata* (red), *C. ramada* (light-blue), *M. cephalus* (dark-blue) and *C. labrosu* (green) from Umm-Hufayn Lagoon presented four distinct phenotypes or forms differing by their body shape. This demonstrates a high level of morphological differentiation between the four Mullet species forms (Fig.3).

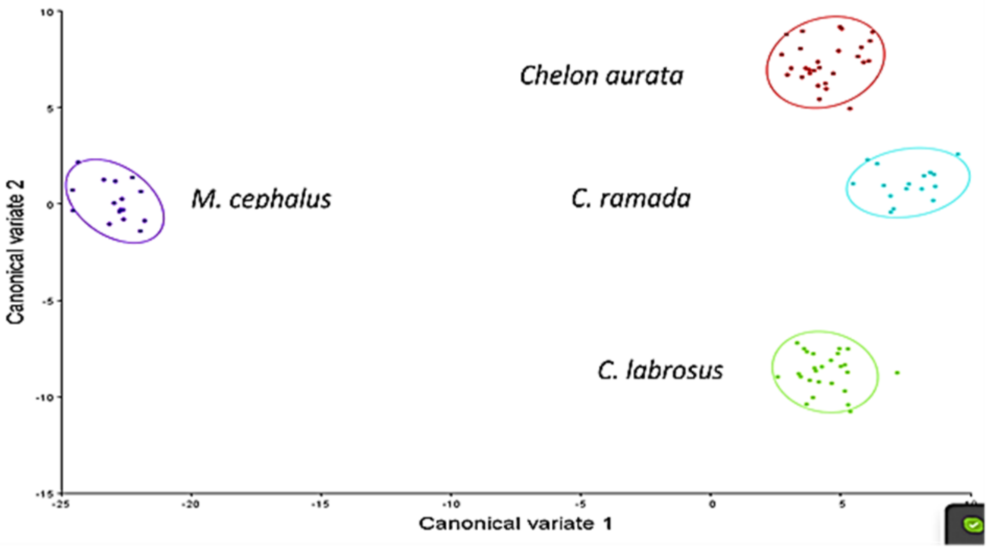

Figure (3). Scatter plot of the first two canonical variates for *Chelon aurata* (Red), *C. ramada* (Light-Blue), *M. cephalus* (Dark-Blue) and *C. labrosus* (Green).

In the mullet species, we used thin-plate splines to create deformation grids that showed how the body shape of the four mullets varied between different species. The deformation grids showed that the different species had different proportions for body shape, with some populations having longer bodies and others having wider bodies (Fig.4). Thin-plate splines are a powerful tool for visualizing variation in body shape. They can be used to visualize the variations in body shape of any organism, and they can be used to identify the factors that contribute to this variation.

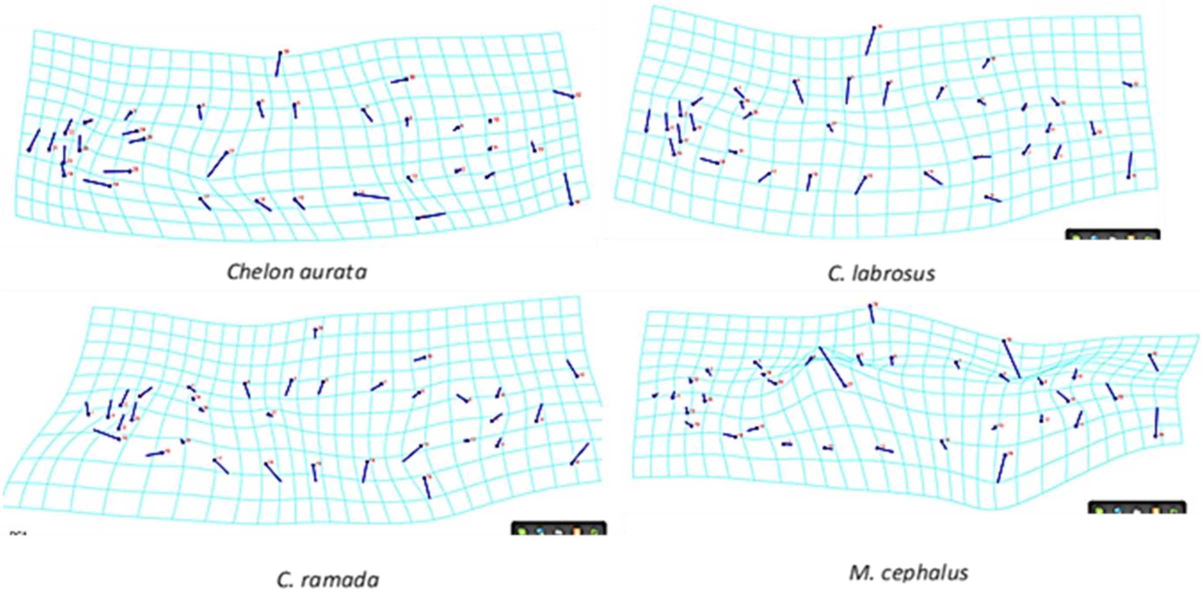

Figure (4). Grids of relative warps with the average shape of *Chelon aurata,C. ramada, M. cephalus and C. labrosus* specimens obtained from 34 landmarks from study area.

The spread of data points for *Chelon labrosus* (green dots) is more extensive, indicating greater variation in body shape. *Chelon aurata* (red), *Liza ramada* (light blue), and *Mugil cephalus* (dark blue) have a more compact distribution of data points compared to *Chelon labrosus*, implying less shape variation. The Principal Component Analysis (PCA) revealed a slight overlap in morphology between *Chelon aurata*, *Liza ramada, and Mugil cephalus* (Fig. 5).The findings of this study align with previous research conducted by (Chien et al., 2018), which demonstrated strong correlations between whole-body growth and various morphometric and meristic characteristics in mullet species from the Karachi coast of Pakistan. This study reinforces the notion that body shape can serve as a proxy for growth and development in mullets.

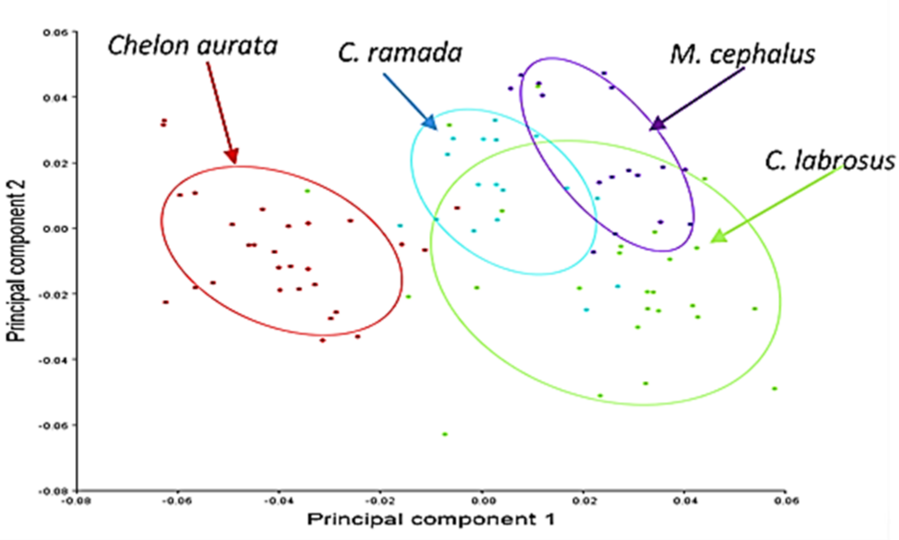

Figure (5). Principal component analyses of morphometric 34 landmarks of *Chelon aurata* (Red), *C. ramada* (Light-Blue), *M. cephalus* (Dark-Blue) and *C. labrosus* (Green).

The effects of habitat on body shape diversification in mullets are complex and vary depending on the specific species and the environment. While (Friedman et al., 2022) examined the effects of habitat on body shape diversification in marine teleost fishes and found that benthic living increases the rate of body shape evolution, leading to extreme body shapes such as wide bodies and elongate forms. Furthermore, the effects of habitat on body shape diversification in mullets are still being studied, but the research that has been done so far suggests that habitat can have a significant impact on the morphology of these fish.

Wetlands can affect the body shape of many fish species, and in Umm-Hufayn lagoon, mullet fish are no exception. The specific effects of wetlands on fish body shape will vary depending on the species of fish and the characteristics of the wetland. The study of how wetlands affect fish body shape is a growing field of research. This research is important for understanding how wetlands function and how they can be managed to protect fish populations.

(Ibañez et al., 2007) discovered that variations in fish scale morphology can effectively distinguish between the congeneric species *Mugil cephalus* and *Mugil curema*, which are distinct from other members of the Mugilidae family. The variation of fish scale morphology to between populations is influenced by their geographic proximity and habitat similarity; it is least effective for populations from nearby areas, improves for populations that are more geographically dispersed, and is most effective for distinguishing between species and genera. Where the level of similarity of habitats and geographical proximity influences morphology divergence among populations and the genetic diversity within populations.

(Asmamaw et al., 2021) reported that the biotic and abiotic factors and genetic structure interactions are believed to be the main cause of the observed morphometric variations, but we suggest that the genetic structure is the main cause of the observed morphometric variations in body shape of mullet species in Umm-Hufayn lagoon because the samples were collected from the same environment, so the biotic and abiotic factors demonstrated a negligible effect on the morphometric variables of mullet species in Umm-Hufayn lagoon.

CONCLUSION

The requirement for correctly estimating population trends, productivity, and fish stocks is to accurately identify fish species, which in turn helps define management strategies for fisheries. In the absence of fisheries data on the Libyan coast, this could will be the primary database; hence, additional studies would be welcome to make more morphological distinctions to estimate population trends for stock fisheries on the Libyan coast.

The current findings provide new tools for studying phenotypes, but the fish of the Libyan coast are still poorly described from a taxonomic standpoint. Future research on the biology and ecology of Umm-Hufayn Lagoon is required. This has additional implications for marine biodiversity conservation in wetlands.

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ETHICS

Author contributions Abdulghani as an author to the methodology section , performed the analytic calculation , Data curation and writing of original draft .Fatma and Sara writing –Review &Editing and Supervision . Fatma Supervised the study.

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

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Research Article **Open Access**

Estimating Reference Evapotranspiration for Shahat Region in Libya Using Genetic Programming

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Abstract: This study was conducted to estimate the reference evapotranspiration (ETo) for Shahat region in Libya using the genetic programming (GP) model compared to the FAO Penman-Monteith equation (FPM56). The climatic data of Shahat Meteorological Station was used for the period from 1963 to 1999. Six different combinations of available meteorological variables were used, such as the average air temperature (T_{mean}), the average relative humidity (RH_{mean}), and the extraterrestrial radiation (R_a). The latter is calculated as a function of the location and time during the year. The GP model was trained using 70% of the climatic data and tested using the remaining 30%. The values of the statistical indicators obtained in this study showed that the root mean square error (RMSE), coefficient of determination (R^2), and Nash-Sutcliffe coefficient of efficiency (NSE) ranged between 0.26 and 0.98 (mm.day^{-1}); 0.67 and 0.98; 0.66 and 0.98, respectively during the testing period. Therefore, GP models represent a great option to estimate ETo, when climatic data are scarce.

Keywords: Reference Evapotranspiration, Genetic Programming, FAO Penman-Monteith Equation, Shahat Region.

تقدير البخر نتح المرجعي لمنطقة شحات في ليبيا باستخدام البرمجة الجينية

المستخلص: أجريت هذه الدراسة لتقدير البخر نتح المرجعي (ETo) لمنطقة شحات في ليبيا باستخدام البرمجة الجينية (GP) مقارنة بمعادلة الفاو بنمان مونتيث (FPM56). حيث تم استخدام البيانات المناخية لمحطة أرصاد شحات للفترة من عام 1963 وحتى عام 1999. تم استخدام ستة تركيبات مختلفة من متغيرات الأرصاد الجوية المتاحة مثل متوسط درجة حرارة الهواء(T_{mean})، ومتوسط الرطوبة النسبية (RH_{mean})، والإشعاع الشمسي فوق الغلاف الجوي (R_a)، هذا الأخير يتم حسابه بمعرفة الموقع والتوقيت خلال السنة. تم تدريب نموذج (GP) باستخدام 70% من البيانات المناخية وتم اختباره باستخدام 30% المتبقية. بينت قيم المؤشرات الإحصائية التي تم الحصول عليها في هذه الدراسة أن الجذر التربيعي لمتوسط مربع الخطأ ومعامل التحديد ومعامل ناش–ساتكليف للكفاءة تراوحت بين 0.26 و0.98 (ملم. يوم$^{-1}$)؛ 0.67 و0.98؛ 0.66 و0.98 على التوالي خلال فترة الاختبار. لذلك تمثل نماذج (GP) خياراً رائعاً لتقدير (ETo)عندما تكون البيانات المناخية شحيحة.

الكلمات المفتاحية: البخر نتح المرجعي، البرمجة الجينية، معادلة الفاو بنمان مونتيث، معادلة الفاو بنمان مونتيث، منطقة شحات.

INTRODUCTION

Evapotranspiration (ET) is an essential hydrological component for the sustainable and efficient management of agricultural water resources, optimum irrigation scheduling, hydrologic water balance and water resources planning and management (Huang et al., 2019; Wu et al., 2019; Yamaç & Todorovic, 2020).

The so-called reference evapotranspiration, denoted as ETo. The reference surface is a hypothetical grass reference crop with an assumed crop height of 0.12 m, a fixed surface resistance of 70 $s.m^{-1}$ and an albedo of 0.23. The reference surface closely resembles an extensive surface of green, well-watered grass of uniform height, actively growing and completely shading the ground (Allen et al., 1998).

Reference evapotranspiration (ETo) is measured by techniques and relatively complex physical principles (Allen et al., 2011), and the most direct and accurate way to estimate it is by water balance in the soil using lysimeters. However, due to limitations associated with the method, the adoption of physical mathematical models has become a practical alternative to ETo estimation.

The FAO-56 Penman-Monteith (FPM56) equation is recommended by the Food and Agriculture Organization (FAO) of the United Nations as the sole standard method for the definition and computation of the reference evapotranspiration (Allen et al., 1998). The FPM56 equation requires air temperature, air humidity, wind speed, and radiation data. These elements are often not available due to the small number of weather stations available in many regions, and when these are present, they may contain insufficient data.

There has been substantial research in recent years focusing on the estimation and prediction of natural phenomena, including the estimation of ETo using machine learning models, e.g., artificial neural network (ANN), fuzzy logic (FIS), genetic programming (GP), multivariate adaptive regression splines (MARS), decision tree (DT), random forests (RFs), support vector machine (SVM), extreme learning machine (ELM), and adaptive neuro-fuzzy inference system (ANFIS) (Adamala et al., 2019) ,(Adeloye et al., 2012) (Chia et al., 2020),(Egipto et al., 2023),(Spontoni et al., 2023),(Raza et al., 2023),(Liu et al., 2022).

A review of the literature shows that applications of GP for modeling evapotranspiration are limited. The study of (Guven et al., 2008) applied GP for modeling daily reference evapotranspiration as a function of solar radiation, mean air temperature, wind speed and relative humidity, and compared the performance of this model with other ETo equations. They found quite satisfactory results and it can be used as an alternative to the conventional models.

The current study is an attempt to develop a genetic programming model based on different combinations of available meteorological variables such as mean air temperature, relative humidity, and extraterrestrial radiation for predicting the ETo at Shahat, Libya.

MATERIALS AND METHODS

The reference evapotranspiration (ETo) in this study was calculated using data from Shahat meteorological station located at the longitude of 21° 51′E, the latitude of 32° 49′N, and mean altitude is 621 meters above sea level. The historical data series includes average monthly maximum (T_{max}), minimum (T_{min}) and mean air temperature (T_{mean})(°C), mean relative humidity (RH_{mean}) (%), and wind speed (U_2) ($m.s^{-1}$), which covered the period from 1963 to 1999. Table (1) shows the statistical parameters of meteorological variables at Shahat weather station.

Table: (1). Statistical parameters of meteorological variables at Shahat weather station

Parameter	T_{max} (°C)	T_{min} (°C)	T_{mean} (°C)	RH_{mean} (%)	U_2 ($m.s^{-1}$)	Sun (hr)
Mean	20.9	12.3	16.6	67.8	4.7	8.0
Standard Error	0.3	0.2	0.2	0.4	0.1	0.1
Standard Deviation	5.9	4.7	5.2	9.3	1.6	2.5
Range	22.1	17.9	19.8	50.0	8.0	11.1
Maximum	31.1	21.2	25.9	89.0	10.0	13.0
Minimum	9.0	3.3	6.2	39.0	2.1	1.9
Count	444	444	444	444	444	444

The REF-ET version 4.1 program (Allen, 2000) was used to calculate the reference evapotranspiration ETo using the Penman-Monteith equation recommended by the Food and Agriculture Organization (FAO) in Bulletin 56. (Allen et al., 1998). This Equation takes the form:

$$ET_o = \frac{\left[0.408 \times \Delta(R_n - G) + \gamma\left(\frac{900}{T + 273} U_2 (e_s - e_a)\right)\right]}{\Delta + \gamma(1 + 0.34\, U_{02})} \qquad (1)$$

Where:
ET_o : is the reference evapotranspiration [$mm.day^{-1}$];
R_n : is the net radiation at the crop surface [$MJ\ m^{-2}\ day^{-1}$];
G : is the soil heat flux density [$MJ\ m^{-2}\ day^{-1}$];
T : is the mean daily air temperature at 2 m height [°C];
U_2 : is the wind speed at 2 m height [$m.s^{-1}$];
e_s : is the saturation vapour pressure [kPa];
e_a : is the actual vapour pressure [kPa];
$e_s - e_a$: is the saturation vapour pressure deficit [kPa];
Δ : is the slope vapour pressure curve [$kPa.°C^{-1}$]; and
γ : is the psychrometric constant [$kPa.°C^{-1}$]

The extraterrestrial solar radiation (R_a) is not measured data but estimated for a certain day and location. One of the outputs of the REF-ET model version 4.1 is extraterrestrial radiation (Allen, 2000). The extraterrestrial radiation, for each day of the year and different latitudes can be estimated from the solar constant, the solar declination, and the time of the year by:

$$R_a = \frac{24\,(60)}{\pi} G_{sc}\, d_r \left[\omega_s \sin(\varphi)\sin(\delta) + \cos(\varphi)\cos(\delta)\sin(\omega_{s)}\right] \qquad (2)$$

Where:
R_a: Extraterrestrial radiation [$MJ\ m^{-2}\ day^{-1}$],
G_{sc} : Solar constant = 0.0820 [$MJ\ m^{-2}\ min^{-1}$],
d_r : Inverse relative Earth-Sun,

ω_s : Sunset hour angle [rad],
φ : Latitude [rad],
δ : Solar declination [rad],
In the northern hemisphere, the latitude, φ, is expressed as a positive value in radians, while in the southern hemisphere, it is expressed as a negative value. The remaining variables in equation (2) can be calculated using the method outlined by (Allen et al., 1998) as follows:

$$d_r = 1 + 0.033\cos\left(\frac{2\pi}{365}\mathrm{J}\right) \qquad (3)$$

$$\delta = 0.409\,\sin\left(\frac{2\pi}{365}\mathrm{J} - 1.39\right) \qquad (4)$$

$$\omega_s = \arccos[-\tan(\varphi)\tan(\delta)] \qquad (5)$$

where J represents the day number in the year, ranging from 1 (1st January) to 365 or 366 (31st December).

Genetic programming

Genetic programming (GP) is a type of evolutionary algorithm (EA) that was introduced by (Koza, 1992). It is based on the principles of natural selection and genetics. GP is a relatively recent addition to the family of EAs, which includes evolutionary programming (Fogel et al., 1966), genetic algorithms (Holland, 1975), and evolution strategies (Schwefel, 1981).

Genetic symbolic regression operates with two sets of variables, namely the functional set and the terminal set (Koza, 1994).

In this study, Genetic programming models were calculated based on the steps mentioned in a field guide to Genetic Programming (Poli et al., 2008), using the open-source program GPdotnet5.1.2 developed by (Hrnjica, 2018).

The steps followed can also be summarized as follows:
1. Determine the external terminals, which are the independent variables, such as (T_{mean}, RH_{mean}, Ra), and the internal terminals, represented by the functions (addition, subtraction, multiplication, division).
2. Determine the fitness function through which strong solutions are selected and weak solutions are excluded.
3. Determine the parameters used in the analysis, such as population size, crossing over, mutation, reproduction, number of constants, and starting method, as shown in Table (2).
4. Determine the stopping point of the program that was achieved after the program reached 500 generations, where the best fitness has not changed more since generation 300 and the execution procedures can be summarized as follows:

1. Randomly initiate populations.
2. Evaluate the fitness of the population
3. Iterate until the solution convergence:
    a. Choose parents from the population:
    b. Generate a new population through crossover.
    c. Apply mutation to the new population.
    d. Compute the fitness of the new population.

The function set consisted of addition, subtraction, multiplication, and division. The data was divided into two parts: 70% of the data for training and 30% for testing. The data was also normalized using the Min-Max Normalization method. The statistical parameters of the climatic variables used in this study are shown in Table (3), and the used models for several scenarios are shown in Table (4).

Table: (2). Genetic programming parameters:

GP parameter		Value
Population	Size	500
	Fitness	Root Mean Square Error (RMSE)
	Initialization	Half and Half
Selection	Elitism	20
	Method	Rank selection
Probability of GP operations	Crossover	0.9
	Mutation	0.05
	Reproduction	0.2
Random constant	Interval	0-1
	Number of random constants	5

Table: (3). Statistical parameters of the climatic variables

Statistical parameters	Climatic Variables		
	T_{mean} (°C)	RH_{mean} (%)	R_a (MJ m^{-2} day^{-1})
Training processes			
Maximum	25.2	89	41.46
Minimum	6.2	39	17.99
Mean	16.44	68.05	30.66
Standard Deviation	5.11	9.47	8.38
Count	311	311	311
Testing processes			
Maximum	25.9	83	41.46
Minimum	7.7	43	17.99
Mean	16.99	67.27	30.53
Standard Deviation	5.57	8.93	8.45
Count	133	133	133

Table: (4). GP models scenarios

Model	Input variables		
	T_{mean}	RH_{mean}	R_a
GP1	✓		
GP2	✓		✓
GP3		✓	
GP4		✓	✓
GP5	✓	✓	
GP6	✓	✓	✓

Performance criteria

Three performance indicators were used to evaluate the model: root mean square error (RMSE)، coefficient of determination (R^2) (Kennedy & Neville, 1986), and Nash-Sutcliffe efficiency (NSE) (Nash & Sutcliffe, 1970), between ET_o using FPM56 and predicted values using GP model. These statistics parameters are defined as follow:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(ET_{GP} - ET_{FPM56})^2}{n}} \qquad (6)$$

$$R^2 = \frac{\left[\sum_{i=1}^{n}\left(ET_{GP} - \overline{ET}_{GP}\right)\left(ET_{FPM56} - \overline{ET}_{FPM56}\right)\right]^2}{\sum_{i=1}^{n}\left(ET_{GP} - \overline{ET}_{GP}\right)^2 \sum_{i=1}^{n}\left(ET_{FPM56} - \overline{ET}_{FPM56}\right)^2} \qquad (7)$$

$$\mathrm{NSE} = 1 - \frac{\left[\sum_{i=1}^{n}\left(ET_{FPM56} - ET_{GP}\right)\right]^2}{\sum_{i=1}^{n}\left(ET_{FPM56} - \overline{ET}_{FPM56}\right)^2} \qquad (8)$$

Smaller values of RMSE and higher values of R^2 indicates higher model performance. The Nash-Sutcliffe (NSE) efficiency is used to evaluate the predictive power of the model and varies from -∞ to 1, with 1 being the perfect fit between the data estimated by the model and the measured data.

Where:
ET_{FPM56} : FPM56 , ($mm.day^{-1}$),
ET_{GP} : Predicted evapotranspiration, ($mm.day^{-1}$),
$\overline{ET}_{FPM56}$: Average FPM56, ($mm.day^{-1}$),
$\overline{ET}_{GP}$: Average predicted evapotranspiration, ($mm.day^{-1}$),
n : Total number of samples.

RESULTS AND DISCUSSION:

Table (1) demonstrates the values of the statistical criteria used in this study. From these results, it can be clearly seen that when using only (T_{mean}) as input to the genetic programming model, which is referred to here as GP1, the values of RMSE and R^2 were equal to 0.77 and 0.81 respectively. Figure (3) illustrates the scatter plot of predicted ETo values by the GP1 model, compared with FPM56 during testing. When added (R_a) to GP1, which is known here as GP2, it significantly increased the performance. The RMSE decreased from 0.77 to 0.46, by 40% and R^2 increased from 0.81 to 0.93, by approximately 15%. Figure (4) illustrates the scatter plot of this relationship.

In GP3, only (RH_{mean}) was used, we notice that the model performs poorly, where RMSE increased to 0.98, and R^2 decreased to 0.67. Figure (5) illustrates the scatter plot of this relationship. GP4 added (R_a) and performed better than GP3. The performance of this model is almost equal to the performance of GP2. Figure (6) illustrates the scatter plot of this relationship.

The results improved significantly when using (T_{mean}), (RH_{mean}) and (R_a) as inputs to GP6. Figure (8) shows the scatter plot of predicted ETo values by the GP6 model, compared with FPM56 during testing. Furthermore, it can be seen from Table (5) that GP6 outperformed the other models in all performance parameters. GP6 was ranked best in the testing process. These results are in accordance with (Liu et al., 2022),(Egipto et al., 2023; Raza et al., 2023) who also indicated that machine learning models represent a great option to estimate ETo.

Table: (5). Performance criteria of the GP models during training and Testing

Model	Input variables	Training			Testing		
		RMSE ($mm.day^{-1}$)	R^2	NSE	RMSE ($mm.day^{-1}$)	R^2	NSE
GP1	T_{mean}	0.84	0.75	0.75	0.77	0.81	0.79
GP2	T_{mean}, R_a	0.53	0.90	0.90	0.46	0.93	0.93
GP3	RH_{mean}	0.93	0.69	0.69	0.98	0.67	0.66
GP4	RH_{mean}, R_a	0.51	0.91	0.91	0.55	0.90	0.89
GP5	T_{mean}, RH_{mean}	0.43	0.94	0.93	0.49	0.93	0.92
GP6	T_{mean}, RH_{mean}, R_a	0.37	0.96	0.95	0.26	0.98	0.98

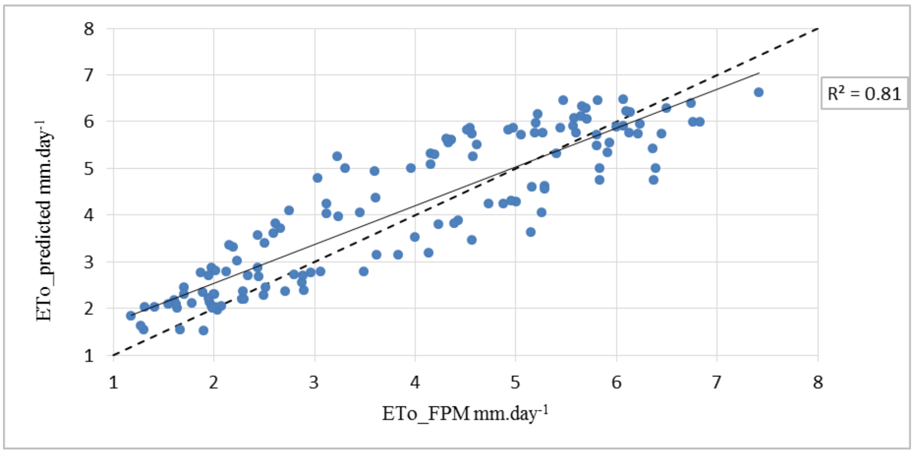

Figure: (3). scatter plot of predicted ETo values by the GP1 model, compared with FPM56 during testing.

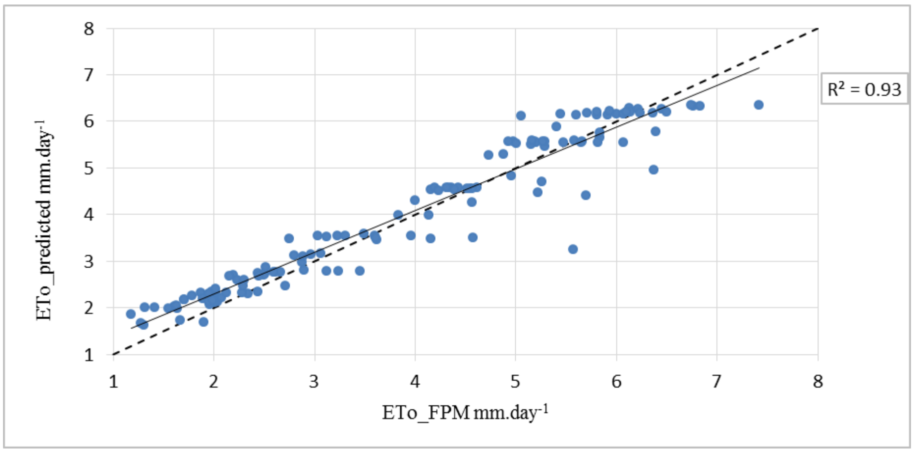

Figure: (4). scatter plot of predicted ETo values by the GP2 model, compared with FPM56 during testing.

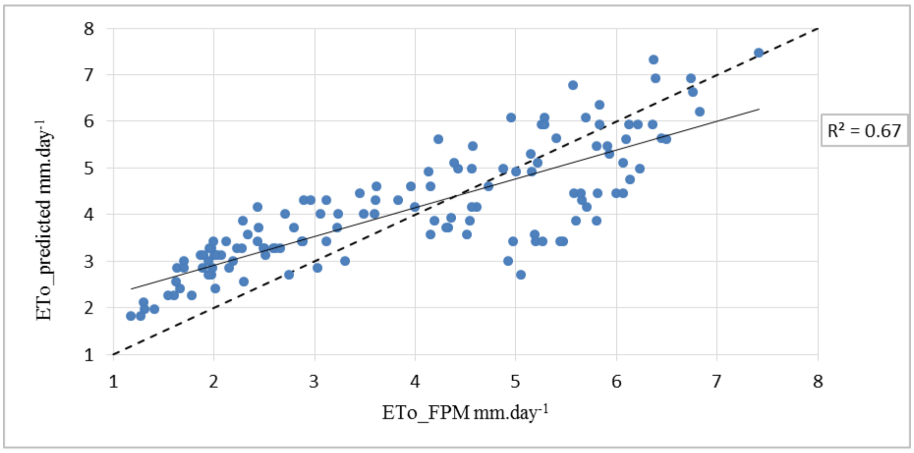

Figure (5). scatter plot of predicted ETo values by the GP3 model, compared with FPM56 during testing.

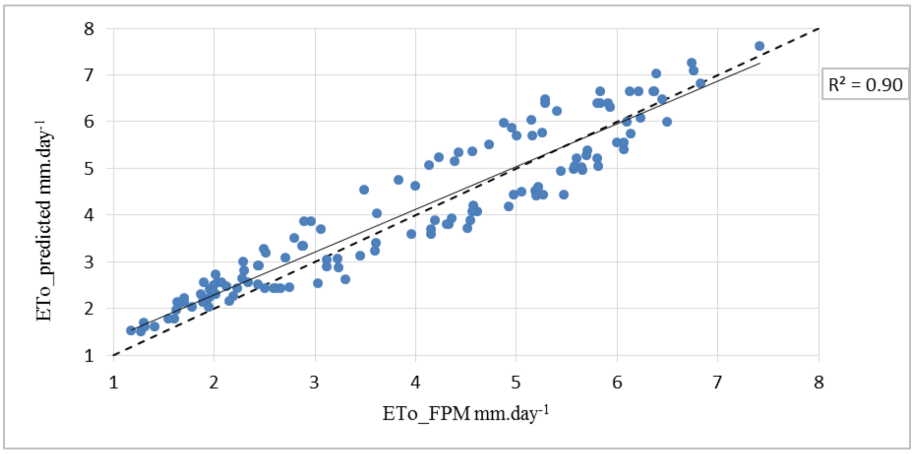

Figure: (6). scatter plot of predicted ETo values by the GP4 model, compared with FPM56 during testing.

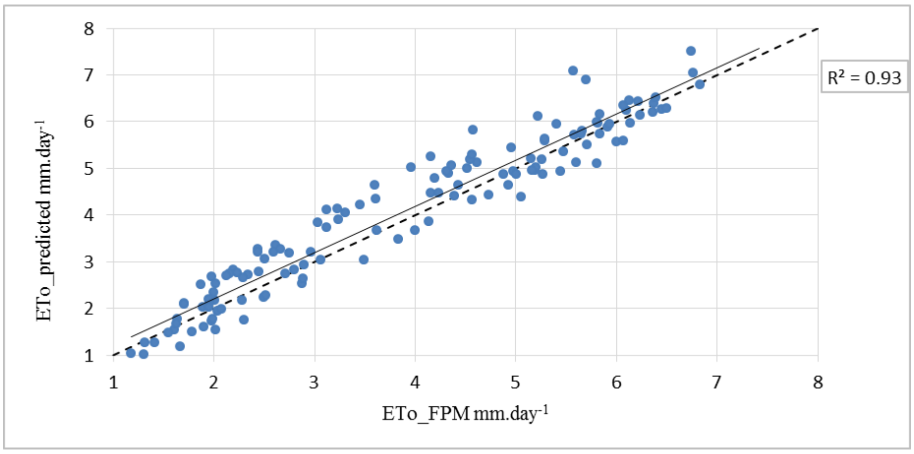

Figure: (7). scatter plot of predicted ETo values by the GP5 model, compared with FPM56 during testing.

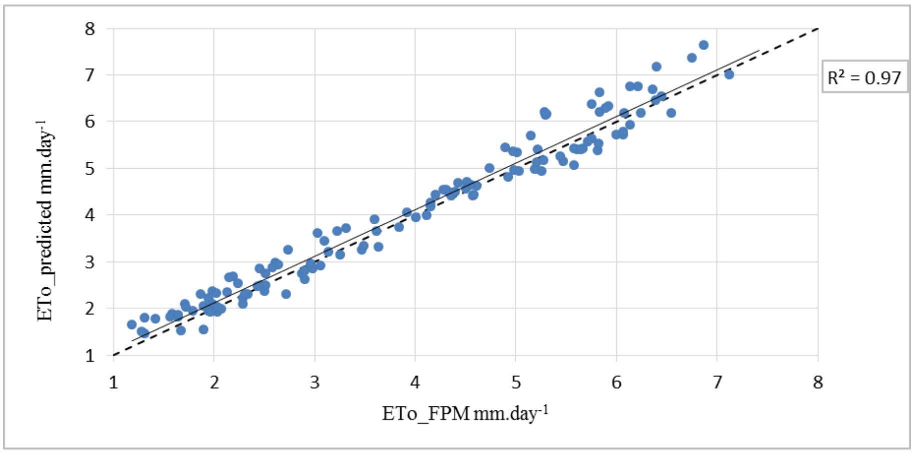

Figure: (8). scatter plot of predicted ETo values by the GP6 model, compared with FPM56 during testing.

CONCLUSION

From the results obtained in this study, It can be concluded that the performance of the GP model is a promising approach and a powerful tool that can be used to calculate reference evapotranspiration when using (T_{mean}), (RH_{mean}) and (R_a) as inputs, especially under the deficiency of complete meteorological data required for the Penman-Monteith equation recommended by Food and Agriculture Organization to calculate the reference evapotranspiration.

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Research Article **Open Access**

Fauna Checklists of Al-Abiar Zone (Zaza, Jeera and Takes) Cyrenaica province – Libya

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Abstract: The annotated checklist, meticulously crafted through a blend of extensive literature review, the author's keen observations, and the wisdom of local communities, unveils the 36 species representing 29 families. The checklist delves into their geographical distribution, mapping their footprints across the zone. This comprehensive picture allows us to understand the delicate dance between each species and its preferred habitat. However, anthropogenic pressure plays a critical role in shaping the distribution and population density of the zone's fauna. It serves as a potent reminder of the urgent need for conservation efforts. The checklist lays the groundwork for identifying and safeguarding crucial habitats and threatened species, ensuring that the symphony of life in this corner of the world continues to resonate for generations to come.

Keywords: Checklists, Fauna, Cyrenaica province, Libya

قائمة الثروة الحيوانية في منطقة الابيار (زازة– جيرة – تاكنس) برقة – ليبيا

المستخلص: تكشف القائمة المرجعية لمنطقة الدراسة، التي تم إعدادها بدقة من خلال مزج الدراسات السابقة من المراجع، والزيارات الميدانية والملاحظات المباشرة للمؤلفين، وملاحظات المجتمعات المحلية، عن 36 نوعًا تمثل 29 عائلة. وتتناول القائمة المرجعية توزيعهم الجغرافي، وتسجل تواجدها في منطقة الدراسة. تتيح لنا هذه الصورة الشاملة فهم العلاقة الدقيقة بين كل نوع البيئات التي تعيش فيها. ومع ذلك، يلعب النشاط البشري دورًا حاسمًا في تشكيل التوزيع والكثافة الانواع للحيوانات في المنطقة. إنه بمثابة تذكير قوي بالحاجة الملحة لجهود الحفظ التنوع الحيوي البري في برقه. تضع القائمة المرجعية الأساس لتحديد وحماية الموائل الحيوية والأنواع المهددة بالانقراض، مما يضمن استمرار صدى سيمفونية الحياة في هذا الركن من العالم للأجيال القادمة.

الكلمات المفتاحية: قائمة الحيوانات البرية. برقة – ليبيا.

INTRODUCTION

The concept of biodiversity includes all biogeography, habitat ecology, legislation and regulation of sustainable development for biodiversity. While the term wildlife includes all wild birds, mammals and reptiles. it is concerned with wildlife management (Caughley, 1994).

Approximately 320 mammal species are native to the Mediterranean basin countries. The majority of mammal species are small volant and non-volant mammals, for example, but not limited to (rodents, bats, shrews, hedgehogs and moles). The Muridae family, which includes rats and mice, is the largest group of mammals in the region. Only 297 of these mammal species have been evaluated, and more than 16% of them, or around 49 species, are currently threatened with extinction. Ungulates, primates, carnivores, and lagomorphs (rabbits and hares) are the groups of mammals with the highest rates of endangerment (Gippoliti, Amori, Castiglia, Colangelo, & Capanna, 2014; Hoffmann *et al.,* 2010) .

Out of the 297 mammal species that have been assessed in the Mediterranean basin, 16.5% are considered threatened, according to (Vié, Hilton-Taylor, & Stuart, 2009). This includes 3% that are critically endangered, 5% that are endangered, and 8% that are vulnerable. An additional 8% of the species are considered near threatened, and 12% are considered data deficient. Seven species, including the lion (*Panthera leo*) and the tiger (*P. tigris*), have been extirpated from the region.

As the report indicated that the terrestrial mammal biodiversity is higher in mountainous parts of the regions in Mediterranean countries. Of the 49 threatened species of Mediterranean mammals, 20 (41%) are unique to the region. As the report noted, the endemic species richness is particularly high in the Maghreb, although the Iberian and Italian peninsulas also hold important concentrations of endemic species, as do the Mediterranean islands.

The first attempts to document Libyan mammals date back to the recent decade, where an Italian researcher (Fraser, 1844) published a list of birds. Studies on Libyan birds continued by (Ghigi, 1913; MoLTONI, 1935; Salvadori, 1922; Giuseppe Scortecci, 1934; Giuseppe Scortecci, 1935); listed , the 38 terrestrial reptile species known in Libya and,(Zavattari, 1937) noted, the 56 species and subspecies.(Al-Awami, 1973) argue that there are not many studies on wildlife vertebrates compared with neighboring countries. The Center for the Studies of Arid Zones and Dry Lands (Dougrameji, 1975) indicated the presence of 26 species of mammals in the AL-Jabal AL-Akhdar zone in particular.

It was also indicated in the latest study (Elkahwage & Jdeidi, 2018) that included a categorical study of mammals in the Msallata National Park Reserve, where the presence of 14 species of mammals was recorded (Wudl, Wobschall, & Hufnagel, 1972).(A. M. Bauer, DeBoer, & Taylor, 2017) collected the Libyan reptiles, where they collected local data from 3350 museum specimens and 163 scientific sources, which resulted in 683 unique sites that were geographically identified and used to create a lexicon. Geographic and maps as well as species maps for each of the 66 reptile species confirmed to exist in Libya. The known reptile fauna of Libya is one of the poorest on the African continent (A. Bauer, 1993) with only 63 terrestrial species recognized.(El Harer, 2014) stated that the fauna in Libya is composed of (65) mammal species and Aves (343) species, While, (A. M. Bauer et al., 2017) stated reptelia are composed of 66 in Libya.

The last comprehensive review was by (Rosendal & Schei, 2012), according to this review, there are no accurate surveys of biological diversity in Libya. In addition, there have been, since 2011, significant human activities in some parts of Libya that have had an impact on biological diversity. Because of this, this report is no longer reliable, and it's crucial that reports be updated to give a more accurate picture. The fourth report provides the most important species of wild animals that are vulnerable to extinction, and indicates that there are 455 vertebrate species and 3,958 invertebrate species. The report also indicates that mammals are one of the most sensitive groups in Libya and that this group has suffered many extinctions.

Location and physiography

The Libya is located in the north of Africa, from 20 to 34° N and 10 to 25° E, stretching on the Mediterranean coast in the north over 2000 Km, and bounded from the east by Egypt and from the west by Tunisia(Agency, 2004) .The total area of Libya is about 1.76 Million km2, more than 95% of Libya is desert due to continental tropical air's year-round dominance (Nelson, 1979) . Little information is still available in recent years about the eco-diversity contexts in Libya (Stokes *et al.*, 2015).

The study area is located on the eastern coast of the Mediterranean Sea in the north-east of Libya on the first terrace, from the Al-Rajma area in the west of the study area to the south of the Tokra area, at a distance of approximately 30 km, on the Mediterranean coast. The study area is located from the plateau southeast of Al-Rajma (N 32.083068 and E 20.350277) to the plain northeast of Al-Rajma (N 32.193021 and E 20.226580) near the Sidi Khalifa area and extends to the northeast of Al-Hamda (N 32.526994 and E 20.653896), which is south of Tokrah .The area is located in wadi Zaza , one of the most important wadies in the zone (N 32.375580 and E 20.544370), which is considered a natural environment with vegetation, biodiversity and also a safe haven for many animals.
There are many wadies, the most important of which is wadi Zaza , which extends to about 38 kilometers to the south, which is about 20 kilometers from Tokrah, and the wadi rises from a large surface level to about 290 meters.

These climatic conditions in Cyrenaica led to biodiversity than the lands that surround it, where the evergreen trees and shrubs of the Cyrenaica region grow on the Mediterranean Sea, the most important of which are juniper, eucalyptus, and shammari. These features made it one of the best environments suitable for the presence of many types of wildlife provides them food and shelter. The occasion is throughout the year, and various forms of wild animals, mammals, reptiles, and resident and migratory birds are endemic to this part of the AL- Jabal AL-Akhdar (Alaib, El-Sherif, & Al-Hamedi, 2017).

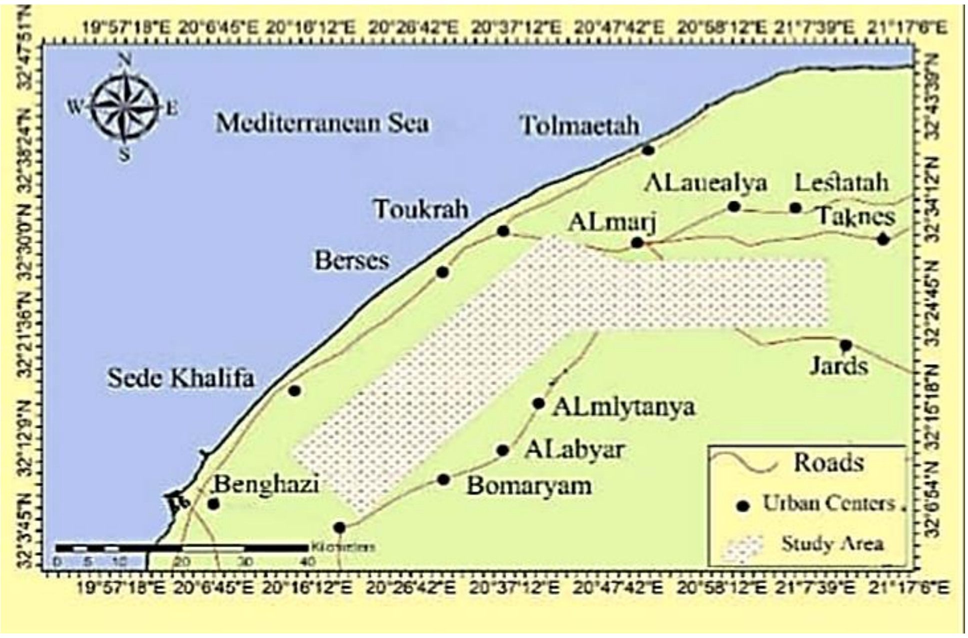

Figure (1) Geographical location of the study zone.

MATERIALS AND METHODS

The mammals present in this study were recorded in in three ways: through field views, field questionnaires and documentation from local residents via photos and videos. The basic information for each species shows the scientific name, English name and local name together. In most cases, the primary data comes from new notes, new recordings.

Wildlife fauna was studied through several field trips, in which many wild species were monitored in the study area in stages, where they were documented and identified according to the classification of Carlos Linnues 1758. Interim schedule for years: (Jeera, Wadi Zaza, Taknes) 2021-2022.

1- April 2021 trip

2-May 2021 trip.

3- July 2021 trip.

4- August 2021 trip.

5 - October 2021 trip.

6- March 2022 trip.

7- April 2022 trip.

8- June 2022 trip

RESULTS AND DISCUSSION

A total of 36 species belonging to 29 families were recorded, with mammalia being the most diverse group, followed by aves and reptilia. This pattern of vertebrate diversity is similar to that observed in other Mediterranean regions (e.g., Greece, Italy, Spain). For instance, a study by (Brotons, Thuiller, Araújo, & Hirzel, 2004) in Greece found that birds were the most abundant vertebrate group, followed by mammals and reptiles. Similarly, a study by (Maiorano, Falcucci, & Boitani, 2006) in Italy found that birds were the most diverse vertebrate group, followed by mammals and reptiles.

The data also noted that, the fauna status in the study zone is considered to range from near threatened to threatened due to the intensity of human activities. This is a common concern throughout the Mediterranean region, where human activities such as urbanization, agriculture, and tourism have led to habitat loss, fragmentation, and overexploitation of wildlife. For example, a study by Blondel *et al.*, (1999) found that habitat loss was the main threat to bird populations in the Mediterranean Basin. Similarly, a study by (Cooney, 2004) found that overexploitation of wildlife was a major threat to biodiversity in the Mediterranean region.

Also, the data pointed out that the species in the study zone represent 8.5% of the total fauna species in Libya (474 species). This suggests that the study zone is an important area for biodiversity conservation in Libya. However, as noted above, the fauna in the study zone is threatened by human activities. Therefore, it is important to implement conservation measures to protect the biodiversity of the study zone.

The differences in the observed distribution of fauna between the current study and a previous study by (Bsisa, 2019) .While Bsisa found rodents to be the most widespread fauna group in the northern eastern part of Libya, the current study found birds, particularly ***Alectoris barbara***, to be more prevalent. Additionally, turtles were found to be the dominant reptile species, especially in residential zones and farms. This difference in observed distribution patterns could be attributed to several factors, including the spatial variation due to habitat differences, climate variation, and anthropogenic factors. Also, temporal variation, this study was conducted over a different time period than Basisa's study, and fauna can fluctuate over time due to environmental factors, human activities, and natural population dynamics. Moreover, the methodology differences, the sampling methods, and the survey techniques employed in the two studies could also contribute to the observed differences. Different methods may have varying sensitivities to detecting different fauna groups, leading to discrepancies in the perceived distribution patterns.

Similar variations in faunal distribution have been observed in other countries and regions. For instance, a study by (dos Anjos et al., 2011) in Brazil found that the abundance of bird species varied significantly across different habitats within the Atlantic Forest biome. Similarly, a study by (Herrmann, Babbitt, Baber, & Congalton, 2005) in the United States demonstrated that the distribution of amphibian species was influenced by a combination of landscape features, climate factors, and anthropogenic disturbances.

The study of Libyan fauna, despite the efforts of previous researchers, remains limited and incomplete. This highlights the need for further research, particularly in the areas of taxonomy, environmental biology, and the factors influencing faunal distribution and abundance. The current study represents an extension of the work initiated by earlier researchers in the past century.

Reptiles are considered one of the least abundant animal groups in the region, with snakes like *Naja haje* and *Macroprotodon cucullatus* being particularly scarce. This observation aligns with the findings of (A. Bauer, 1993) who noted that the reptile fauna of Libya, particularly when compared to that of other African regions, is relatively impoverished.

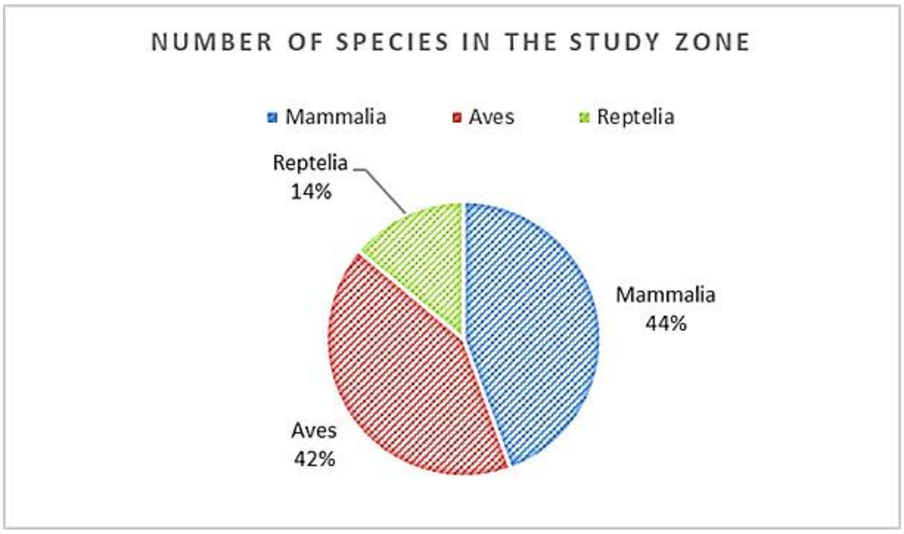

Figure (2) Percentage of species in the zone.

The study also highlights the negative impacts of human activities, such as urban expansion, hunting, and deforestation, on mammal populations. These activities have caused direct threats to mammal survival, as evidenced by their observed migration towards the southern marginal zones. This observation is consistent with the findings of (Panel, 2011) ,who emphasized the sensitivity of mammals to direct anthropogenic disturbances. The limited knowledge of Libyan fauna is not unique to this region. Similar situations have been observed in other countries, particularly those with challenging environmental conditions or limited research resources. For instance, a study by (Antonelli et al., 2022) on the herpetofauna of Madagascar revealed significant gaps in knowledge regarding the distribution and conservation status of many reptile species. Similarly, a study by(Peres & Nascimento, 2006) on the Amazonian rainforest highlighted the need for comprehensive biodiversity surveys to adequately assess the conservation status of the region's rich fauna.

We conclude that there is a pressing need for increased scientific efforts to study Libyan fauna, given the various anthropogenic pressures it faces. These pressures are continuously intensifying and pose a threat to the habitats of faunal species in the study area. The encroachment of human activities into Cyrenaica has impacted its biodiversity, leading to faunal loss and habitat degradation. This has altered mammal fauna communities in the region. As a result, Cyrenaica's fauna can be considered a valuable source of biodiversity data for Libya, both for conservation purposes and for reconstructing the population history of mammalian species in Libya and the wider Mediterranean region.

The situation in Libya is not unique. Similar scenarios have been observed in other countries, particularly those with fragile ecosystems or limited research resources. A study by (Ceballos, Ehrlich, & Dirzo, 2017) on the global mammal decline concluded that human activities are the primary driver of mammal extinction. Similarly, a study by(Ripple et al., 2015) highlighted the alarming decline of vertebrate populations worldwide, with anthropogenic factors being the main cause of this trend.

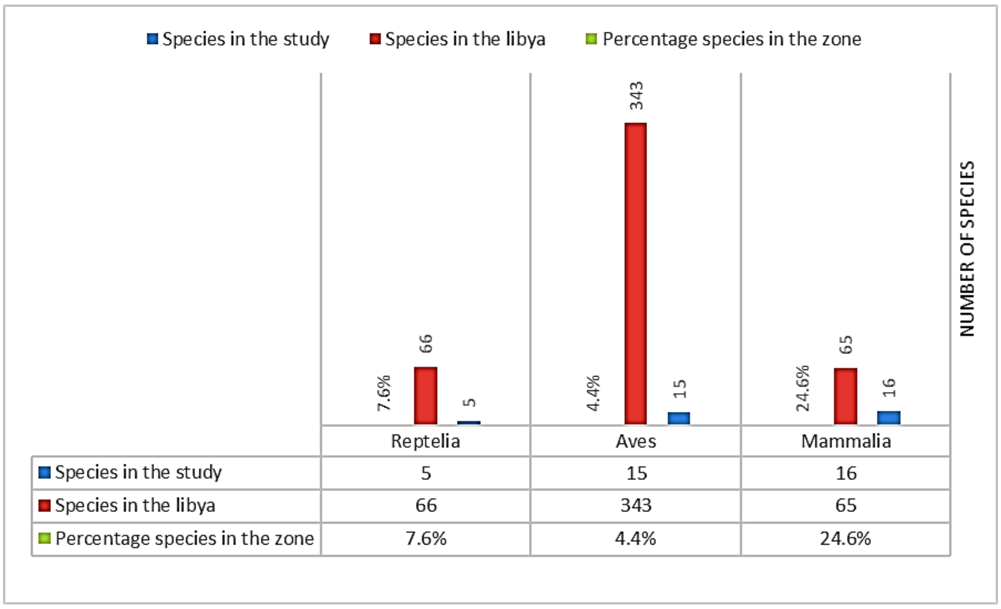

Figure (3) percentage of species in the zone compared with the number of species in Libya.

list of fauna species:

Mammalia

1- Pipistrellus kuhlii (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Chiroptera
Family	Vespertilionidae

2- Lapus capensis (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Lagomorpha
Family	Leporidae

3- Hystrix cristata (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Rodentia (bowdich, 1821)
Family	Hystricidae (fischer de Waldheim,1817)

4- Spalax ehrenbergi (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Rodentia
Family	Spalacidae

5- Hemiechinus auratus libycus (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Erinaceommorpha
Family	Erinaceidae (fischer,1814)

6- Jaculus jaculus (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Rodentia (bowdich, 1821)
Family	Dipodidae (fischer de Waldheim,1817)

7- Mus musculus domesticus (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Rodentia (bowdich, 1821)
Family	Muridae (llliger,1811)

8- Microtus guentheri (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Rodentia (bowdich, 1821)
Family	Cricetidae (fischer ,1817)

9- Fennec vulpes zerda (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Carnivora
Family	Canidae

10- Hyaena hyaena (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Carnivora (bowdich, 1821)
Family	Hyaenidae (gray,1821)

11- Vulpes vulpes (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Carnivora (bowdich, 1821)
Family	Canidae (fischer,1817)

12- Canis anthus (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Carnivora (bowdich, 1821)
Family	Canidae (fischer,1817*)*

13- Poecilictis libyca (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Carnivora (bowdich, 1821)
Family	Mustelidae

14- Felis Caracal caracal (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Carnivora (bowdich, 1821)
Family	Felidae

15- Felis lybica (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Carnivora (bowdich, 1821)
Family	Felidae

16- Gazella dorcas (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Mammalia
Order	Artiodactyla (owen,1848)
Family	Bovidae (gray,1821)

List of fauna species.

Aves

1- Hirundo rustica rustica (Linnaeus, 1758)

Superclass	*(Tetrapoda)*
Class	*Aves*
Order	*Passeriformes*
Family	*Hirundioidae*

2- Strptopelia turtur (Linnaeus, 1758)

Superclass	*(Tetrapoda)*
Class	*Aves*
Order	*Columbidae*
Family	*Columbidae*

3- Columba livia (Linnaeus, 1758)

Superclass	*(Tetrapoda)*
Class	*Aves*
Order	*Columbiformes*
Family	*Columbidae*

4- Coturnix coturnix (Linnaeus, 1758)

Superclass	*(Tetrapoda)*
Class	*Aves*
Order	*Galliformes*
Family	*Phasianidae*

5- Upupa epops (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Aves
Order	Bucerotiformes
Family	Upupidae

6- Galerida cristata (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Aves
Order	Passeriformes
Family	Alaudidae

7- Alectoris barbara (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Aves
order	Galliformes
family	Phasianidae

8- Eremophila bilopha (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Aves
Order	Passeriformes
Family	Alaudidae

9- Chlamydotis undulata (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Aves
Order	otidiformes
Family	otididae

10- Athene noctua (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Aves
Order	strigiformes
Family	Strigidae

11- Aquila chrysaetos (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Aves
Order	Accipitridae
Family	Accipitridae

12- Corvus corax (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Aves
Order	Passeriformes
Family	Corvidae

13- Lanius excubitor (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Aves
Order	Passeriformes
Family	Lanidae Rafinesque (shrikes, 1815)

14- Ardea purpurea (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Aves
Order	Pelecaniformes
Family	Ardeidae (leach.1820)

15- Coccothraustes coccothraustes (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Aves
Order	Passeriformes
Family	Fringillidae (leach.1820)

list of fauna species.

Reptalia

1-Naja haje (Linnaeus, 1758)

Superclass	*(Tetrapoda)*
Reptilia	*Class*
Order	*Squamata*
Family	*Elapidae*

2- Testudo kleinmanni (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Reptilia
Order	Testudines (batsch,1788)
Family	Testudinidae (batsch,1788)

3- Mabuya vittate (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Reptilia
Order	Squamata
Family	Scincidae

4- Macroprotodon cucullatus (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Reptilia
Order	Squamata (oppel,1811)
Family	Colubridae (oppel,1811)

5- Chamaeleo chamaeleon (Linnaeus, 1758)

Superclass	(Tetrapoda)
Class	Reptilia
Order	Squamata
Family	Chamaeleonidae

CONCLUSION

Scientific reports on Libyan mammals are scarce and provide only a partial overview of the country's mammalian diversity. Previous checklists of Libyan mammals have been limited in scope, with only a few covering the entire fauna. While the report by the General Authority for the Environment focuses on the most endangered Libyan wild animal species, its coverage of other Libyan species is incomplete. Due to the incompleteness of previous checklists, it was deemed necessary to compile a new and updated checklist for select Libyan regions.

This checklist is based on the author's original field observations during the study period. It provides a more accurate picture of the distribution of various species within the Al-Abiar Zone (Zaza, Jeera, and Taknes) than was previously possible. The lack of comprehensive faunal studies is a common challenge faced by many countries, particularly those with vast and diverse ecosystems or limited research resources. For instance, a study by (Schipper et al., 2008) on African mammal diversity revealed significant gaps in knowledge regarding the distribution and conservation status of many species. Similarly, a study by (Wilson & Reeder, 2005) on global mammal diversity highlighted the need for urgent conservation efforts to protect the world's mammal fauna from extinction.

Finally, the authors hope that the new reviews and additional data on some of the less well-known species found within Cyrenaica's habitat boundaries will encourage various competent bodies in the country to utilize this information for distribution and conservation purposes. The present checklist includes several species and subspecies not mentioned in previous reports.

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

Author contribution: A, B conducted the theoretical formalism, A, B, C, D, performed the field work and analytical calculations, A, B, C, D, E, contributed to the final version, A, B, supervised the work.

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Research Article **Open Access**

Examining the Influence of Respiratory Disorders on Some Hematobiochemical Parameters in Al-Jabal Al-Akhdar Libyan Sheep

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Abstract: The aim of this study was to evaluate some changes in blood indicators and selected biochemical variables in the serum of infected sheep. Respiratory diseases are indistinguishable in terms of cause. This study was carried out on 60 native sheep in Elgabal Alakhdar province in Libya. Samples were divided into two groups. The first group included 20 clinically healthy sheep, while the second groups, the experimental group, included 40 sheep. The animals showed symptoms of respiratory disease, including a higher than normal temperature, an increase in pulse and respiratory rate, and the presence of nasal discharge. The animals aged for more than six months and weighed between 35-60 kg. Two blood samples were collected: whole blood samples for a hematological picture and serum samples for biochemical analysis, which included glucose, Total protein (TP), albumin (Alb), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Serum concentrations of sodium (Na), potassium (K) and chloride. Significant changes between diseased animals and healthy animals were observed in values of WBCs, RBCs, HG and in biochemical parameters, including glucose, AST, ALT ,Total protein (TP), albumin (Alb), and Serum concentrations of sodium (Na), potassium (K) and chloride.

Keywords: Biochemical parameters , Sheep, Respiratory diseases,

دراسة تأثير الاضطرابات التنفسية على البعض المؤشرات الدموية والكيميائية في الأغنام الليبية بالجبل الأخضر

هدفت هذه الدراسة إلى تقييم بعض التغيرات في مؤشرات الدم وبعض المتغيرات البيوكيميائية في مصل الأغنام المصابة بأمراض الجهاز التنفسي والتي لا يمكن تمييزها من حيث السبب. أجريت هذه الدراسة على 60 رأساً من الأغنام المحلية في محافظة الجبل الأخضر في ليبيا. تم تقسيم العينات إلى مجموعتين المجموعة الأولى اشتملت علي 20 من الأغنام السليمة سريرياً ،اما المجموعة الثانية، المجموعة التجريبية، فضمت، 40 حيواناً. ظهرت على الحيوانات أعراض أمراض الجهاز التنفسي، بما في ذلك ارتفاع درجة الحرارة عن الطبيعي وزيادة في النبض ومعدل التنفس، ووجود إفرازات من الأنف. أعمار الحيوانات أكثر من ستة أشهر ووزنها من 35−60 كغم. تم جمع عينتين من الدم: عينات دم كاملة للصورة الدموية، وعينات مصل للتحليل الكيموحيوي والتي شملت الجلوكوز، البروتين الكلي (TP)، الألبومين (Alb)، إنزيم ناقلة أمين الألانين (ALT)، ناقلة أمين الأسبارتات (AST) وتركيزات مصل الصوديوم (Na) والبوتاسيوم (K) والكلوريد. وقد لوحظت تغيرات كبيرة بين الحيوانات المريضة والحيوانات السليمة في قيم الدموية والقيم البيوكيميائية بما في ذلك كريات الدم البيضاء، أظهرت كرات الدم الحمراء، والهيموجلوبين HG تغيراً معنوياً في المعايير البيوكيميائية وكذلك الجلوكوز، و AST، وALT،و البروتين الكلي (TP)،والألبومين (Alb)، وتركيزات المصل من الصوديوم (Na)، البوتاسيوم (K) والكلوريد.

الكلمات المفتاحية: القيم البيوكيميائية ، الأغنام، الأمراض التنفسية.

INTRODUCTION

Small ruminants, such as goats and sheep, are highly prized in countries across the Mediterranean, Africa, and Southeast Asia. These animals have great potential to provide a variety of valuable resources, including meat, milk, and wool (Kumar et al., 2014). Respiratory disease is a common issue that sheep farmers often come across. It can affect either a whole group of sheep or individual animals. This type of disease is usually caused by a combination of infectious factors and certain management practices that make the sheep more susceptible to getting sick. Unfortunately, these respiratory diseases can result in significant losses for farmers (Bell, 2008)**.**

The disease has multiple factors that contribute to its development. It is often caused by a combination of infectious agents, along with environmental and managerial factors that make animals more susceptible to the disease (Barghouth, 2000). Pneumonia typically occurs when the bronchioles and alveoli in the lungs become inflamed in response to certain agents, leading to the consolidation of lung tissue. When exposed to a sufficient amount of microorganisms, combined with factors such as their susceptibility and weakened general defense mechanisms, their lung's ability to fight off diseases becomes compromised, allowing pneumonia to develop (Bruere et al., 2002)**.**These injuries not only impact the health of animals, but also have a significant effect on their overall well-being. This can be seen through various symptoms such as weight loss, decreased milk production, poor quality wool, difficulties with birth control, and reduced reproductive efficiency. Additionally, these injuries also result in economic losses due to the need for veterinary health services and the considerable effort required to address these issues (Durkot et al., 1986). Hematological tests have been extensively utilized to diagnose different diseases and assess the nutritional status of animals (Carlos et al., 2015). Diagnosing respiratory diseases in small ruminants early, quickly, and effectively is a difficult task in African and Southeast Asian countries. These regions have limited access to laboratory resources, which makes it challenging to identify and address the respiratory disease outbreaks that often lead to a significant loss of a small ruminant population (Chakraborty et al., 2014).When the body is faced with a disease, the liver kicks into action and starts producing a group of plasma proteins called acute-phase proteins (APP).

These proteins can either increase or decrease in concentration depending on factors like infection, inflammation, and other internal or external challenges (González et al., 2008).Farm animals can suffer from various diseases that affect the balance of body fluids, electrolytes, and acid-base levels(Sen & Constable, 2013).Respiratory diseases in sheep have significant clinical and economic implications, making them a subject of interest for many researchers in the field of small ruminant practice. Therefore, the purpose of this study was to examine various hematological and biochemical changes in order to compare healthy sheep with those affected by respiratory diseases.

MATERIALS AND METHODS

Animals

This research was conducted on 60 native Libyan sheep, which were divided into two groups.
The first group consisted of 20 apparently healthy sheep served as the control group.
The second group included 40 sheep that were diagnosed with respiratory disease based on clinical
All animals were over six months old and weighed between 35 and 60 kg. The clinical .symptoms history of the sheep was obtained from their owners.

Sampling

Blood samples were taken from both groups and divided into two parts. The first part was collected using disodium ethylene diamine tetracetic acid (EDTA) to analyze the blood cells. The second part was placed in plain centrifuge tubes to separate the serum, which was then stored at -20ºC until it could be tested for other biochemical parameters.

Analytical methods

Hematological analysis

In this study, the researchers looked at certain blood measurements to evaluate the health of the participants. These measurements included the number of red blood cells (RBC), the concentration of hemoglobin (HG), and the count of white blood cells (WBC).

Serum biochemical analysis

The serum samples were analyzed to measure the levels of glucose, total protein (TP), albumin (Alb), and the enzymatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Additionally, the concentrations of sodium (Na), potassium (K), and chloride (CL) in the serum were also determined. These measurements were conducted using a spectrophotometric method with commercially available test kits, following the instructions provided by the manufacturer.

Statistical analysis

The data were examined using an independent t-test analysis with equal variance. The means of two groups were compared, and IBM (SPSS) software 20 was used to represent the values as mean ± SD. All differences were deemed statistically significant at a p-value<0.05.

RESULTS

The total leukocyte count or TLC (WBC) count, showed a significant increase (P≤0.05) in the second group (diseased animals) compared to the control group. The values were 21.57 ± 0.44 and 6.09 ± 0.08 (×10^3 /µl) in the diseased animal and control group, respectively, as shown in Table 1**.** The second group of animals (diseased animals) showed a significant decrease in red blood cell (RBC) count compared to the control group (P≤0.05). The RBC count values were 7.56 ± 0.04 and 10.92 ± 0.11 (×10^6 /µl) in the diseased animal and control group, respectively, as shown in Table **1**. The hemoglobin content (HGB) in the second group (diseased animals) was significantly decreased (P≤0.05) compared to the control group. The values were 7.35 ± 0.06 and 12.69 ± 0.12 (g/dl) in the diseased animal and control group, respectively, as shown in Table 1.

Table (1). Hematological parameters (Mean± SD) in Healty and diseased sheep (N= 60)

Parameters	Control Group I (healthy sheep n=20)	Diseased sheep n=40
WBC	6.09 ± 0.08^{b}	21.57 ± 0.44^{a}
RBC	10.92 ± 0.11^{a}	7.56 ± 0.04^{b}
HG	12.69 ± 0.12^{a}	7.35 ± 0.06^{b}

Means having different letters are significantly different (P<0.05)

The second group of animals (diseased sheep) showed a significant decrease in serum glucose levels (P≤0.05) compared to the control group. The values were 45.83 ± 0.20 and 76.24 ± 0.38, respectively, as shown in Table 2. The levels of total protein and albumin in the serum were significantly lower (P≤0.05) in the second group (diseased animals) compared to the control group. The values of total protein were 3.41 ± 0.09 and 4.35 ± 0.08 (g/dl), and the levels of albumin were1.64 ± 0.04 and 1.88 ± 0.02 in the diseased animal and control groups, respectively, as show in Table 2. The serum levels of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were significantly increased ($P \leq 0.05$) in the second group of animals with the disease compared to the control group. The values of ALT were 55.73 ± 0.16 IU/L and 34.3 ± 0.4 IU/L, AST levels were 371.8 ± 0.85 IU/L and 220 ± 1.54 IU/L, and ALP levels were 315.25 ± 3.88 IU/L and 86.78 ± 4.11 IU/L in the diseased animal and control group, respectively, as shown in Table 2. The serum levels of sodium (Na), potassium (K), and chloride (CL) were significantly decreased in the second group (diseased animals) compared to the control group. The values of sodium (Na) were 107.7 ± 0.77 and 136.9± 1.68 Eq/l, and the levels of potassium (K) were 2.65 ± 0.05 and 4.47 ± 0.05 Eq/l. Meanwhile, the chloride (CL) levels were 82.74 ± 0.16 and 103.8 ± 0.56 Eq/l in the diseased animal and control group, respectively, as shown in Table 2.

Table (2). Some Biochemical Parameter analysis Mean± SD) in Healty (control) and diseased sheep (N= 60)

Parameters	Control (Healthy sheep)	Diseased sheep
Glu mg/dl	76.24 ± 0.38^{a}	45.83 ± 0.20^{b}
T.P g/dl	4.35 ± 0.08^{a}	3.41 ± 0.09^{b}
ALB g/dl	1.88 ± 0.02^{a}	1.64 ± 0.04^{b}
Na mEq/l	136. ± 1.68^{a}	107.7 ± 0.77^{b}
K mEq/l	4.47 ± 0.05^{a}	2.65 ± 0.05^{b}
ALP U/I	86.78 ± 4.11^{b}	315.25 ± 3.88^{a}
CL mEq/l	103.8 ± 0.56^{a}	82.74 ± 0.16^{b}
ALT U/l	34.3 ± 0.4^{b}	55.73 ± 0.16^{a}
AST U/l	220 ± 1.54^{b}	371.8 ± 0.85^{ab}

Means having different letters are significantly different ($P<0.05$)

DISCUSSION

Small ruminants, such as sheep and goats, are highly valuable in regions like Southeast Asia, Africa, and the Mediterranean due to their ability to provide meat, milk, and wool. However, they are particularly susceptible to respiratory illnesses, which account for almost 50% of their deaths. Respiratory infections are responsible for 5.6% of all small ruminant diseases, affecting both sheep and goats (Donia et al., 2018)**.** Sheep anatomical structure makes them susceptible to respiratory processes. Furthermore, a variety of illnesses, particularly in older animals, can damage the respiratory system and result in large financial losses. The financial losses brought on by the illnesses are connected to both output losses and death (Lacasta et al., 2019)**.** The blood parameter profile is determined in order to assess the individual's health and keep an eye on the animals' nutritional and metabolic status (Diaz Gonzalez & Silva, 2017).

In our study, white blood cell (WBC) counts significantly increased ($P \leq 0.05$) in diseased animals compared to those in the control group. These changes could be attributed to the rise in acute inflammatory illnesses, especially those brought on by bacterial infections. This may be explained by the fact that various cells are stimulated by infectious agents and tissue damage products to release growth factors, cytokines, and other inflammatory mediators**.** These mediators function as prompt stimuli and are all involved in increasing the number of white blood cells overall as well as the production, proliferation, maturation, and release of mature and immature neutrophils from the bone marrow (Sayed et al., 2002)**.**This study's observations of the impact of pneumonia on red cell parameters included a marked decline in the diseased group's RBC count, Hb, which suggested the existence of microcytic hypochromic anemia**.** These modifications may be explained by the fact that in inflammatory conditions, the mononuclear phagocytic system becomes hyperplastic, trapping free iron and increasing the amount of iron stored in phagocytic cells, reducing the amount of iron transferred to growing erythrocyte cells in the bone marrow, which lowers the synthesis of Hb and microcytic hypochromic RBCs (El-Naser & Khamis, 2009).

This is similar to result of (Sadeghian et al., 2011), which observed that while the infected goats' PCV, RBC counts, and Hb concentrations were lower than those of the healthy goats, only the hemoglobin drop was statistically significan**t.** Serum biochemical and haematological indices are crucial panels used in the diagnosis, prognosis, and treatment of diseases in livestock (Onasanya et al., 2015).In our study, serum glucose was significantly ($P \leq 0.05$) decreased in diseased animals compared to those in the control group. This result was consistent with result observed by (Cole et al., 1988).

Due to discomfort from respiratory symptoms, nutrition has also decreased, resulting in lower glucose levels. It's possible that sheep's response to bacterial endotoxin increases peripheral glucose uptake because of a rise in insulin concentration in the blood, and this reaction happens even in the absence of fever (Southorn & Thompson, 1986).This might be the outcome of prolonged malnutrition and low energy intake during illness. Calves with chronic pneumonia also showed decreased glucose concentrations, as reported by (Blum et al., 1996).Serum levels of total protein and Albumin were significantly ($P \leq 0.05$) decreased in diseased animals compared to those in the control group , the same result observed by(EI-Seidy et al., 2003) hypoalbuminemia may result from starvation and the liver's inability to synthesize proteins. It has been proposed by others that specific bacteria or their toxins can increase capillary permeability, allowing plasma proteins to escape from tissues. This results in an increase in the osmotic pressure of proteins in tissue fluids while simultaneously decreasing it in blood(Omran et al., 2005).As a negative acute phase protein, albumin often and noticeably decreases in value during inflammation (Cerón et al., 2005).In our study, serum Levels of sodium ((Na) ,potassium (K) and chloride(CL) were significantly ($P \leq 0.05$) decreased in diseased animals compared to those in the control group. This result was in agreement with result observed by (Novert, 2002). Certain researchers linked the alterations in serum electrolyte levels during a respiratory disease's acute phase to hyperpyrexia and the subsequent spread of infection to the liver and kidneys, which causes dysfunction in the liver and kidneys. Changes in plasma Na and Cl concentrations often result from adjustments in relative water content (Leaf, 1962)**.**

Reduced nutritional intake and gastrointestinal involvement can lead to hypokalemia(Smith & Smith, 2009)**.**Serum levels of alanine transaminase (ALT),aspartate transferase(AST) and alkaline phosphatase (ALP) were significantly ($P \leq 0.05$) increased in diseased animals compared to those in the control group. These alterations may be related to the failure of several organs, including the liver, which is brought on by bacterial infection and hepatic necrotic and degenerative changes (Aytekin et al., 2011). AST, ALT, and ALP serum enzymatic activity were noticeably elevated in the sick group. These alterations in the liver's antioxidant capacity could be related to cellular damage that modifies the liver's transport function and membrane permeability, causing enzymes to seep out of the cells. According to it, the comprehensive hepatic tissue membrane injury is indicated by the noticeable release of AST and ALT from liver cytosol into circulation (Sarhat et al., 2016).

CONCLUSION

Sheep with clinical signs of respiratory disease show hematobiochemical alteration characterized by some biochemical changes, including changes in glucose. ,total protein, albumin, AST, ALT, ALP, and a change in electrolyte balance, a decrease in serum of sodium ,potassium and chloride ,as well as hematological changes, including RBCs, WBCs, HB. The changes in these parameters can be used as indicators of the healthy status of sheep. It may help in the early detection or correct diagnosis of respiratory diseases in sheep flocks.

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