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Detection and Quantitative Estimation of Toxic Acrylamide Levels in Selected Potatoes Chips and French Fries from the Libyan Market Using HPLC-UV Method

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Abstract: Acrylamide is a potential health hazardous compound occurring in baked and fried food as a result of excessive dry heating during the preparation and/or processing of foods. Exposure to a high level of acrylamide may cause cancer, neurotoxicity, and mutagenicity. In this study, an isocratic reversed-phase high-performance liquid chromatographic (HPLC) method using a C18 column was used for the determination of acrylamide in selected food. The mobile phase consisted of 0.1% formic acid in water: acetonitrile (98:02), and the flow rate was 1.0 mL min^{-1}, elution was monitored at 200 nm. Validation in selected conditions showed that the chosen method is sensitive, selective, precise, and reproducible with a linear detector response for the determination of acrylamide. The limit of detection (LOD), and the limit of quantification (LOQ), were achieved at 0.41μg mL^{-1} and 1.25 μg mL^{-1} respectively. The proposed method was also applied after validation to the most popular six brands of chips and French fries available in the Libyan market. Acrylamide was extracted by a simplified extraction method avoiding cleanup by solid-phase extraction (SPE), then analyzed by HPLC-UV. The highest level of acrylamide was found in one brand of chips with a concentration of 16.33 μg mL^{-1}, whereas only one of the French fries products analyzed exhibited an acrylamide concentration of 10.26 μg mL^{-1}.

INTRODUCTION

Acrylamide is a vinyl monomer widely used in industrial applications and drinking water purification (Pedersen & Olsson, 2003). It has long been known as a neurotoxic agent (Stadler & Scholz, 2004). It is rapidly absorbed following oral administration in all species. Moreover, orally ingested acrylamide is able to reach most human tissues. It can cross the blood/placenta barrier in a human placenta in an in vitro model, as well as the blood/breast milk barrier in vivo of lactating mothers (Sorgel et al., 2002). Also, it is a natural by-product of carbohydrate-rich foods at high temperatures above 120°C, and it is formed by a reaction between amino acid asparagine and reducing sugars such as potatoes when exposed to high temperature during the process of frying, roasting, or baking (Stadler

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& Scholz, 2004). On the other hand, fried products with a low level of acrylamide were not a serious concern with regards to the potential health issue associated with ingested acrylamide as it is readily absorbed, metabolized, and excreted via urine. It was only considered as a contaminant in water sources (Rommens et al., 2008).

Acrylamide is considered one of the most common toxins in food containing high concentrations of hydrocarbons that have been subjected to high temperatures (Mottram et al., 2002). It is present in the daily food of most people's diet. It may be found in food products such as potato chips, fried potatoes, cornflakes, or bread. Some of these products are attractive to children and young people which makes matters worse (Altunay et al., 2016).

Many studies have shown that the average daily intake of acrylamide for adults was estimated to be in the range of 0.2 to 1.04 µg/kg of body weight per day (Hartmann et al., 2008; Konings et al., 2003; Mojska et al., 2010; Svensson et al., 2003). However, higher exposure is assumed in children and adolescents, reaching up to 3.4 µg/kg body weight daily (Vainio, 2003). The European Union has set a limit of 0.1 µg/L residual acrylamide in drinking water (Binet et al., 2015). Whereas FAO/WHO have agreed that the "no observed adverse effect level" (NOAEL) for acrylamide neuropathy is 0.5 mg/kg body weight/day, while that for fertility changes is four times higher than for peripheral neuropathy. The estimated average chronic human dietary intake is in the order of 1 µg/kg body weight/day (Kapp, 2002). Acrylamide is metabolized to form the epoxy derivative glycidamide which is conjugated with glutathione and converted into mercapturic acid as by-products in urine, which is the primary route of acrylamide elimination in humans (Fuhr et al., 2006). Both acrylamide and glycidamide could bind covalently to nucleophilic sites of biological macromolecules. The major targets of these compounds seem to be the SH and NH_2 groups of proteins and nucleic acid nitrogen (Fuhr et al., 2006), and its metabolites react with nucleophilic sites of blood protein haemoglobin (Hb) and form stable reaction products (adducts). Hb Adducts from acrylamide and glycidamide can be quantified to measure the level of human exposure to acrylamide (Wenzl et al., 2003).()

Epidemiological evidence on the relation between dietary acrylamide and the risk of several cancers has continued to accumulate during the last years (Khan et al., 2017). Many studies have confirmed that human exposure to acrylamide may lead to neurological effects in humans (Calleman et al., 1994). Also, experimental animals that were administered acrylamide orally experienced carcinogenic effects such as tumours of mammary glands in female rats, testicular tumours in male rats, and increased rates of tumours of the thyroid gland, central nervous system, uterus, clitoral gland, and oral tissues (Fuhr et al., 2006).

Acrylamide is classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC). It has been demonstrated to have carcinogenic properties in animals and humans (Stadler & Scholz, 2004; Virk-Baker et al., 2014), and it has been recognized as a carcinogen in rodents and classified as probably carcinogenic in humans (Bull et al., 1984). The mutagenicity of acrylamide was also confirmed at low concentrations in different mammalian cell lines (Robinson et al., 1984). Two separate independent studies have confirmed that acrylamide induced lung and skin tumours in mice at a dose of 2mg/kg per day, administered in drinking water (Liu et al., 2019).

Consequently, many researchers investigating acrylamide content have mainly utilized gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS) for the determination and quantification of acrylamide

in various foods (Elbashir et al., 2014; Galuch et al., 2019). Although, during the routine analysis of acrylamide by GC–MS, the derivatization step may usually be carried out before the clean-up step' which was found to be expensive and time-consuming (Gertz & Klostermann, 2002; Nemoto et al., 2002). Calbiani *et* al. (2019) developed and validated a rapid method for the determination of acrylamide in cooked food samples by reversed-phase LC-MS coupled with electrospray ionization. A simplified extraction step with acidified water and without cleanup was developed in this method (Calbiani et al., 2019).

At present, several analytical methods are available for determining acrylamide content in snack food based on high-performance liquid chromatography (HPLC). These methods involve the extraction of acrylamide and utilized solid-phase extraction SPE cartridges for clean-up. The main advantage to use HPLC is that the removal of water after aqueous extraction is not necessary (Can & Arli, 2014; Gokmen et al., 2005; Muthaiah et al., 2018; Oroian et al., 2015).

The objective of this study is to quantify and determine the level of acrylamide in chips and French fries collected from the Libyan market by assessing dietary acrylamide exposure and its effects on health in humans.

MATERIALS AND METHODS

Chemicals, reagents and solutions: All chemicals, analytical standards, reagents, and solvents used throughout this study were analytical grade and highly pure. Acrylamide (Purity 99.0%) was purchased from (Novachim-Tunisia). Also, other chemicals and solvents were used, including acetonitrile (Carlo Erba Reagents S.A.S., France) with purity 99.9 % (for HPLC) as a solvent; hexane 95% was purchased from (Sigma-Aldrich, Germany). Formic aid (Riedel-Dehaen AG Seelze Hannover) with purity 98-100 %, Magnesium sulfate Anhydrous was purchased

from FLUKA, Germany. Primary secondary amine (PSA) was purchased from VARIAN (USA), and Acetic Acid (96%) was purchased from PANREAC QUIMICA (Spain). Water was obtained in-house by Water Distillation Unit.

A stock solution of acrylamide (100 µg mL^{-1}) was prepared by dissolving 0.01 g in 100 mL of distilled water (0.10 mg mL^{-1}). Working standard solutions were prepared by appropriate dilution of the stock solution with distilled water (2.5, 5.0, 10, 15, and 20µg mL^{-1}).

Sample Extraction: The extraction procedure of the potato chips sample entailed the following steps: weigh 7.5 g of thoroughly homogenized sample into a 50 mL centrifuge tube, then add 7.5 ml acetonitrile, 0.75 ml acetic acid, and 5ml hexane. Vortex the tube thoroughly for one min to solubilize/disperse the sample in hexane. Centrifuge the tube for 5 min at 3700 rpm using an RT6000B centrifuge from Sorvall. Subsequently, discard the hexane layer. The supernatant was transferred into a clean centrifuge tube and add 375 mg of anhydrous Mg SO4 and 125 mg of PSA. Immediately, seal the tube and shake vigorously for 30 seconds, and centrifuge at 3700 rpm for 5 min. The supernatant was evaporated by a vacuum rotary evaporator. The dry residue was reconstituted in 2 mL acetonitrile for HPLC analysis (Anastassiades et al., 2003).

The recovery experiment was performed by spiked 5 mg of acrylamide standard to 7.5 mg of thoroughly homogenized potato chips samples and extracted then dried. Then 10 mL of acetonitrile was added to reach a concentration of 100 µg mL^{-1}. The percentage recoveries were calculated using the following equation: percentage of recovery = [C_E/C_M× 100], where CE is the practical concentration obtained from a standard solution of 100 µg mL-1 of each compound in acetonitrile and CM is the spiked concentration after extraction (Chowdhury et al., 2012).

Instrumentation (HPLC): The HPLC system consisted of Thermo Series P2000 Analytical Pump, The Series 200 Autosampler, Series 200 UV/Vis Detector (from 190 to 1000 nm), and 20 μL loop injector. The stationary phase represents the analytical column which was a Brownlee Bio C18 column of 250x4.6 mm and 5 μm particle size.

HPLC operating conditions used Mobile Phase consisted of water containing 0.1% Formic acid (A) and acetonitrile (B), the flow rate was 1.0 mL min-1, the injection volume 20 μL, and it was detected at UV wavelengths of 200 nm.

Statistical analysis: Data were analyzed using SPSS version 25 software. Statistical differences were tested using one-way ANOVA. Differences were considered significant at p values ≤ 0.05 and then Tukey HSD analysis (Honest Significant Difference) was performed to determine whether there were statistically significant differences at p values ≤ 0.05 between the concentrations of the different samples.

RESULTS AND DISCUSSION

Validation method: To determine the linearity of the HPLC response, a standard solution of acrylamide was prepared as described. Good linear correlations were obtained between peak areas and concentration in the selected range of 2.5– 20 μg mL^{-1}. Characteristic parameters for regression equations and correlation coefficients are given in (Table 1). The linearity of the calibration curve was validated by the high value of correlation coefficients of the regression graph.

A peak tailing (As ~1.01) was being observed in chromatograms of acrylamide at all concentrations analyzed. Calibration standards were prepared and then demonstrated a linear response (R^2 =0.999) over a 2.5 -20μg mL^{-1}. The limits of detection and quantification were determined to be 0.41 and 1.25μg

mL^{-1}for acrylamide respectively, which is considered quite sensitive in comparison with most of the previous methods.

Intra-day precisions were determined using five concentration levels. The data showed that the method provided high levels of precision when used by the same analyst on the same day. Thus, the precision of the proposed method was determined by running calibration series solutions at 2.5– 20 μgml^{-1} and then was evaluated in terms of repeatability and expressed as the relative standard deviation (RSD, %). The result of precision was ranged between 0.45 and 1.79 %, indicating good repeatability. All validation parameters for acrylamide are summarized in Table 1.

Table: (1). Summary of validation data for the quantification of acrylamide.

Parameter	Acrylamide
t_R (min) (t^0 = 1.95 min^a)	3.85
Capacity factor (k')	0.17
Symmetry factor (A_s)	1.01
LOD (μg mL^{-1})	0.41
LOQ (μg mL^{-1})	1.25
N (plates)	246.94(987.76) *N expressed in plates per meter*
Coefficient of regression (R^2)	0.999 (y = 209459x-93643)
Precision (%RSD) N=3 20 μgmL^{-1} 15 μg mL^{-1} 10 μg mL^{-1} 5.0μg mL^{-1} 2.5 μg mL^{-1}	0.45 0.96 1.79 0.34 1.42

Accuracy of the proposed method was also determined, the results indicated that the accuracy of the method performed in triplicate using 20μg mL^{-1} concentration level, showed that in most cases, the recoveries of acrylamide ranged from 89.05% to 92.13%, with RSDs less than 2.50% for the sample extraction procedure. Chromatogram of overlapped six concentration series obtained at the desired retention time is shown in Figure: 1.
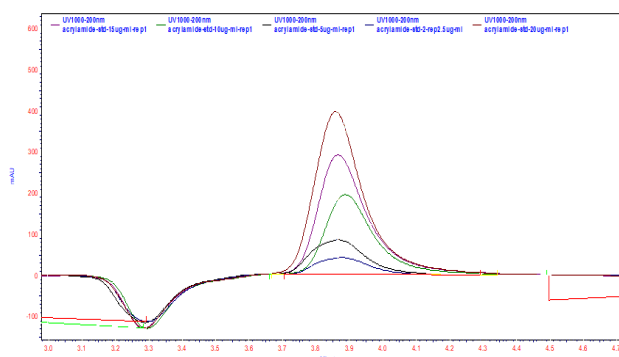
Figure: (1). HPLC chromatograms of acrylamide standards at wavelengths of 200 nm were 2.5;5.0; 10;15;20 µg mL-1

Quantification of Acrylamide in selected potato chips and French fries: The validated method of acrylamide was applied to analyze six food items include potato chips and French fries (Table 2). qualitative analysis of acrylamide content with HPLC was done by comparing the retention time of acrylamide standard at 20ug/mL with samples, using the simplified sample treatment procedure, without the SPE step, it was possible to identify and determine acrylamide without overlapping between acrylamide peak and the overlapping compounds and produced minimum interfering peaks at the UV wavelength (200 nm) detection.

The regression equation of linearity was used to calculate the concentration of extracted samples, and five standards were prepared to build the calibration curve that was varied according to the expected level of acrylamide in food samples. All the analyzed samples have returned a positive level of acrylamide content. The Tiger brand was found to have the highest concentration of acrylamide when compared with the other potato chips products selected from the Libyan market with a statistically significant difference ($p < 0.001$) in comparison with other brands, whereas the Doritos brand showed the lowest concentration. The qualitative analysis of acrylamide content with HPLC was done by comparing the retention time of acrylamide standard at 20µgmL^{-1} with sample retention times. The

qualitative analysis of acrylamide was analyzed by HPLC-UV. After validation and standard calibration series were carried out, the analysis of six products collected from the Libyan local market was performed. Results of acrylamide analysis showed the presence of a significant amount of acrylamide in all samples analyzed with different ranges (Table2). There was a statistically significant difference at the $p \leq .05$ level within the levels of acrylamide for the samples. This strongly shows the presence of acrylamide in the samples. The results indicate that acrylamide was detected in all samples, but its concentration varied significantly from (2.85 −16.33 ppm). The statistical analysis indicated that the concentration of acrylamide in selected samples was positive and significant ($p < 0.001$). Data have shown that the acrylamide levels significantly differed among the brands. All the samples had acrylamide concentrations high acrylamide content above the permissible limits.

Table: 2. Acrylamide content in foods selected from Libyan market using HPLC-UV

Product Name	Acrylamide Contents
Pringles sour cream and onion (Poland)	4.65 ppm
Doritos sweet chili pepper (Egypt)	2.85 ppm
Tiger (Egypt)	16.33ppm
Lay's with chili and lemon flavor (Egypt)	6.91 ppm
Mr. Crunch ketchup (Libya)	3.25 ppm
French fries	10.26 ppm

In the present study, the acrylamide quantity of the potato chips of different brands and French fries ingested in humans was determined. The data shows that the tested brands contain a relatively high level of acrylamide. The highest level was about 1104 µg/66g detected in the Tiger brand, followed by French fries 627 µg /60g, then Lays 301 µg/43g, Mr crunch 210 µg/65g, Pringles 185/40g, and the lowest was Doritos with 89 µg/32g (Figure 2).

The most recent toxicological data and exposure levels estimated the average human exposure at 1 µg/ kg of body weight /day, with high-intake consumers at 4 µg/kg of body weight/ day (Fuhr et al., 2006). In light of this data, there is a clear hazard of acrylamide from long-time exposure, particularly with young age people, who are more attracted to chips and fries and ingest a high amount on a daily basis. Moreover, acrylamide can be found in most daily diet products such as baked food, cornflakes, and coffee, which increases the chance of more exposure and also, toxicity. With acrylamide, there is no doubt about the existence of a hazard. The International Agency for Research on Cancer of the WHO has evaluated acrylamide as possibly carcinogenic to humans (Stadler and Scholz, 2004). In view of these results, the consumption of acrylamide in the diet should be controlled to limit its hazards to human health.
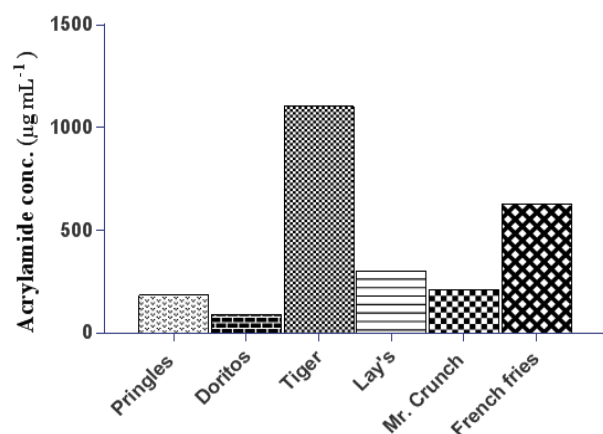
Figure: (2). Concentration of acrylamide per small package unit of selected brands that are marketed in the local Libyan market.

CONCLUSION

A quantitative HPLC–UV method has been developed which is rapid, sensitive, and reproducible. HPLC-UV is a method for the analysis of acrylamide in potato chips and French fries from the Libyan market using a simple sample preparation procedure. The LOQ and LOD were $1.25 \mu g m L^{-1}$ and $0.41 \mu g m L^{-1}$ respectively. The present HPLC method was successfully applied for the determination of acrylamide content of six potato chips and French fries products. Moreover, sample preparation comprising simplified extraction provides an effective and simple method to extract acrylamide and remove most oily substances in samples before HPLC analysis. Validation results reveal that this method is suitable for routine analysis of acrylamide residues in food products at low levels because detection and quantitation limits in the low concentrations and excellent repeatability were shown.

More measures should be made to lower and control the level of acrylamide in food items, especially in those consumed by susceptible groups such as children, young adults, and pregnant women.

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الكشف والتقدير الكمي لمستوى مادة الأكريلاميد السامة في رقائق البطاطس، والبطاطس المقلية المختارة من السوق الليبي باستخدام طريقة HPLC-UV

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المستخلص : مادة الأكريلاميد هي مركب ضار بالصحة ، يحدث في الأطعمة المخبوزة، والمقلية نتيجة التسخين الجاف المفرط أثناء تحضير أو معالجة الأطعمة. قد يؤدي التعرض لمستوى عالٍ من مادة الأكريلاميد إلى الإصابة بالسرطان، والتسمم العصبي، والطفرات الجينية. في هذه الدراسة، تم استخدام طريقة الكروماتوغرافيا السائلة عالية الأداء (HPLC) ذات الطور المعكوس باستخدام عمود C18 لتقدير مادة الأكريلاميد في الأطعمة المختارة. يتكون الطور المتحرك من 0.1% حمض الفورميك في الماء: أسيتونيتريل (98:02)، ومعدل التدفق 1.0 مل/ دقيقة، تم الكشف عن طريق الكاشف فوق البنفسجى عند طول موجى 200 نانومتر. أظهر التحقق من صحة الطريقة في الظروف المختارة أن الطريقة المختارة حساسة، وانتقائية، ودقيقة، وقابلة للتكرار مع استجابة خطية للكاشف لتحديد مادة الأكريلاميد. وتم تحقيق حد الكشف (LOD) عند 0.41 ميكروغرام /مل، و حد التقدير(LOQ) 1.25 ميكروغرام /مل على التوالي. تم تطبيق الطريقة المقترحة أيضًا بعد التحقق من صحتها على العلامات التجارية الستة الأكثر شيوعًا لرقائق البطاطس، والبطاطس المقلية في السوق الليبي. وتم استخلاص مادة الأكريلاميد بطريقة استخلاص مبسطة تتجنب التنظيف باستخلاص الطور الصلب (SPE)، ومن ثم تم تحليلها بواسطة HPLC-UV. حيث تم العثور على أعلى مستوى من مادة الأكريلاميد في علامة تجارية واحدة من رقائق البطاطس بتركيز 16.33 ميكروغرام/ مل، بينما أظهر منتج واحد فقط من منتجات البطاطس المقلية التي تم تحليلها تركيز أكريلاميد قدره 10.26 ميكروغرام /مل.

الكلمات المفتاحية: أكريلاميد، سرطان، رقائق البطاطس، بطاطس مقلية، جهاز الكروماتوجرافي السائل عالي الأداء.

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Evaluation of Efficacy of Collagen-Calcium Alginate Protectants on Chronic Wound Healing in Horses

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Abstract: This study aimed to evaluate the efficacy of collagen-calcium alginate as an accelerator of wound healing in a clinical case of ten horses attended at the University Veterinary Hospital (UVH) at Omar Al-Mukhtar University, having non-healing chronic wounds. The pattern of wound healing was evaluated both clinically and by planimetry. All cases showed healing progress after the application of the collagen-calcium alginate film. The study results suggested that collagen-calcium alginate film can be a better wound healing biomaterial in horses. It can be used as a less expensive skin substitute in order to stimulate and promote wound healing in animals especially in the case of chronic non-healing wounds.

Keywords: Biomaterial, Collagen-Calcium Alginate, Wound Healing, Chronic Wound.

INTRODUCTION

One of the most complicated biological events after birth is wound healing (Gopinath *et al.*, 2004; Schäffer *et al.*, 2004). It is a complex process of replacing dead tissue with vital tissue (Khaled & Ashraf, 2008; Khaled *et al.*, 2014). The aims of any wound treatment are to alleviate animal pain and discomfort, functional and cosmetic recovery, economical and time-efficient procedures, and prompt decision-making in case of signs of delayed healing. (Ashraf & Khaled, 2008; Ballard & Baxter, 2000). In chronic wounds, the main focus of wound healing has been on the relationship between tissue degradation by excess inflammation and tissue synthesis induced by a pro-healing environment. Due to their biocompatibility, biodegradability, and nontoxicity, natural polymers have been increasingly studied for health care applications (Mali *et al.*, 2006). The collagen based film is a potentially useful biomaterial since it is the main component of the connective tissue and enables the release of regulated drugs within the target tissues (Gopinath *et al.*, 2004). Alginates are highly absorbent gel-forming materials with haemostatic properties (Blaine, 1947), and it has long been recognized that when a gel is produced on the wound surface dehydration is prevented, and rapid wound healing occurs (Winter, 1962). Alginates are known to break down to simple monosaccharide type residues in contact with body fluids and be fully absorbed. The wound exudates turn the calcium into sodium salt, allowing the dressing to dissolve by dissolution. Any remaining residual fibers inside the wound are biodegraded, thereby eliminating the need for full removal (Burrow *et al.*, 1983). With collagen-calcium alginate biomaterial, early wound healing can be encouraged (Khaled *et al.*, 2014). The present research was carried out to test the efficacy of calcium alginate film for wound healing.

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MATERIALS AND METHODS

In the Central Leather Research Institute, Chennai, calcium alginate film was developed at a thickness of 0.2 mm (Figure1) (Khaled *et al*., 2016; Khaled *et al*., 2014; Rafi *et al*., 2008). Ten horses suffering from unhealed wounds that attended the University Veterinary Hospital (UVH), Omar Al-Mokhtar University, were chosen. Wound characterizations for all horses are given in Table 1. Animals were treated with regular saline and povidone-iodine with a course of antibiotics for a period of 26 days without signs of wound healing. Wounds were treated for one to two minutes with calci-um alginate film soaked in Gentamicin solution (Dutch Farm Veterinary Pharmaceuticals, the Netherlands).

The treatment was conducted once every three days, and four to five applications were administered to treat the wounds. Clinical observations, bacteriological analysis, and wound planimetry were determined for wound healing by square counting procedures (Richard *et al.*, 2000) at the boundary between the normal skin ,the wound and the outlined region. As defined by Bohling *et al.* (2004), the percentage of epithelialization, wound contraction, and total wound healing were determined.
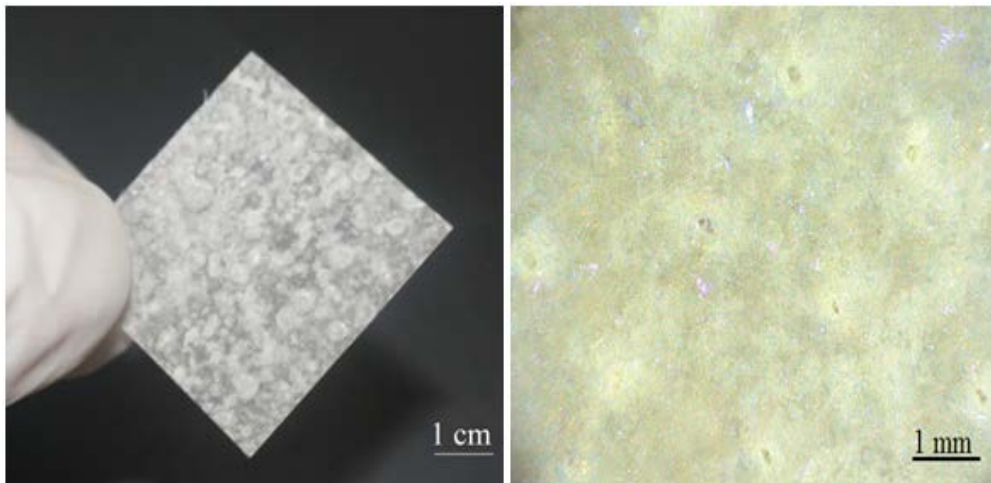
Figure: (1). Appearance of collagen-calcium alginate film (2 x 2cm sheet).

Table: (1). Characterization of the wounds in examined horses

Case No	Age in Months	Sex	Nature and site of wound	Exudate	Wound Size (cm^2)	Duration of Illness (days)
1	22	F	Right hand	Purulent	12.6	20
2	36	F	Left hand	Serosanguinous	7.3	11
3	53	M	Left elders from the neck	Exudates	14.3	7
4	18	F	Left hand	Exudates	16	12
5	29	F	Right elders from the neck	No exudate	8.2	7
6	48	M	Left man	Purulent	4.8	24
7	66	F	Left hand	Serosanguinous	17.8	18
8	40	F	Right hand	No exudate	9.8	26
9	33	F	Left hand	No exudate	11.6	20
10	50	M	Right hand	Serosanguinous	8.1	9

M= Male, F= Female

RESULTS

Collagen-calcium alginate film with therapeutic ultrasound massage was well tolerated in all the cases. No animals showed any intolerance or bandage disturbance throughout the treatment. The formation of healthy granulation tissue was observed in all cases without any side effects. After the first application of collagen-calcium alginate film, there was a marked reduction in wound discharge and the development of new shiny and bright red granulation tissue, which indicated angiogenesis and the healing of the wound. *Staphylo-coccus aureus Pseudomonas*, and *Klebsiella species* were isolated from cases 1 and 7 and *Pseudomonas* from cases 5 and 6. No organisms were isolated from cases 2, 3, 4, and 8. *Staphylococcus aureus, Pseudomonas*, and *Klebsiella species* were the common organisms isolated from infected wounds. Wound planimetry indicated that all the cases showed progress in epithelialization, contraction, and wound healing. Contraction ceased when the epithelialization of the wound is completed (Table2 and Figure 2).

Table: (2). Wound planimetry

Case No	Characteristics	Wound healing (%)				
		1st (day 3)	2nd (day 6)	3rd (day 9)	4th (day 12)	5th (day 15)
1	Epithelialization	9.32	24.12	45.15	83.54	
	Contraction	13.98	32.78	73.89	89.38	NR
	Wound healing	23.09	51.12	89.43	90.58	
2	Epithelialization	11.67	32.10	56.41	89.32	
	Contraction	22.10	41.90	82.10	90.53	NR
	Wound healing	34.78	49.67	76.28	79.97	
3	Epithelialization	14.82	36.91	60.21	82.51	
	Contraction	24.18	51.88	90.16	96.32	NR
	Wound healing	22.81	46.09	88.01	82.39	
4	Epithelialization	9.89	47.32	65.28	83.05	96.21
	Contraction	32.98	63.11	82.84	93.82	94.01
	Wound healing	39.85	39.09	79.89	79.98	89.19
5	Epithelialization	18.09	31.98	67.95	83.27	89.65
	Contraction	28.97	73.52	73.92	85.36	90.27
	Wound healing	27.11	42.45	79.43	92.52	93.78
6	Epithelialization	11.98	46.98	63.63	79.54	
	Contraction	21.98	53.97	69.34	82.98	NR
	Wound healing	34.50	39.48	78.43	85.29	
7	Epithelialization	19.46	31.89	61.86	80.12	89.84
	Contraction	34.98	58.65	79.49	89.63	92.87
	Wound healing	25.98	41.96	81.95	79.54	86.37
8	Epithelialization	13.87	42.97	63.94	79.73	
	Contraction	27.52	63.52	75.89	88.89	NR
	Wound healing	29.89	52.90	79.85	84.95	
9	Epithelialization	10.86	35.89	59.12	78.23	87.97
	Contraction	35.89	61.90	81.13	91.99	96.78
	Wound healing	32.97	41.97	72.89	82.12	92.98
10	Epithelialization	11.98	38.96	60.87	75.89	
	Contraction	33.87	58.93	78.93	92.98	NR
	Wound healing	39.53	60.19	80.31	90.23	

Figure: (2) Wound characterization and healing percentages for fifteen days

DISCUSSION

In all cases, the application of collagen-calcium alginate film was well tolerated by the animals. The collagen-calcium alginate film is easy to apply on the wound without any adverse reaction and was well accepted by all the animals. The application of collagen-calcium alginate film did not show any adhesion of the gauze during wound dressing.

The colour of the wound bed in all cases was red while cases 2 and 5 showed a bright beefy red colour, which indicates healthy granulation tissue with neovascularisation (Taylor & Bayat, 2003), and resistance to infection until the epithelial barrier is re-established (Hosgood, 2003; Pope, 1993). The bright red colour observed is due to the microvascular network throughout the granulation tissue (Tonnesen *et al.*, 2000). The basic fibroblast growth factor sets the stage for angiogenesis during the first three days of wound repair (Schäffer *et al.,* 2004) and plays an important role in granulation tissue formation and the wound healing process (Takehara, 2000). Granulation in all the cases

was flat without any exuberant nature; granulation tissue with a smooth surface facilitates the migration of epithelial cells (Pope, 1993). In cases 1,3,4,6,7,8,9, and 10 the granulation tissue was red in colour due to neovascularisation (Taylor & Bayat, 2003). Mal-odour was observed up to Day 12 in all cases (Taylor & Bayat, 2003). The presence of bacterial infection was the common cause for the mal-odour observed, because all the wounds were infected. Serous discharge was noticed up to Day 12 in cases 2 and 5, and mild serous discharge was noticed up to Day 8 in cases 4 and 9; subsequently, the discharge was reduced because healthy vascular granulation tissue is resistant to infection (Hosgood, 2003; Pope, 1993). On Day 4, epithelialisation was significantly better in all cases, because collagen from the wound margins began to migrate to form new epithelium (Rangaraj *et al.,* 2011).

The percentage of wound contraction on post wound Days 4 and 8 of all cases showed no significant difference, this may be because of an inherent property of fibroblasts that appears early in the process of wound contraction after

some time, do not contract as forcefully as those that appear later (Bohling *et al.*, 2004). All the cases showed a maximum mean percentage of total wound healing from Days 4 to 8; this is in concurrence with the results of Bohling *et al.* (2004) but differs from the findings of (Swaim *et al.*, 1993). *Staphylococcus aureus*, *Psudomonous, Klebsiellasp Escherichia coli* is the common infection in wounds (Kumar *et al.*, 2006). All wounds can be contaminated regardless of the precautions taken (Arul Jothi *et al*., 2006). Collagen-calcium alginate film is a biocompatible protein that does not interfere with the body's normal immunologic response and can be used in non-healing chronic wounds, which require a trigger to stimulate the normal healing process.

CONCLUSION

The present study has shown that collagen-calcium alginate film can be used as a wound-healing stimulant to promote the healing of chronic wounds in animals. This is a promising finding because collagen-calcium alginate film is an inexpensive biomaterial as a skin substitute to stimulate wound healing in animals where the cost of treatment is a major consideration.

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تقييم فعالية واقيات الكولاجين والكالسيوم ألجينات في التئام الجروح المزمنة في الخيول

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المستخلص : هدف الدراسة تقييم فعالية الكولاجين الكالسيوم ألجينات؛ لتسريع التئام الجروح في الخيول التي تعاني جرحا مزمنا غير قابل للشفاء، هذه الخيول كانت موجودة في المستشفى البيطري الجامعي (UVH) جامعة عمر المختار. تم تقييم نمط التئام الجروح على المستوى الإكلينيكي. أظهرت جميع الحالات تقدماً جيداً في الشفاء بعد دهن غشاء الكولاجين الكالسيوم ألجينات. اقترحت نتائج الدراسة أن فيلم الكالسيوم الكولاجين ألجينات يمكن أن يكون مادة حيوية أفضل في التئام الجروح في الخيول، ويمكن استخدامه بديلا جلديا أقل تكلفة لتحفيز التئام الجروح المزمنة التي لا تلتئم في الحيوانات.

الكلمات المفتاحية : المادة الحيوية ، الكولاجين الكالسيوم ألجينات ، التئام الجروح ، الجروح المزمنة.

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Carbon Stock Evaluation and Potential Carbon Market Value Determination of Ashaavieen Nature Reserve, Msallata, Libya

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Abstract: This study targets to assign the total carbon stock of Ashaavieen Nature Reserve, Msallata, Libya, assisting its potential carbon market value based on different global market sources. Northern and Southern sites were adopted representing the dominant tree species; (*Pinus halepensis, Ziziphus lotus,* and *Ceratonia siliqua*). The samples were conducted across the summer and winter of 2020 to get an annual average. These samples were classified into leaves, stems, bark (aboveground), and roots (belowground). The soil samples were collected at 0-10 cm, 10-20 cm, and 20-30 cm depth. Organic carbon content was assessed based on the Loss on Ignition method (LOI). The results have shown that the bark has recorded the highest carbon content rate, followed by the stem, leaf, and root. Also, the carbon in soil samples has a direct relationship with depth in the order of (0-10 cm) > (10-20 cm) > (20-30 cm). Total carbon stock was 870.47 (t/ha/yr) and 1858.21 (t/ha/yr) in Northern and Southern sites. Total tree coverage of the study area was estimated at 91.26 hectares, while the potential carbon market value ranged from 0.72 t0 32.09 million USD.

INTRODUCTION

Forest ecosystems could play a vital role in global carbon regulation and climatic change mitigation (Li et al., 2019). But, the rapid growth in social and economic levels frighteningly harms those ecosystems as it shrinks at alarming rates. Forestlands in the Arab Region cover nearly 6% of the total forest coverage worldwide. About 217 thousand ha. Libya containing 6 million tons of carbon in forest living biomass alone (El-Baha et al., 2010).

(FAO & UNEP, 2020) has emphasized that approximately 420 million hectares of forestlands were lost since 1990 due to conversion to other land uses. Mathematically, if this repulsive stress continues, all forest ecosystems would disappear in 290 years.

The significance of carbon stock evaluation comes due to the direct relationship between forest regression and carbon stock retraction. (Van der Werf et al., 2009) have reported that deforestation contributes approximately one-fifth of the yearly GHGs emissions.

Ashokri (2020) find out that *Pinus halepensis* is the most dominant species with an Importance Value Index (IVI) of 66.64%, followed by *Ziziphus lotus* and *Ceratonia siliqua* about 11.78% and 11.77%, respectively. The highest biomass value was *Pinus halepensis* at 7888.27 t ha⁻¹.

Aboveground, belowground biomass, and soil carbon are the most significant contributors to the total ecosystem carbon. The forest soil participates in more than two-thirds of the total carbon stored in the forest ecosystem due to a strong relationship between plant

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biomass and terrestrial carbon (Ahmed, 2018).

The global carbon market establishment is vital as an effective tool for global climatic change mitigating and testing the efficiency of climate protection. It is also to confirm that the significance of forest ecosystems is not limited by the goods they provide. Based on (Lin, 2020), the traded volume of global carbon markets has reached 8.7 billion tons worth about 260 billion USD. This study aims to determine the total carbon stock and its potential carbon market value of the study area.

MATERIALS AND METHODS

This study was carried out at the Nature Reserve of Ashaavieen, Msallata, Libya (32.589128º N, 13.865004º E) fig (1), which is about 90 km far from the capital city. The total area of Ashaavieen Reserve is about 496 ha (Ashokri, 2020). Two sites (N and S) were chosen based on the presence of the selected species. The samples were collected through summer and winter.

These samples were taken out from the living parts of four trees. These parts are the leaf, stem, bark, and root for each species (*Pinus halepensis, Ziziphus lotus,* and *Ceratonia siliqua*). The leaf samples were taken by the leaf-cutter (Corona TP 6881).

The stem samples were obtained via drilling until the stem center, while the bark samples were stripped with a keen knife at the chest height (1.5 m). These samples were dried at 70 ºC to ensure stable readings of weight. Then the samples were grinded and placed into plastic containers to be transferred to the Muffle Furnace. Plant biomass total carbon stock was estimated using the following equation:

*Organic Carbon Content (t ha⁻¹) = mean organic carbon content of the highest two living parts (%) * total Biomass (t ha⁻¹).*
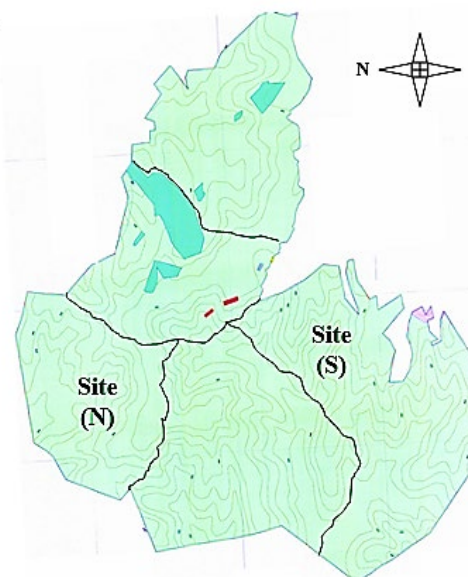
Figure: (1) Study Area (Alhusein et al., 2017).

The soil samples were collected using 5 cm in diameter PVC pipe from three depths (0-10 cm), (10-20 cm), and (20-30 cm) as scarce differences after the depth of 30 cm (Balesdent et al., 2018). The soil samples were dried and sieved using a 2 mm sieve. These samples were then kept in plastic containers before being placed into the Muffle Furnace. Soil Carbon content was estimated using the following formula:

Soil Carbon (Mg ha⁻¹) = bulk density (g cm⁻³) soil depth interval (cm)*C%.*

According to (Perera & Amarasinghe, 2013), the total carbon was calculated by adding up the mean carbon content of the living parts and the total soil carbon of the chosen year (t ha C yr⁻¹). The total carbon stock for the whole study area was estimated based on the following equation:

*Total Ecosystem Carbon Stock of a targeted area (Mg) = total carbon (Mg ha⁻¹) * Area (ha).*

Total tree coverage of the study area was estimated using ArcGIS 10.8

The total carbon stock of the ecosystem was converted into CO_2 equivalents (as CO_2 is the most common GHG in the atmosphere), and the following formula was utilized:

*Total Potential CO_2 Emissions per hectare (CO_2 e) = total carbon stock of the ecosystem * 3.67 (conversion Factor) based on (Kauffman & Donato, 2012).*

The potential carbon market value was calculated by multiplying the total ecosystem carbon stock with carbon unit price (based on the carbon unit price of the market used) as follows:
*Potential Carbon Market Value (USD) = total ecosystem carbon stock (Mg) * carbon unit price (USD)*

RESULTS & DISSCUSSION

The results indicated that the bark has the highest organic carbon content within living parts, around $61.12\% \pm 2.5$ at the Northern site and about $66.00\% \pm 4.01$ at the Southern site. In comparison, the roots have the lowest at $42.77\% \pm 1.12$ and $45.30\% \pm 1.82$ at Northern and Southern sites, respectively. The carbon content was high in the bark, followed by the stem, leaf, and root, respectively (Figure 2); this agreed with (Madeira et al., 2002).
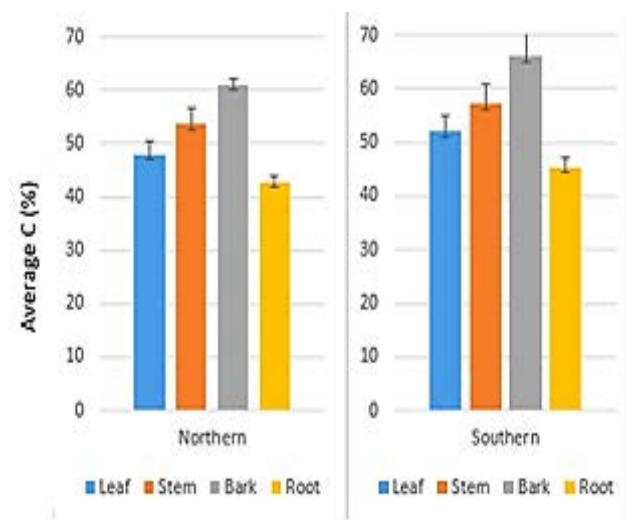
Figure: (2). Carbon Distribution in Living Parts.

Total biomass carbon stock was higher in the

Southern site than in the Northern Site at 322.50 and 190.42 (t/ha/yr). This could be a result of the positive relationship between biomass and carbon stock of an ecosystem (Wassihun et al., 2019).

The results also show that approximately 72% and 79% of the total carbon stock was stored in the soil. It is estimated at roughly 680.07 in N and 1535.71 (t/ha/yr) in S (Table: 1). This result is similar to (Edmondson et al., 2015) that indicates soil contributes around 75% of the total ecosystem carbon stock.

The contain of carbon storage in soil was high in upland soil (0-10 cm), then depth (10-20 cm) and depth (20-30 cm) as a consequence of the carbon content goes in parallel with depth (Lawrence et al., 2015).

The results illustrate that the assessment of carbon distribution over seasons was higher in summer than winter in both sites as primary productivity is related to temperature levels (Ontl & Schulte, 2012).

Table: (1). Ecosystem Carbon Stock in both Study Sites.

Carbon Stocks	Northern Site (t/ha/yr)	Southern Site (t/ha/yr)
Carbon Stock in Living Parts (ABG & BG)	190.42	322.50
Soil Carbon Stock	680.07	1535.71
Total	870.49	1858.21

The potential carbon market value is ranged from 0.72 to 23.09 million USD (Table 2) based on the estimation of the total tree coverage, which was around 91.26 ha.

Table: (2). Price of Carbon Stocks Based on Some Global Markets.

Ecosystem Carbon Stock (T C yr⁻¹)	Voluntary Market	GHGs Initiative	EU ETS
(N Site) 870.49*91.26 ha = (79440.91)	79440.41*6 USD = (746642.46)	79440.41*9.69 USD = (769777.57)	79440.41*191.80 USD = 15.23 mil)
(S Site) 1858.21*91.26 ha = (169580.24)	169580.24*6 USD = (1,02 mil)	169580.24*9.69 USD = (1.68 mil)	169850.24*191.80 USD = (32.57 mil)
(Average) 1319.35*91.26 ha = (120403.88)	120403.88*6 USD = (722423.28)	120403.88*9.69 USD = (1.16 mil)	120403.88*191.80 USD = (23.09 mil

CONCLUSION

The study concludes that organic carbon content in three parts was high in the bark, then stem, leaf and root. The soil carbon constitutes about three-quarters of the total ecosystem carbon stock and the highest amount in the third layer (20-30 cm). The carbon storage amount over seasons was higher in summer than in winter. The potential carbon market value of the study area was in the range of 0.72 – 32.09 million USD.

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تقدير مخزون الكربون وتحديد قيمته السوقية لمحمية الشعافيين الطبيعية، مسلاتة، ليبيا

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المستخلص: تهدف الدراسة إلى تحديد إجمالي مخزون الكربون في محمية الشعافيين الطبيعية، مسلاتة، ليبيا، وتحديد قيمتها المحتملة في سوق الكربون بناءً على مصادر السوق العالمية المختلفة. تم تحديد موقعين شمالي، وآخر جنوبي ويرمز لها بـ(N وS) لتمثلها أنواع الأشجار السائدة (*Pinus halepensis* و*Ziziphus lotus* و*Ceratonia siliqua*). تم أخذ العينات الموسمية خلال صيف، وشتاء لسنة 2020 للحصول على متوسط للسنة. كما تم تصنيف العينات المجمعة إلى أوراق، وسيقان، ولحاء (فوق الأرض)، وجذور (تحت الأرض). جمعت عينات التربة على عمق 0-10 سم، 10-20 سم، و20-30 سم. قدير محتوى الكربون العضوي بناءً على طريقة الخسارة في الاحتراق (LOI). أظهرت النتائج أن محتوى الكربون في الأجزاء الحية سجل اللحاء أعلى قراءة ثم ساق ورقة وجذر على الترتيب. كما أوضحت النتائج أن نسبة الكربون في عينات التربة له علاقة مباشرة مع العمق، وكان اعلى في عمق (0-10 سم) ويليه العمق (10-20 سم) ثم (20-30 سم). بلغ إجمالي مخزون الكربون في الموقعين الشمالي، والجنوبي 870.47 (طن / هكتار / سنة) و1858.2 (طن / هكتار / سنة) على التوالي. وقدرت الغطاء الشجري الإجمالي لمنطقة الدراسة بـ 91.26 هكتار بينما تراوحت القيمة السوقية المحتملة للكربون من 0.72 إلى 32.09 مليون دولار أمريكي.

الكلمات المفتاحية: محمية الشعافيين الطبيعية، مخزون الكربون، القيمة السوقية للكربون، الخسارة في الاحتراق.

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Cognitive Radio Networks Based on Users' Reputation Scheme

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Abstract: Cognitive Radio (CR) can be defined as a technology that allows users to change the transmission parameters as required to increase the spectrum efficiency. Because of this mechanism, some threats emerge. Two major threats are found in CR. The first is the Primary User Emulation Attack (PUEA), where the attacker is able to transmit at a forbidden time slot effectively, emulating the signals of the primary user. This makes all the system users believe that the spectrum is occupied by a good primary user. The second threat is known as the spectrum sensing data falsification attack (SSDF). In this case, the attackers send false observation information, intentionally or unintentionally, to the fusion center (FC), causing it to make the wrong decision. In this study, the scheme presented was based on a users' reputation for secure spectrum access in cognitive radio networks. Each Secondary User (SU) performs local sensing and then forwards the sensing results to the main fusion center FC. The FC makes the final decision about the presence of the primary user based on the proposed approach. The schemes substantially reduce the effect of cognitive users with low reputation values while improving the impact of cognitive users with the high reputation values on the final decision. It has been verified that the proposed approach can improve the sensing performance under the impact of a different number of reliable and unreliable users in a CR network. Results based on simulation show that the proposed scheme outperforms the traditional majority scheme despite a high number of malicious users.

INTRODUCTION

Spectrum sensing and spectrum sharing are important functionalities of CR, which enables the secondary users to monitor the frequency spectrum and detect vacant channels to use (Yadav et al., 2012). A procedure in the CR known as cooperation spectrum sensing involves many users that sense this spectrum and send reports to a base station known as a fusion center FC. The FC is able to process and manipulate those reports to make a final decision about the absence or presence of primary users. This kind of cooperation gives a chance to some adversary nodes that aim to falsify the results of the sensing (Pawelczak et al., 2006).

The disadvantages of cooperation spectrum sensing that compromise and limit the cooperation outcome are the control channel bandwidth, consumption of the energy, and reporting delay. Malicious users presented in the cooperation system will decrease the overall system performance (Cabrić et al., 2005; Zhao, 2007).

There are some previous contributions related to this work. For example, a method known as clustering and soften hard combination is

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presented to perform a significant trade-off between the overhead saving and performance increase. The cluster cooperative spectrum sensing has some disadvantages, such as in the case when the SUs that have good location correlation are grouped into the same cluster in order to decrease the consumption of the energy for data transition to the cluster-head CH. So, it is very likely that many SU within a cluster can be affected by shadowing or attacker's distribution. Hence CH may take incorrect group decisions about the primary's user availability and then send it to the fusion center misleading the final decision (Cabric et al., 2004; Yucek & Arslan, 2009). This paper uses both the terms 'trust' and 'reputation' to refer to the same meaning.

The mentioned problems are discussed in this paper and tackled by considering a reputation value for each user in the network, where the final decision is taken at the FC to increase the performance of the network. Based on this mechanism, the security of the cognitive network is improved by increasing the sensing performance.

COOPERATIVE SPECTRUM SENSING CHALLENGES

The challenge in cooperative spectrum sensing is to combine the detections of many nodes that might have different sensing times and different sensing results to make accurate detection of the spectrum. This leads to the development of a powerful sharing algorithm for cooperative spectrum sensing crucial to increase the detection performance in cognitive radio networks. A strong and smooth CR communication that combats malicious behaviors of users, trust management is important for SUs to assess the trustworthiness of users (Tkachenko et al., 2006; Yucek & Arslan, 2009).

R_i (reputation values) are performed by the FC and these values represent the trustworthiness for the ith CR user based on local

sensing difference D_i, sensing location factor P_i, and control channel condition C_i. This targets to reduce the impact of malicious CR users on the final fusion decision and improve the performance of cooperative spectrum sensing in CR networks. These values reduce the effect of cognitive users with low reputation values while increasing the impact of cognitive users with the high reputation values on the final decision.

CR SIMULATED NETWORK STRUCTURE

The scenario of the simulated CR network is presented in Figure 1. The network consists of:
- A number of good cognitive users (secondary users).
- A number of malicious users (attackers).
- A Fusion Center FC. The main task of the FC is to use the reports sent from the good and malicious users to decide on whether the primary user is present or not (Dutta & Arora, 2018).
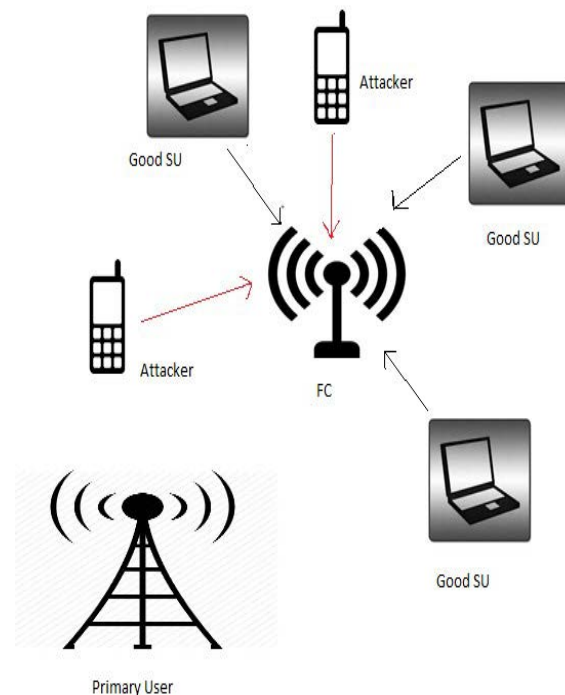
Figure (1). Cognitive radio simulated model

USERS' REPUTATION PROCEDURE

The reputation scheme makes a final decision about the presence of malicious users based on both the users' reputation values and the number of users, rather than the number of users alone. The algorithm in this scheme considers that highly trusted users contribute more to the final decision in order to make more accurate sensing. This approach presents a flexible reputation model for distributed cooperative spectrum sensing against malicious users by benefiting from the users' reputation that describes the behavioral characteristics of both malicious and good users. The algorithm is shown below. Each user has a different weight (reputation) depending on the type of CU. Good CUs are assigned with higher weights. Users with a reputation value over a predefined threshold are considered reliable users (good users).

Two hypotheses are presented in this work. If the primary user is present (H_1) or not present (H_0).
Some variables used in this algorithm are as follows:
L_{di} =Local decision of user i
N = The number of Secondary Users

MODEL ALGORITHM BASED ON USERS' REPUTATION SCHEME

The following algorithm represents the main steps of the proposed approach:
Begin
R_i = Reputation of user i, λ   Threshold of trustworthiness
L_{di} = Local decision of user i
 if L_{di} = 1 spectrum is occupied
 if L_{di} = -1 spectrum is unoccupied
R_i , i=1: to N
For i =1: to N do
If R_i >= λ then
User i is a reliable SU
        Add user i to reliable SU list
Else User i is a malicious user
        Add user i to Malicious users' list

End if
End for
For i=1: to N do
Take a final decision according to the equation below

$$decision = \begin{cases} H_1 \ if \ \sum_{i=1}^{N} L_{di} \ R_i \ > 0 \\ H_0 \ if \qquad otherwise \end{cases}$$

 End for

RESULTS AND DISCUSSION

MATLAB Program is used to simulate and test the proposed algorithm. First, it is important to compare our proposed scheme with the traditional majority scheme that takes the final decision based on a majority vote. Figure 2 shows the maximum number of malicious users the CR network can tolerate to make a correct decision based on the traditional scheme.

It's clear that in the traditional majority scheme, the maximum number of malicious users does not exceed 50% of all users. Thus, it is vital to design a procedure that can tolerate more malicious users in the network to make better decisions regarding the presence of the primary user.

Additionally, results based on our proposed scheme are presented. Let the reputation value of good SUs be denoted by α, and let the reputation value for malicious users be denoted by β. M is the total number of users, and finally, K is the number of malicious users.
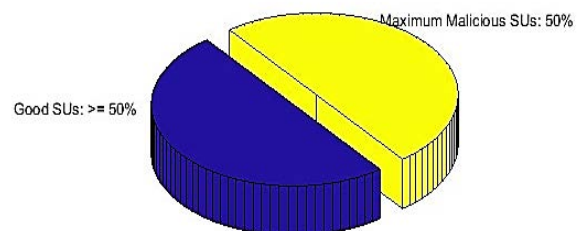
Figure (2). Users' ratio based on conventional role

Figure 3 shows the relationship between M and K. α and β in this case are chosen to evaluate the network under low-value parameters (α=0.6 and β=0.2). It's crucial to check how many malicious users the network can tolerate. For example, from figure 3, if the total number of users M is 29, it's found that the maximum number of malicious users the system can tolerate to make a correct decision is 22, which means there are only 7 good users who were able to make a correct sense.
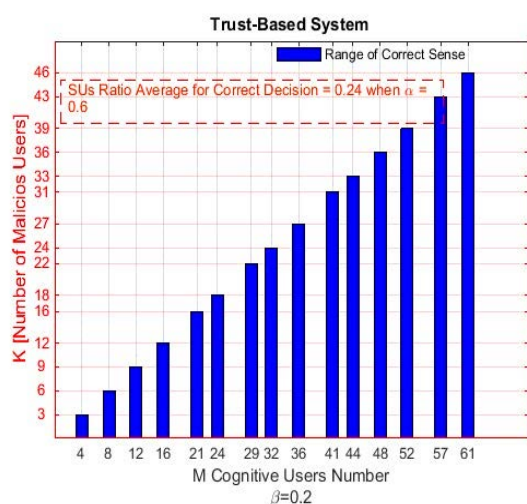
Figure (2)، Users number when α=0.6 and β=0.2

It is clear from figure 3 that the ratio of good SUs the system needs to make a correct sense is at least 24% of all users. This means that the system can tolerate about 76% of malicious users. Figure 4 shows the good users' ratio when α=0.6 and β=0.2 based on the proposed algorithm.
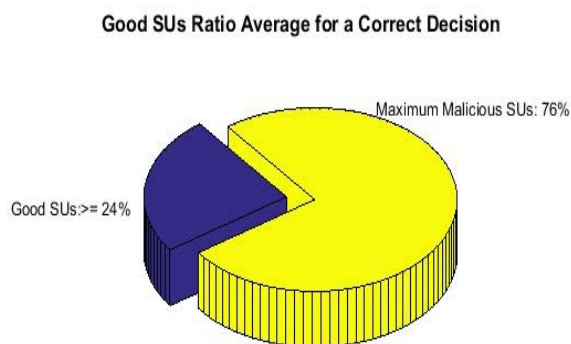
Figure (4). Users' ratio based on proposed trust approach

CONCLUSION

This paper has studied and focused on the users' trust mechanism because the users' trustworthiness is a crucial factor in the CR detection system. CRNs have a unique security problem that is not faced by a conventional wireless network. The main objective of any preventive security mechanism is to reduce and eliminate the impact of some operations performed by adversary users.

The proposed scheme outperforms the traditional majority scheme in terms of malicious user toleration. In fact, the traditional majority scheme can tolerate only about 50% of all users to make a correct decision while in the reputation-based scheme the system can tolerate about 76% of malicious users to make a correct final decision.

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شبكات الراديو المعرفية المعتمدة على ثقة المستخدمين

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المستخلص: يمكن تعريف شبكات الراديو المعرفية الذكية المعروفة بـ (CR) Cognitive Radio بأنها تقنية تسمح للمستخدمين بتغيير بيانات الإرسال على النحو المطلوب لزيادة كفاءة التردد. هذه الآلية تنتج بعض التهديدات الأمنية، هناك هجومان رئيسيان في CR ، الأول هو هجمات محاكاة المستخدم الأساسية (PUEA) Primary User Emulation Attack حيث يكون المهاجم غير الآمن، والمسمى بغير الشرعي قادرًا على الإرسال في فترات زمنية محظورة بحيث يحاكي بشكل فعال إشارات المستخدم الأساسي الشرعي مما يجعل جميع المستخدمين في النظام يعتقدون أن التردد مشغول من قبل مستخدم أساسي شرعي. والهجوم الثاني المعروف بهجمات تزوير بيانات استشعار التردد (SSDF) Spectrum Sensing Falsification Attack حيث يرسل المهاجمون معلومات استشعارية خاطئة قد تكون عن قصد، أو عن غير قصد إلى مركز الاندماج الرئيسي Fusion Center (FC) وهذا يجعل FC يتخذ قرارا خاطئا.في هذا العمل ، تم تقديم مخطط يستند إلى سمعة المستخدمين للوصول الآمن إلى التردد في شبكات الراديو المعرفية. يقوم كل مستخدم ثانوي (Secondary User SU) باستشعار محلي، ثم يقوم بإعادة توجيه نتائج الاستشعار إلى مركز الاندماج الرئيسيFC ، لذلك يتخذ FC القرار النهائي بشكل جماعي بشأن إذا ما كان التردد مشغولا من قبل مستخدم شرعي، أو غير شرعي. تقلل هذه المخططات المقترحة في هذا العمل بشكل كبير من تأثير المستخدمين الإدراكيين ذوي قيمة السمعة المنخفضة، مع تحسين تأثير المستخدمين الإدراكيين ذوي القيمة العالية للسمعة على القرار النهائي. تم التحقق من أن هذا النهج المقترح يمكن أن يحسن أداء الشبكة تحت تأثير عدد مختلف من المستخدمين الموثوق بهم وغير الموثوق بهم في شبكة CR. تظهر النتائج المستندة إلى المحاكاة أن المخطط المقترح يتفوق على مخطط الأغلبية التقليدي على الرغم من وجود عدد كبير من المستخدمين الضارين في الشبكة.

الكلمات المفتاحية: الراديو المعرفي، ثقة المستخدمين، المستخدم الأساسي، هجوم محاكاة المستخدم الأساسي، الاستشعار عن التردد التعاوني.

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Effect of pH, Sucrose Concentrations and Medium States on *in vitro* Rooting of Pineapple (*Ananas comosus* (L) Merr) cv Queen

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Abstract: The effect of medium states (liquid, semi solid, solid), pH (5.0, 5.5, 6.0, 6.5) and sucrose concentrations (10, 20, 30, 40 g/l) on *in vitro* rooting of pineapple cultured in full strength MS enriched with IBA at 0.5 mg/l were investigated. According to average overall sucrose concentrations, overall pH adjustments, and at each combination of equal sucrose and pH, liquid medium was always super than solid and semisolid. The tallest plantlets (66 to 71 mm) obtained in liquid medium enriched with sucrose at 10 and 20 g/l both adjusted to pH 6.0; sucrose at 20 g/l and adjusted to pH 6.5 and sucrose at 30 g/l and adjusted to pH 5.0. All of the above combinations except sucrose at 20 and pH 6.0 resulted in 100% rooting. Sucrose at 30 g/l and pH 5.0 resulted in two times more (11 roots per shoot) and three times longer roots (39 mm) than the other treatments (5 roots each about 14 mm long). Each rooting parameter had different optimum combinations of medium state, sucrose and pH adjustment. For any parameter, proper pH adjustment could reduce the optimal sucrose enrichment from 30 to 20 and even to 10 g/l. Hence, pH adjustment is suggested as an important approach for reduction of *in vitro* rooting medium cost.

INTRODUCTION

In vitro rooting of pineapple were reported in medium solidified with phytagel at 2.5 g/l (Ko et al., 2006), agar at 6 (Rahman et al., 2001), agar at 7 (De Almeida et al., 1997) and 8 g/l (Akbar et al., 2003), in liquid medium using filter paper bridged (Soneji et al., 2002), sponge matrix (Gangopadhyay et al., 2005), Luffa matrix (Dutta et al., 2013), in static liquid medium; (Be & Debergh, 2006; Hamad et al., 2013 ; Hamad, 2019) and *ex vitro* in potting mix (Escalona et al., 1999; Liu et al., 1989). The effect of both of liquid and solid medium at full and half strength enriched with different auxin types and concentrations on the *in vitro* rooting of pineapple were compared (Hamad et al., 2013). Several other factors such as different combination of hormone types, concen-trations and medium strength (Bhatia & Ashwath, 2000; Firoozabady & Gutterson, 2003), shoot ages

and cultivars (Hamad et al., 2013) , mix of commercial sugar with sucrose (Dutta et al., 2013) were found to induce significant effect on the *in vitro* rooting responses of pineapple. Medium pH adjustment in all of these cases was kept fixed at pH 5.7, and sucrose enrichments, on the other hand, most of the cases kept fixed at 30 g/l. In some cases, sucrose at 20 (Ko et al., 2006; Sunitibala Devi et al., 1997) and 40 g/l (De Almeida et al., 1997) in solid medium and at 20 (Soneji et al., 2002) and 35 g/l \ (Kofi & Adachi, 1993) in liquid medium was used for rooting.

In few cases the sucrose effect on rooting was compared at 2 \ (Mengesha et al., 2021), 3 (Zaied, 2007) and 4 (Almobasher, 2016; Hassan et al., 2018) different concentrations in solid medium and at 2 (Be & Debergh, 2006) and 6 (Hamad, 2019) different concentrations in liquid medium. (Hassan et al., 2018; Zaied, 2007) reported that rooting occurred in solid MS medium with no sucrose

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enrichment but the best rooting was in medium enriched with sucrose at 20 g/l. On the contrary, (Mengesha et al., 2021) reported that no rooting could be obtained and shoots died in solid MS devoid of sucrose and (Almobasher, 2016) not only reported no rooting in solid MS medium devoid of sucrose but also in medium enriched with sucrose at 10 g/ l and both found best root formation and length in medium enriched with sucrose at 20 g/l. (Dutta et al., 2013) recommended mix of commercial sugar at 2% with sucrose at 1 % in liquid with luffa support and Be and (Be & Debergh, 2006) recommended sucrose enrichment of 30 g/l for liquid MS medium. Hamad (2019) found that no rooting could be obtained in liquid MS medium enriched with sucrose at 5 g/ l and the optimum concentration ranged from 10 to 30 g/ l depending on the rooting parameters used for evaluation and varied according to the length of incubation period. In all of these studies of pineapple *in vitro* rooting, the medium pH was fixed at 5.7. The effect of different combinations of sucrose, medium states and pH adjustments still yet to be tested.

Cost of rooting stage (Hamad, 2019) was expected to be three times cost of multiplication stage (Hamad, 2017a and b). In large scale production, the cost of medium during rooting could be decisive factor for feasibility of micropropagation. Sucrose and agar made the largest portion of the medium components and are very important items of the medium cost. Rooting could occur in solid and liquid medium. That is complete elimination of agar cost is possible. On contrary, sucrose is an obligatory requirement for *in vitro* rooting and indispensable component of the medium (Almobasher, 2016; Mengesha et al., 2021 ; Hamad, 2019). Reduction of sucrose cost could be done either by using of cheap sucrose alternatives (Dutta et al., 2013; Mengesha et al., 2021; Nelson et al., 2015) or by using the most possible minimum concentration. In fact, (Dutta et al., 2013) reported

that compared to solid media enriched with sucrose at 30 g/l using of mix of commercial sugar with sucrose in liquid media with luffa matrix reduced the chemical cost of multiplication by 15 % and rooting cost by 62 %. Sucrose is just a one of several factors that could have independent and interaction effect on rooting (Hamad, et al 2013; Hamad, 2019). Minimum sucrose concentration that would maintain highest response for each rooting parameter is expected to be different at different combinations of other root effecting factors such as medium state and pH adjustments. The objective of this study is to compare the effect of different pH adjustments (5.0, 5.5, 6.0 and 6.5) and agar concentrations (0.0, 3.5, 7.0 g/l) on the rooting responses of Queen pineapple to different concentrations of sucrose (10, 20, 30, 40 g/l). The goal is to determine the pH of liquid medium that maintained the highest rooting responses at lowest sucrose concentration.

MATERIALS AND METHODS

One and half liter of MS medium (Murashige & Skoog, 1962) was enriched with IBA at 0.5 mg/l and divided into 4 beakers each received 312 ml and marked A, B, C, and D. Sucrose at 10, 20, 30 and 40 g/l were added to beakers A, B, C and D respectively. The content of each beakers divided equally (78 ml) into 4 glass jars marked with the same beaker symbol plus 1, 2, 3 and 4. The content of jars numbered 1, 2, 3 and 4 adjusted to pH 5.0, 5.5, 6.0 and 6.5 respectively. Then the content of each jar divided equally (26 ml) into another 3 glass jars marked with same symbol plus H, S and L. Agar at 3.5 and 7 g/l added to glasses marked with H and S respectively, and the glass marked L without agar. The glasses covered with autoclavable plastic lid and the medium was then sterilized at 121° C and 1.5 kg/cm^2 for 25 minutes. The content of each glass dispensed under laminar into 3 culture tubes marked as glass. Three shoots from stock cultures were cultured per each culture tube. The cultures incubated un-

der photo-period of 16 hours of light and constant temperature of 25° C. After 60 days of incubation, the cultures removed from the incubation room, the shoots picked out of the cultures and placed over squared paper, for counting of the roots and measuring the root and shoot length. Each tube was considered as one replicate and the data except shoot length (plantlet height) were transformed to square roots (x+1) before analysis of variance. Analysis of variance and Duncan Multiple Range Test for significant of treatments at $p \le 0.05$ were computed using SPSS statistical package No. 11.

RESULTS

Analysis of variance (Table 1) showed that sucrose had significant independent (direct) and dependent (indirect) effect via interaction with pH in all rooting parameters, and dependent (indirect) effect via interaction with agar (medium state) in two of the rooting parameters (root number and plantlet height). But the interaction in case of the other two parameters (rooting percentage and root length) was not significant. Medium state (agar) had significant independent effect (direct) in all rooting parameters except rooting percentage and dependent (indirect) effect via interaction with sucrose in two of rooting parameters (root number and plantlet height). Medium pH, on the other hand, did not have significant independent (direct) effect on any of the rooting parameters and did not interact significantly with agar concentrations (medium state). However, medium pH did have dependent (indirect) significant effect via interaction with sucrose in all rooting parameters, and via collective interaction with both of sucrose and agar (medium states) on all rooting aspects except the plantlets height. Overall sucrose concent-rations and pH adjustments (Table 2), liquid medium resulted in taller plantlets (51.06 mm), more (5.38 roots) and longer roots (18.25 mm) than solid medium (39 mm tall plantlets, 3.13 roots per shoots, 10.96 mm long each) but the rooting

percentage of liquid (69.4 %) and solid (59 %) medium were not significantly different (Table 2). Similar, overall medium states and pH adjustments, the largest number (5.4 roots), longer roots (16.25 mm) and highest rooting percentage (76.3 %) obtained in medium enriched with sucrose at 20 and 30 g/l. However, sucrose at 20 g/l resulted in tallest plantlets (52.8 mm) and sucrose at 30 g/l resulted in an intermediate plantlet height (48.08 mm). Sucrose at 10 and 40 g/l resulted in the lowest rooting percentage (47.0 %), fewest (2.1 roots) and shortest (6.7 mm long) roots, but the plantlets on medium enriched with sucrose at 10 g/l was taller (42.33 mm) than that in medium with 40 g/l (31.9 mm). Overall medium states and sucrose, the pH adjustments of medium had no significant effect on any of the rooting parameters. On the other hand, overall pH adjustments, liquid medium enriched with sucrose at 30 g/l resulted in highest rooting percentage (88 %) and more (8.8 roots) and longest roots (29 mm) while semi solid enriched with sucrose at 10 g/l resulted in lowest rooting percentage (31.3 %), and fewest (1 root per shoot) and shortest roots (5.3 mm). However, the tallest plantlets (63 mm), on the other hand, obtained in liquid medium enriched with sucrose at 20 g/l while the shortest plantlets (28 and 30 mm) obtained in semi solid and liquid medium enriched with sucrose at 40 g/l. No significant different on plantlet height on solid medium enriched with sucrose at 10 and 40 g/l and semi solid enriched with sucrose at 10 g/l

Comparing of all combinations of sucrose, pH and medium states (Table 2) showed that the tallest plantlet (70 and 71 mm) was obtained in liquid medium enriched with sucrose at 10 and 20 g /l and adjusted to pH 6.0 and the most stunted plantlets (15.0 mm) obtained on semi solid enriched with sucrose at 40 g/l and adjusted to pH 5.0 and pH 6.0. On the other hand, all shoots (100 %) could be rooted in liquid media enriched with sucrose at 10 g/l and adjusted to pH 6.0; sucrose at 20

g/l and pH 5.0 and pH 6.5; sucrose at 30 g/l and pH 5.0 and on semi solid media enriched with sucrose at 20 g/l and adjusted to pH 6.5; sucrose at 30 g/l and pH 5.0 and sucrose at 40 g/l and pH 6.5. However, 100 % rooting obtained in liquid medium could be reversed to 22 % if enriched with sucrose at 10 g/l and adjusted to pH 5.5 and that of semi solid reversed to 11 % if enriched with sucrose at 40 g/l and adjusted to pH 5.0 and 6.0. Contrary, none of the combinations in which solid medium was used irrespective of sucrose and pH and none of the combination in which the media adjusted to pH 5.5 irrespective of sucrose and medium states resulted in 100 % rooting. The highest rooting percentage in solid media was 89 %, obtained in medium enriched with sucrose at 20 g/l and adjusted to pH 5.0 and sucrose at 30 g/l and pH 5.0 and pH 5.5. Highest root formation (10-11 roots) obtained in liquid medium enriched with sucrose at 20 and 30 g/l and adjusted to pH 5.0 and pH 5.5 and in solid medium enriched with sucrose at 30 g/l and adjusted to

pH 5.0. The lowest root formation (1 root) obtained in almost all (75 %) of the combinations in which the sucrose enrichment was 10 g/l and in 50 % of the combinations in which sucrose enrichment was 40 g/l. The longest root (39 mm) obtained on liquid medium enriched with sucrose at 30 g/l and adjusted to pH 5.0 and the shortest (3.0 mm) on semi solid medium enriched with sucrose at 10 g/l and adjusted to pH 6.5; sucrose at 40 g/l and adjusted to pH 5.0 and pH 6.0 and on solid medium enriched with sucrose at 10 g/l and adjusted to pH 5.5.

Table. (1). Significant of main and interaction effect of medium states, sucrose concentrations and pH of full strength MS medium on the *in vitro* rooting of Queen pineapple.

Factors	df	Rooting parameters (p values)			
		Plantlet height	Rooting %	Root No.	Root length
Medium states	2	0.0001**	0.1310	9.09E-07**	5.14E-05**
Sucrose conc.	3	5.19E-08**	0.0001**	1.53E-12**	7.59E-09**
pH	3	0.8404	0.5973	0.4395	0.1909
State*Sucrose	6	0.0103*	0.4861	0.0055**	0.1382
State*pH	6	0.5682	0.2698	0.2666	0.4854
Sucrose*pH	9	1.07E-05**	0.0238 **	1.23E-06**	0.0004**
State*Sucrose*pH	18	0.2315	0.0085**	2.39E-05**	0.0038**
Error	96				
Total	144				

Table (2). Effect of medium states, sucrose concentrations and pH of full strength MS medium on the *in vitro* rooting of Queen pineapple

Medium states (Agar/l)	pH	Sucrose (g/l)				
		10	20	30	40	Average
Plantlet height (mm)						
Liquid	5	55 bc	56 bc	66 ab	22 ef	49.75 AB
(0.0 g/l)	5.5	61 abc	57 bc	60 abc	22 ef	50 AB
	6	70 a	71 a	56 bc	31 cdef	57 A
	6.5	38 cde	68 ab	38 cde	46 cd	47.5 AB
	Average	56 AB	63 A	55 AB	30.25 D	51.06
Semi solid	5	52 bcd	53 bc	56 bc	16 f	44.25 AB
(3.5 g/l)	5.5	32 cdef	42 cd	55 bc	24 ef	38.25 B
	6	38 cde	65 ab	48 bcd	15 f	41.5 AB
	6.5	27 def	52 bcd	27 def	58 abc	41 AB
	Average	37.25 CD	53 AB	46.5 BC	28.25 D	41.25
Solid	5	33 cdef	36 cde	51 bcd	32 cdef	40.5 AB
(7 g/l)	5.5	28 def	42 cd	56 bc	27 def	38.25 B
	6	30 cdef	43 cd	27 ef	42 cd	35.5 B
	6.5	44 cd	39 cde	37 cde	48 bcd	42 AB
	Average	33.75 CD	42.5 BCD	42.75 BCD	37.25 CD	39.06
	Grand aver	42.3	52.8	48.08	31.91	43.7
Rooting %						
Liquid	5	44.3 abcde	100 a	100 a	33.3 abcde	69.4 NS
(0.0 g/l)	5.5	22.3 de	89 abc	96.3 ab	37 abcde	61.2 NS
	6	100 a	44.3 abcde	77.7 abcd	78 abcd	75 NS
	6.5	33.3 abcde	100 a	78 abcd	78 abcd	72.3 NS
	Average	49.98 BCD	83.08 AB	88.0 A	56.58 ABCD	69.41
Semi solid	5	66.7 abcde	77.7 abcd	100 a	11 e	63.8 NS
(3.5 g/l)	5.5	22 bcde	55.7 abcde	55.7 abcde	44.3 abcde	44.4 NS
	6	22 bcde	89 abc	77.7 abcd	11 e	49.9 NS
	6.5	14.7 de	100 a	55.7 abcde	100 a	67.6 NS
	Average	31.3 D	80.6 AB	72.3 ABC	41.6 CD	56.42
Solid	5	33.3 abcde	89 abc	89 abc	44.3 abcde	63.9 NS
(7.0 g/l)	5.5	22 bcde	77.7 abcd	89 abc	55.3 abcde	61 NS
	6	44.3 abcde	77.7 abcd	44.3 abcde	89 abc	63.8 NS
	6.5	78 abcd	33.3 cde	33.3 cde	44.3 abcde	47.3 NS
	Average	44.4 CD	69.4 ABC	63.9 ABCD	58.3 ABCD	59
	Grand Aver	41.89	77.69	74.78	52.16	61.63
Root number						
Liquid	5	1 hi	10 ab	11 a	1 hi	5.75 AB
(0.0 g/l)	5.5	1 hi	10 ab	11 a	3 defgh	6.25 A
	6	5 abcdef	3 defgh	7 abcd	4 cdefg	4.75 B
	6.5	1 hi	6 abcde	6 abcde	6 abcde	4.75 B
	Average	2 CDE	7.3 AB	8.8 A	3.5 CD	5.38
Semi solid	5	1 hi	3 defgh	8 abc	0 i	3 CD
(3.5 g/l)	5.5	1 hi	2 efghi	3 defgh	2 efgh	2 D
	6	0 i	7 abcd	6 abcde	1 hi	3.5 BC
	6.5	0 i	5 abcdef	2 efgh	4 cdefg	2.75 CD
	Average	0.5 E	4.25 CD	4.75 BC	1.75 DE	2.81
Solid	5	1 hi	5 bcdef	11 a	1 hi	4.5 B
(7 g/l)	5.5	1 hi	2 efgh	7 abcd	4 cdefg	3.5 BC
	6	2 efgh	2 efgh	1 hi	6 abcde	2.75 CD
	6.5	4 cdefg	1 hi	1 hi	1 hi	1.75 D
	Average	2 CDE	2.5 CDE	5 BC	3 CD	3.12
	Grand aver	1.5	4.68	6.18	2.75	3.77

Medium states	pH	Sucrose (g/l)				
(Agar/l)		10	20	30	40	Average
Root length (mm)						
Liquid	5	14 bcd	24 abc	39 a	8 cde	21.3 A
(0.0 g/l)	5.5	9 cde	27 abc	28 abc	6 cde	17.5 AB
	6	16 abcd	12 bcd	37 ab	12 bcd	19.3 A
	6.5	4 de	20 abcd	12 bcd	25 abc	15.3 AB
	Average	10.8 CD	20.5 AB	29 A	12.8 BCD	18.25
Semi solid	5	9 cde	14 bcd	24 abc	3 e	12.5 AB
(3.5 g/l)	5.5	4 de	11 bcd	8 cde	7 cde	7.5 B
	6	5 de	25 abc	14 bcd	3 e	11.8 AB
	6.5	3 e	25 abc	8 cde	15 bcd	12.8 AB
	Average	5.3 D	18.75 AB	13.5 BC	7 CD	11.15
Solid	5	10 bcd	15 bcd	26 abc	6 cde	14.3 AB
(7.0 g/l)	5.5	3 e	14 bcd	24 abc	7 cde	12 AB
	6	7 cde	11 bcd	6 cde	14 bcd	9.5 AB
	6.5	12 bcd	7 cde	7 cde	6 cde	8 B
	Average	8 CD	11.8 BCD	15.75 BC	8.3 CD	10.96
	Grand aver	8.03	17.01	19.41	9.36	13.45

Means of each rooting parameter followed by small letters and overall average followed by capital letters were not significantly different at p ≤ 0.05 according to Duncan Multiple Range Test.
NS. (Not significant).

DISCUSSION

Previous reported studies of *in vitro* rooting assessed the rooting treatment based on one parameter, rooting percentage (Bhatia & Ashwath, 2000), root number (Danso et al., 2008). In some cases two parameters, root number and length (Almobasher, 2016; Aydieh et al., 2000; Khatun et al., 1997) and in few cases three parameters: rooting percentage, root number and length (Amin et al., 2005; Soneji et al., 2002) were used for assessment of rooting treatments. Results (Table, 2) showed that assessment of rooting treatment based on one or two parameters could not be claimed as best rooting treatment. Different combinations of sucrose, pH and medium state could be recommended based in which parameter was used for assessment of treatments. Out of 48 combinations, seven resulted in 100 % rooting, five in tallest plantlets (65 to 71 mm), five in highest root number (10 – 11 roots) and two in longest roots (37, 39 mm). Not only at any fixed pH and medium state, different rooting parameters have different optimal sucrose enrichment and each single rooting parameter could have several optimum combinations of pH, medium state and sucrose enrichment but also combination which optimum for one parameter could suppress or promote another one, two or three rooting parameter. Two combinations (liquid enriched with sucrose at 10 g/l, pH 6.0; sucrose at 20 g/l, pH 6.5) was optimal for rooting percentage (100 %) and plantlet height (68 and 70 mm) but suppressed root number from 11 to 5 and 6 roots and root length from 39 to 16 and 20 mm respectively. Other two combinations (liquid enriched with sucrose at 20 g/l pH 5.0 and sucrose at 30 g/l, pH 5.5) was optimum for rooting percentage (100 and 96 %) and root number (10 and 11 roots) but suppressed the plantlet height from 70 to 56 and 60 mm and root length from 39 to 24 and 28 mm respectively. Semi sold medium enriched with sucrose at 20 g/l and adjusted to pH 6.5, sucrose at 30 g/l and pH 5.0 and sucrose at 40 g/l and pH 6.5 was optimum only for rooting percentage (100 %). Liquid medium enriched with sucrose at 20 g/l and adjusted to pH 6.0 was optimum only for plantlet height (71 mm). Liquid medium enriched with sucrose at 20 and 30 g/l and adjusted to pH 5.5 and solid medium enriched with sucrose at 30 g/l and adjusted to pH 5.0 was optimum only for root number (10 to11 roots). Liquid medium

enriched with sucrose at 30 g/l and adjusted to pH 6.0 was optimum only for root length (37 mm).

Generally, *in vitro* rooting is done for propagation purposes or physiological studies and best treatment should be judged based on parameters that serve the goals of propa-gators and physiologist. For propagation purposes selection of rooting treatment will be in favor of low cost and particular parameter quality which is very essential for higher survival of acclimatization. Assess-ment based on one parameter will be in favor of liquid over solid medium; lower over higher sucrose; shorter and fewer over longer and more roots for easy handling of acclimatization stage. If assessment based on rooting percentage (100 %) the cheapest combination was sucrose at 10 g/l and pH 6.0 and if for tallest plantlets (70 mm) the cheapest was also liquid medium enriched with sucrose at 10 and pH 6.0. For highest roots number (10 roots), the cheapest one was liquid medium enriched with 20 g/l and adjusted to pH 5.0 and 5.5. Two treatments resulted in longest roots, 39 and 37 mm. (liquid enriched with sucrose at 30 g/l adjusted to pH 5.0 and pH 6.0) and both were of equal cost (equal sucrose enrichment). If, more than one parameters included for assessment of rooting treatments, the choice will be compromise between possible best response of both parameters and low cost of the treatment. For both of rooting percentage (100 %) and plantlet height (70 mm), the best conciliation for both parameters, and low cost would be liquid medium enriched with sucrose at 10 g/l and adjusted to pH 6.0. For rooting percentage (100 %) and root number (10 roots), the best compromise between best results and low cost would be liquid medium enriched with sucrose at 20 g/l and adjusted to pH 5.0. None of the combinations was best for three and only one combination (liquid medium enriched with sucrose at 30 g/l and adjusted to pH 5.0) was the best compromise for all of four rooting parameters (100 % rooting, 66 mm tall plantlets, 11

roots, 39 mm long). It is clear that in liquid medium, simple manipulation of medium pH could reduce the optimum sucrose from 30 g/l to 20 g/l by adjusting the medium to pH 6.5 and even to 10 g/l by adjusting to pH 6.0. Obtaining 100 % of rooting and 70 mm tall plantlets with 5 roots each 16 mm long in liquid medium enriched with sucrose at 10 g/l by adjusting the medium to pH 6.0 is very important approach for reduction of *in vitro* rooting cost. It is simpler and easier approach than using of cheap sucrose alternative at fixed pH of 5.7 as mean of cost reduction in micropro-pagation of pineapple (Dutta et al., 2013; Mengesha et al., 2021; Nelson et al., 2015), banana (Kodym & Zapata-Arias, 2001) and several plant species (Gangopadhyay et al., 2002).

Future investigation of combinations of low concentration range of 5 to 20 g/l of cheap sucrose alternative and wider pH range of 3.5 to 8.0 may lead to optimum sucrose enrichment lower than 10 g/l, and substantial reduction in cost of rooting medium and worth being tested. Since cost of rooting (Hamad, 2019) is expected to be about three times cost of multiplication (Hamad, 2017a and b) any effort for minimizing the cost of rooting will be very important for reduction of total cost of micropropagation. In large scale production any small reduction of sucrose enrichment per liter of medium could be turning point for commercially feasible micropropagation system.

In most of *in vitro* rooting studies the relation between the rooting parameter and survival during acclimatization was not tested. A little attention was paid to determine which rooting parameter is more important than the others for survival, and what are the lower limit of the parameter required for survival. Nevertheless, (Escalona et al., 1999) reported that the survival percentage of *ex vitro* acclimatized rootless shoots increased from 20 to 100 % as the size of the shoots increased from 20 to 80 mm long (Be & Debergh, 2006; Dal

Vesco et al., 2001; DeWald et al., 1988; Ko et al., 2006; Soneji et al., 2002) respectively reported that over 90 % of 35, 50, 60, 70 and 80 mm tall plantlets survived acclimatization stage. For pineapple, plantlet height is probably more crucial for acclimatization survival. However, the effect of different rooting treatment on the plantlet height was rarely reported (Hamad, 2019; Be & Debergh, 2006; Hamad et al., 2013; Hassan et al., 2018), and was not tested as factor for survival of acclimatization stage. For better selection of treatments and proper micropropagation system, rooting and acclimatization treatments should be evaluated in connection with each other. After rooting stage the plantlets should be separated into groups according to root number, root length and plantlet height before being subject to acclimatization treatments. Assessment of rooting. Treatments should not only base on comparison of parameters, but in which rooting treatment and which rooting parameter resulted in highest acclimatization survival. Selection of best rooting treatment should be based on particular parameter with specific quality, which result in highest survival than the other parameters.

Medium pH adjustment determined the optimum sucrose enrichment for each rooting parameter on different medium states (Table, 2) and could switch the nature of sucrose and medium state interaction from enhancing to retarding rooting responses. In all medium states (liquid, semi solid and solid) in which the pH was adjusted to 5.0 and 5.5, the optimum sucrose for rooting %, root number, root length and plantlet height was 30 g/l. However, if the medium pH was adjusted to 6.0, the optimum sucrose for rooting responses in solid and semi solid was 20 g/l, while in liquid medium the optimum sucrose was 10 g/l. If the medium pH adjusted to 6.5, the optimum sucrose for rooting response in liquid and semi solid was 20 g/l, while in solid medium was 10 g/l. Adjustment of semi solid medium pH to (5.0 and 6.0) and enrichment

with low (10 g/l) and high sucrose (40 g/l) promoted rooting percentage but retarded root number while in liquid medium improved both process of rooting % and root number. Solid medium, on the contrary, suppressed root number if enriched with sucrose at 10 and 40 g/l and suppressed rooting percentage but improved root number if enriched with sucrose at 20 and 30 g/l. Semi solid, on the other hand, improved both process of rooting percentage and root number. All shoots in liquid medium enriched with sucrose at 10 g/l and adjusted to pH 6.0 and sucrose at 30 g/l and adjusted to pH 5.0 rooted (100 %) and developed into 66 and 70 mm tall plantlets. However, the second treatment resulted in two times (11 roots) more and longer (39 mm) roots than the first treatment (5 roots, 16 mm). Shoots cultured in liquid medium enriched with sucrose at 20 g/l and adjusted to pH 6.0, and in medium enriched with sucrose at 10 g/l and adjusted to pH 5.5 developed in taller plantlets (71 and 61 mm) but these treatments failed to induce more than 45 % rooting. Being seven combinations resulted in 100 % rooting but in different number and length root, implied that root initials might have been formed in all of these combinations, but its growth arrested under some combinations of sucrose and pH and promoted under others. Low and high sucrose did not support root development. Histological study of apple *in vitro* rooting showed that initiation of root initials depended on IBA while development and growth of roots depended on sucrose enrichment (Harbage et al., 1993).

At fixed sucrose of 10, 20 and 30 g/l, increasing pH up to 6.5 decreased rooting percentage. while at fixed pH of 5.0, 5.5, 6.0 and 6.5 increasing sucrose enrichment up to 30 g/l increased rooting percentage. However, on the contrary at fixed sucrose of 40 g/l increasing medium pH up to 6.5 cause an increase of rooting percentage. This indicated that higher sucrose enrichment suppressed rooting and that suppression could be allevi-

ated by increasing medium pH. Increasing of rooting in medium enriched with sucrose at 40 g/l by increasing the medium pH indicated that the pH interference kept sucrose accessibility and /or absorbed amount of sucrose below the limit that retard root initiation and development. Statistical analysis (Table 1) showed that sucrose had independent significant effect on rooting percentage and interacted significantly with pH, while pH did not had significant independent effect on rooting percentage. Medium state, on other hand, neither had independent effect nor interacted with sucrose or with pH. Root number and root length, on the other hand, was under independent effect of both sucrose and medium state and interaction of sucrose with pH and sucrose with medium state.

On the same time, all of the three parameters was not effected by pH and interaction of pH with medium state but all of the three rooting parameters (rooting %, root number and root length) was under collective significant interaction of the three factors (sucrose medium state and pH). This indicated that the collective interaction occurred in two steps: First, the sucrose interacted with pH to produce an intermediate product or condition that trigger initiation process of root premordia (significant effect in rooting percentage). Second, medium state interacted with the product or condition resulted from sucrose pH interaction and facilitate root growth and development. Neither the effect of medium state alone nor the interaction of medium state with sucrose or pH on the rooting percentage was significant (Table, 1) Medium states. did not effects the process of root premordia initiation, but facilitated root growth and penetration of internal tissues of the shoots. Once emerged, the root elongation in liquid was faster than in solid media. The importance of medium pH for *in vitro* rooting is not understood yet. The effect varied among different plants and media. Lowering of pH from 5.7 to 4.7 reduced the rooting percentage of Geraldton wax from 63 to 20 % (Page &

Visser, 1989), while lowering the pH from 5.7 to 4.0 increased the rooting percentage of Australian woody plants from 28 to 100 % (Williams et al., 1985). In a solid WPM medium enriched with sucrose at 30 g/l, lowering the pH from 5.7 to 3.5 decreased the rooting of *Choisya ternata* by up to 60 % and Delphinium by 15 % (Leifert et al., 1992). At fixed concentration of sucrose (30 g/l), using of solid and liquid MS medium and adjusting the pH to range of 4.2 up to 6.2 did not affect the rooting percentage of *Maranta leuconeura* cv Kerchoviana (Bennett et al., 2003; Ebrahim & Ibrahim, 2000) reported that the lower rooting percentage (62 %) and few roots (4 roots) of *Eucalyptus glabulus* were mainly due to presence of NHNO. In medium devoid of NHNO, rooting increased to 94% and roots to 7 roots per shoot over pH range of 4.0 to 6.0. (Harbage et al., 1993) noticed that the optimal pH for root formation of Gala apple varied at different concentration of IBA. Increasing the sucrose concentration shifted the *in vitro* rooting dose-response curve of Jork 9 apple to auxin to the right (Calamar & De Klerk, 2002). Our results indicated that for *in vitro* rooting of pineapple, the rooting dose response curve to sucrose could be shifted by medium pH and the sucrose concentration could be minimized by adjusting the medium to proper pH.

Most of the reported rooting studies used either solid or liquid medium at full or half strength at fixed sucrose of 30 g/l and pH 5.7 and recommended different concentration and combination of rooting hormones. For assessment of rooting treatments some used only one parameter and others used two or three rooting parameters and come up with different recommendations for *in vitro* rooting depending in which parameter was used for assessment and which factors was included in testing of rooting. In this study, liquid medium enriched with sucrose at 30 g/l and adjusted to pH 5.0 was optimum for all rooting parameters, (100 % rooting, 66 mm tall plantlet with11 roots per shoot each 39 mm long.

However, none of the treatments recommended in previous studies or in this study could be adopted as a universal treatment for rooting unless the cost of rooting kept at its lowest. The crucial rooting parameter for survival of acclimatization identified, and the mode of the factor effect on each of the three physiological steps of rooting elucidated. Future studies of *in vitro* rooting should focus in determining the relation between rooting parameters and percentage of survival during acclimatization. The two stages should be studied in connection with each other. Determining of optimum combination for each single rooting parameter as done in this study will help in selection of the most proper treatment and best timing for histological, physiological and biochemical study of root formation steps.

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تأثير درجة الحموضة وتركيز السكروز وحالة الوسط على التجذير المخبري للأناناس صنف كوين

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المستخلص: تم بحث تأثير حالة الوسط (سائل، صلب وشبه الصلب)، ودرجة الحموضة (5.0، 5.5، 6.0 و6.5)، وتركيز السكروز (10، 20 ، 30 و40 جرام / لتر) على تجذير عزلات أناناس مزروعة في وسط موريشيوس، وسكوج (MS) يحتوي على هرمون حمض اندول بيوتيرك (IBA) بتركيز 0.5 مللي جرام / لتر. طبقا للمتوسط العام لكل تركيزات السكروز، ودرجات حموضة الوسط، والمتوسط عند كل توليفة متساوية المحتوى من السكروز، ودرجة الحموضة فإن الوسط السائل كان أفضل من الوسط الصلب، والوسط شبه الصلب. أقصى طول للنبيتات الناتجة (66 – 71 ملم) تم الحصول عليها باستعمال الوسط السائل المحتوي على سكروز 10 جرام / لتر، ودرجة حموضة 6.0، والوسط السائل المحتوي على سكروز 20 جرام / لتر ودرجة حموضة 6.5، والمحتوي على سكروز 30 جرام / لتر، ودرجة حموضة 5.0. كل من هذه التوليفات السابقة الذكر ما عدا توليفة سكروز 20 جرام / لتر، ودرجة حموضة 6.0 أدت إلى تجذير كل العزلات (100 %). توليفة سكروز 30 جرام، ودرجة حموضة 5.0 ضاعفت عدد الجذور مرتين (أحد عشر جذرا بالعزلة)، وطول الجذور ثلاث مرات (29 ملم) مقارنة بالتوليفات الأخرى، والتي أدت إلى تكوين خمسة جذور بمتوسط طول 11 ملم. كل مقياس من مقاييس التجذير له متطلبات مثالية من توليفة خاصة من السكروز، ودرجة الحموضة، وحالة الوسط، وتختلف من مقياس تجذير، ومقياس تجذير آخر. لكل مقياس من مقاييس التجذير توجد درجة حموضة أكثر ملاءمة من غيرها، والتي تؤدي لخفض تركيز السكروز المطلوب من 30 إلى 20 وحتى إلى 10 جرام / لتر. لهذا فإن اختيار درجة حموضة وسط التجذير تعتبر مدخلا مهما لخفض تكلفة التجذير.

الكلمات المفتاحية: حالة الوسط، درجة الحموضة، السكروز، التجذير، الأناناس.

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The Incidence of Isolated Ventricular Septal Defect in Libyan Newborns

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Abstract: This study aimed to evaluate the incidence and spontaneous closure of ventricular septal defects in a randomly selected newborn population, using color Doppler echocardiographic screening. Color flow Doppler echocardiographic screening was performed in 635 neonates within the first week of life. Patients with a ventricular septal defect were also followed up for 6 months to detect spontaneous closure rate and its timing. The incidences of a ventricular septal defect in all neonates, preterm neonates, and term neonates were found as 48.8/1,000, 64/1,000, and 48.1/1,000 live births, respectively. Only three patients were symptomatic. 21 cases had a muscular ventricular septal defect and 10 cases had a perimembranous ventricular septal defect. Most of them had a small ventricular septal defect (≤ 3 mm). Spontaneous closure was observed in 64.5% of ventricular septal defects within 6 months Closure rate was found as 80% for preterm infants and 66.8% for term infants ($p > 0.05$). The incidence of a ventricular septal defect was considerably high in neonates when routine color flow Doppler echocardiographic examination was performed. Despite the increased incidence of ventricular septal defect, spontaneous closure rate was remarkably high within the first 6 months of life. These defects may result from delayed physiologic development and have a good prognosis.

Keywords: Echocardiography, Newborn, Ventricular Septal Defect, Incidence

INTRODUCTION

A ventricular septal defect is the most common form of CHD and accounts for 15% to 20% of all such defects, not including those occurring as part of cyanotic CHDs. The defects vary in size, ranging from tiny defects without hemodynamic significance to large defects with accompanying CHF and pulmonary hypertension (Park, 2014). After using color Doppler echocardiography, the incidence of ventricular septal defects (VSDs) has been reported in some studies to be higher than 7/1000 live birth (Hiraishi et al., 1992). In some of these studies, the incidence of muscular VSD was examined in full-term (Roguin et al., 1995) or preterm neonates (Du et al., 1996) and neonates having a low risk for the development of VSD (Sands et al., 1999). They concluded that the incidence of VSD might have been underestimated in the past. The increasing preterm delivery, environmental factors, or better echocardiographic imaging due to evolving technology may be responsible for the increase in the reported incidence of VSD. Data regarding the epidemiology of VSD is very limited and depends on a few epidemiological studies (Clark, 2001).

Thus, this study was designed to evaluate the incidence of VSD in a randomly selected preterm and full-term newborn population, using color Doppler echocardiographic screening. Also, the study intended to follow-up patients with VSD for a year to detect the rate and timing of spontaneous closure.

MATERIALS AND METHODS

All live newborns delivered in the Obstetrics Department of Albayda Hospital and kept in

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the nursery for more than one day in the period from April 2018 to December 2018 were included in the study group. Parental consents were obtained from the parents of each neonate. A detailed history of the neonates, parents, and family members was taken by interviewing the parents. Clinical characteristics of cases with VSD were compared with neonates without congenital heart defect (CHD). 635 (320 M, 315 F) were included in the study. There were 540 (85%) full-term and 77(12.1%) preterm (gestational age<37 weeks) infants. Their gestational ages ranged from 30 to 42 weeks Echocardiographic studies were performed in all neonates within the first week in the Department of Pediatric in Albayda Hospital. Complete trans-thoracic two-dimensional, M-mode, continuous wave and pulsed wave Doppler and color Doppler echocardiographic examinations were performed in all cases followed by a thorough physical examination. Echocardiographic examination was performed using Toshiba Xario cardiac imager.

The diagnosis of a VSD was established based on the presence of a mosaic image passing anywhere through the ventricular septum from the left ventricle to the right ventricle, and a turbulent systolic flow jet recorded on the right surface of the VSD by pulsed or continuous wave Doppler. The diameter of VSD in two-dimensional and/or color flow mapping was measured in all planes and the largest diameter of VSD was recorded. Apical four-chamber, parasternal long- and short-axis, and subcostal positions were used to image the defect. The defects were classified according to previously defined criteria (Snider et al., 1997). Infants who had associated complex structural cardiovascular defects were excluded from the isolated VSD group in neonates with VSD, echocardiography was repeated at the 1st, 3rd, 6th months of age or until the spontaneous closure of the VSD was confirmed. The chi-square test was used to compare the differences where appropriate. A p value less than 0.05 was considered statistically significant.

RESULTS

A ventricular septal defect was found in 31 of 635 neonates, at an incidence of 48.8 in 1,000 live births. 26 of them were full-term and 5 were preterm infants. The incidences of VSD in full-term and preterm neonates were 48.1 in 1,000 live births (26/540 cases) and 64 in 1,000 live births (5/77 cases), respectively. There was no statistical difference in the incidence of VSD between full-term and preterm infants (p=0.52). By echocardiographic screening, other CHDs were identified in 27 neonates. Atrial septal defect, bicuspid aortic valve, and patent ductus arteriosus were detected in 13, and 7 cases, respectively.

Tetralogy of Fallot (3 cases), Transposition of great arteries (2 cases), Double outlet right ventricle (1 case), single ventricle (1 case) were also identified. In this study, the first echocardiographic examination of babies was performed within the first week after birth. Patent ductus arteriosus was also detected in 213 babies within the first week after birth. At the end of the first year of life, it was observed that patent ductus arteriosus persisted in only 7 of them. Gestational age, birth weight, and sex distribution of cases with VSD were not different from neonates without CHD (Table 1). By echocardiography, 21cases (67.7%) had a muscular VSD (14 located at the apical region, 7 located at the mid-muscular region and 10 cases (32/2%) had perimembranous VSD. 25 cases had single and 6 cases (19.3 %) had 2 VSDs. Thus, 37 VSDs were detected in 31 neonates. Defect sizes ranged from 1 to 7 mm in diameter VSDs detected in preterm neonates were all small-sized and located in the muscular region. Only 7 of 37 VSDs were detected by using two- dimensional imaging solely. However, all defects were easily recognized after using color Doppler echocardiographic imaging. 2 cases did not present for follow-up examinations after the first visit. The remaining 29 cases with VSD were followed up during 6 months. 9 of them had a peri-membranous and 20 of them had a muscular VSD. 6 of them had 2 VSDs.

Except for 3; all had small-sized VSD. Spontaneous closure was recorded in 20 of 31 (64.5%) of VSDs.

Table: (1) Clinical Characteristics of Neonates with Ventricular Septal Defect

	neonates with ventricular septal defect(n=31)	Neonates without ventricular heart defect (n= 604)	
	No. of cases(%)	No. of cases(%)	p value
Gestational age (weeks)	38.3 ± 1.8	38.2±1.9	0.5
Sex (female/male)	1.31	0.80	0.11
Birth weight (gram)	3125 ± 540	3150 ±550	0. 66
Maternal age (year)	28.7± 4	28.3 ± 5	0.8
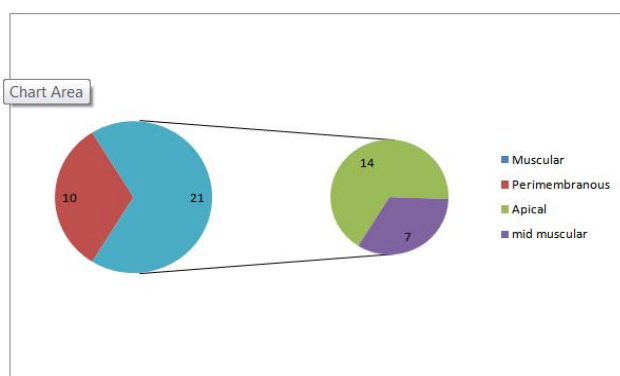
Figure: (1). Ventricular Septal Defect Location
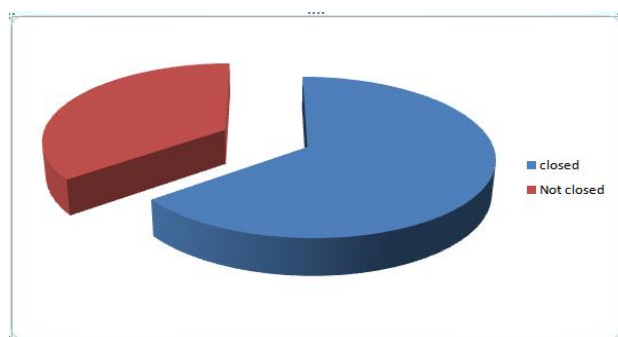
Figure: (2). Spontenous closure in first 6 months of life

DISCUSSION

The incidence of VSD in term newborn infants varies from 20.4 to 53.2/1,000 live births (Sands et al., 1999) Like these studies, it was observed that the incidence of VSD was con-siderably high in full-term infants when routine color flow Doppler echocardiographic examination was performed. Du reported that the prevalence of muscular VSD in preterm infants was 56.6/ 1,000 live births (Du et al., 1996), in this work, the incidence of VSD in all neonates and full-term neonates was similar to rates in Sands and Roguin studies. The incidence of VSD in preterm infants in the current study was also higher than the rate reported by Du (Du et al., 1996). In the current study, the incidence of VSD in full-term and preterm newborns was not found to be statistically different. Therefore, in contrast to previous thought, it could not be shown that increasing preterm delivery was responsible for the increasing incidence of VSD. Color flow Doppler echocardiographic screening is the most reliable method for assessing true incidences of VSD and detects neonates with VSD even in cases without murmur.

CONCLUSION

It was concluded in this study that 64.5% of cases closed spontaneously within six months of life. If newborns were preselected by physical examination for echocardiographic examination, the incidence of VSD would have been lower than what was determined. Major factors influencing the spontaneous closure rate of VSDs are the patient's age at the first examination, localization, and size of the defects. By color flow Doppler echocardiography, Closure was seen in 86.5% of muscular VSDs. These results were similar to those of (Du et al., 1998; Miyake et al., 2004)'s studies. The other 13.5% were not closed at the time of this study and followed beyond that time. The current study also could not show a difference in spontaneous closure rate of VSD between full-term and preterm neonates, as in (Roguin et al., 1995)'s study. The current study showed that the incidence of VSD was considerably high in full-term and preterm neonates when routine color flow Doppler echocardiographic examination was performed. Despite the increased incidence of VSD, most patients' VSD was clinically insignificant and the spontaneous closure rate

was remarkably high within the first six months of life. Thus, this study concludes that these defects may be the consequence of delayed physiological development and have a fairly good prognosis. Indeed, since the majority of tiny VSDs may either close spontaneously or never cause medical problems, patients with small VSD do not need specialized cardiologic care (Hoffman & Kaplan, 2002). The current study also conclude that routine echocardiographic screening in all neonates without clinical findings is not necessary, except in neonates with a familial history of CHD or having well-defined risk factors.

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نسبة حدوث ثقب الجدار البطيني المتفرد في الأطفال الليبيين حديثي الولادة

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المستخلص : هدفت هذه الدراسة إلى تقييم معدل حدوث عيوب الحاجز البطيني، والإغلاق التلقائي لعيوب الحاجز البطيني في فئة حديثي الولادة تم اختياره عشوائيًا باستخدام فحص صدى القلب دوبلر الملون. تم إجراء فحص تخطيط صدى القلب دوبلر لتدفق اللون على 635 مولودًا خلال الأسبوع الأول من العمر. وتمت متابعة مرضى عيب الحاجز البطيني لمدة 6 شهور للكشف عن معدل الإغلاق التلقائي، وتوقيته. تم العثور على حالات عيب الحاجز البطيني في جميع المواليد، والخدج، والمواليد الناضجين على أنها 48.8 / 1000 و 1000/64 و 48.1 / 1000 ولادة حية على التوالي. ثلاثة مرضى فقط يعانون من الأعراض. 21 حالة بها عيب عضلي و 10 حالات بها عيب في الحاجز البطيني حول الغشاء. كان لدى معظمهم عيب صغير في الحاجز البطيني (3 مم). لوحظ الإغلاق التلقائي في 64.5٪ من عيوب الحاجز البطيني خلال 6 أشهر تم العثور على معدل الإغلاق بنسبة 80٪ للخدج، و 66.8٪ للرضع الناضجين ($P > 0.05$) كان معدل حدوث عيب الحاجز البطيني مرتفعًا بشكل كبير عند المواليد عند إجراء فحص صدى القلب بتدفق دوبلر اللوني الروتيني. على الرغم من زيادة حدوث عيب الحاجز البطيني ، كان معدل الإغلاق التلقائي مرتفعًا بشكل ملحوظ خلال الستة شهور الأولى من العمر. قد تنجم هذه العيوب عن تأخر التطور الفسيولوجي، ويكون لها تشخيص، وتوقعات جيدة للمستقبل.

الكلمات المفتاحية : تخطيط صدى القلب ، حديثي الولادة ، عيب الحاجز البطيني ، معدل الإصابة.

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Genotypes Environment Interaction for Seven-Week Body Weight of Poultry Breeds

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Abstract: This study investigated the interaction of poultry breeds x environment under Libyan conditions and compared with that under a temperate zone for a seven-week bodyweight. Data were collected from the weekly records during the rearing period for three grandparent breeds Hypeco, Avian, and Shaver, at Ghotsultan and Tawargha from 1986 to 2001. Traits studied were daily feed consumption, cumulative feed consumption, body weight, feed consumption ratio, and mortality. The model used to analyze the data for the rearing period was included: overall mean, the effects of location, breeds within the year, the flock, seasons, and the age. Breeds x seasons and breeds x locations interaction were found in weight at seven weeks of age. Avian showed consistent performance under both hot and cold seasons for seven weeks bodyweight (1308.3-1304.4g), while Hypeco and Shaver perform well under cold seasons but not in hot seasons in Libya. Hypeco and Avian perform less under Libyan conditions compared to temperate zone conditions, whereas Shaver performs a little better under Libyan conditions. In addition, Hypeco had lower mortality, which was about 2.84%, compared with Avian 3.0%, and Shaver, which was 4.38% during the rearing period. This study could conclude that the performance of grandparent breeds under Libyan conditions was affected by weather and management.

Keywords: Genotype; Poultry breeds; Interaction; Body weight; Libyan conditions.

INTRODUCTION

Local chicken production in Libya is regarded as low production because they were produced only in small scale (family) farms and lacked regular health control programs, unimproved housing, and scavenging to meet the nutritional needs. Nevertheless, local chickens are a significant protein source for consumers and a substantial source of income for poultry keepers with steadily increasing poultry produce. Environment change and animal production are related, and the effects of environmental change on livestock and poultry production are apparent worldwide (Mengesha, 2011).

Poultry growth performance is not only inherited, but it is also greatly affected by the environment (Babinszky et al., 2011). The increasing indication for G × E interactions in recent broilers and various suboptimal conditions confirm the need for breeding programs to improve performance under particular stressful environments (Pakdel et al., 2005). One of the primary reasons for low production in the hot tropical climate is heat stress. In poultry production, broiler growth and meat yield have been strongly and negatively affected by high ambient temperatures (Ahaotu et al., 2019; Okonkwo and Ahaotu, 2019). It appears that broiler stocks reared for a high growth rate and meat yield in optimal environments cannot entirely express their genetic potential when bred in hot climates unless their selection programs include breeding for heat tolerance (Adeyinka et al. , 2006).

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Due to the low performance of the local breed, many projects were established by the Libyan government to cover the increasing demand for broiler meat. These projects used standard broiler parents, selected for temperate climates, whereas Libyan weather is hot, dry, and humid during summer, moderate-warm in winter with some variations between different locations. The use of standard broiler grandparents in hot climates may respond differently to different climate and management conditions. Therefore the objective of this study was to test the interaction of three imported breeds with location and season udder Libyan conditions and compared with that under the temperate zone for seven-week body weight.

MATERIALS AND METHODS

Location : The study was conducted in two different climatic zones: in Libya, which is characterized by a subtropical climate and two projects (Ghot-Al-Sultan and Tawargha) were selected to be investigated in Libya, and in a temperate zone which is characterized by relatively moderate mean yearly temperatures, with average monthly temperatures above 10°C in their warmest months and above −3°C in their colder months (Trewartha and Horn, 1980). Most areas with a temperate climate present four seasons, and temperatures can cause variation considerably between summer and winter (McColl, 2005). Ghot-Al-sultan is located on the east side of Libya on the width line 32 and longitude line 21. The temperature range from 6 to 17 C° and 18 to 33 C° in winter and summer respectively. Tawargha is located on the west side, on the width line 32 and longitude line 15. The range of temperature ranges from 9 to 17 C° winter and 19.57 to 29.24 C° in summer.

Data: Three breeds were used in the two projects simultaneously, Hypeco from 1986 to 1991, Avian from 1993 to 1996, and Shaver from 1998 to 2001. Every breed had 12 flocks in each project. Every species had Male Lines (A and B) and female lines (C and D).

Data were collected from the weekly records for several birds for each breed during rearing, as shown in Table 1.

Table (1): Total number of birds in each breed during rearing.

	Ghot-Al sultan	Tawargha
Hypeco	16590	16734
Avian	14103	12561
Shaver	15633	16699

In this study, the hen housing system is a Non-cage system floor system. The area was divided into floor space, feed space, water space, and nests. The feed program consists of (corn 54.1% - soybean meal 19.3% - limestone (Big) 5.34% - Limestone(small) 3.56% - Wheat Barn 16% - DCP 0.9% - Salt 0.3% - Lysine 0.05 – Methionine 0.1%). One-day chick provided 24-hour light and then decreased gradually to reach 8 hours light and 14 hours dark. By week 18, pullets were subjected to the 2-hour impulse to enhance sexual maturity. Under the brooders, the temperature was 35 C°. Afterwards, the temperature may slowly decrease to 26 C°, and an average temperature in the house of 18 to 20 C°. To prevent dehydration of the chicks during the first week it is important to maintain a relative humidity of 70%.

Traits studied: The traits studied were daily feed consumption (DFC), cumulative feed consumption (CFC). 7 Week body weight, feed consumption ratio (FCR), and mortality.

Statistical Analysis: To test breeds interaction with location and season for body weight at seven weeks, this model included year-season, breeds, and location. The following statistical model using Stat Soft Inc (2007):

$Y_{ijkl} = \mu + L_i + S_j\text{-}Y_k + B_l / S_j\text{-}Y_k + B_l * L_i + B_l * S_j\text{-}Y_k + E_{ijkl}$

Whereas:

μ = overall mean, L_i = the effect of ith location (fixed) i (i = Ghot-Al sultan, Tawargha), S_j -Y_k

= the effect of Sj^{th} -Yk^{th} year (fixed) Bl/Sj Bl^{th} = the effect of Bl^{th} breed / Sj^{th} season-Yk^{th} year, Bl *Li = the interaction between Bl^{th} with Li^{th} location Bl * Sj-Yk = the interaction between Bl^{th} breed with the Sj^{th} season - Yk^{th} year, Eijkl=the unexplained residuals.

RESULTS

There were significant (P<0.001) differences in bird daily feed intake between the breeds (Table.2). Hypeo breed had higher daily feed intake compared with other breed and had higher body weight (1569 g) than Avian (1501g) and Shaver (1533g). In contrast, Shaver had higher mortality (4.38 %) than Avian (3.0%) and Hypeco (2.84%) Table 2. Mean and standard error of feed traits, live body weight, and percentage of mortality for different breeds (P. Rearing Period).

Interactions between breed and locations for the bodyweight at seven-week age are presented in figure (1). The avian breed had a higher body weight (1393.83g) in the Ghot AL sultan project than the Tawargha project (1252.3g). In contrast, Shaver had a lower body weight (1072.5g) in the Gout AL sultan project than the Tawargha project (1320.1g). In addition, Hypeco is quite similar in both locations.

Interactions between seasons and breeds for the body weight at seven weeks age and seasons are presented in figure (2). Shaver had a higher body weight (1274.6g) in cold seasons than in hot seasons (1106.3g). Hypeco had a higher body weight (1314.5g) in cold seasons than in hot seasons (1238.1g). In addition, Avian showed quite similar body weight during all seasons. Interactions of breeds under Libyan and temperate zone for the body weight at seven weeks age are presented in figure (3). Shaver had a lower body weight (863g) (1196.05g), whereas Hypeco and Avian had a higher body weight in the temperate zone (2350g) (2695g) than Libya (1263.7g) (1323.06g) respectively.

Table (2): Feed consumptions and growth traits and mortality for breed during rearing Prevalence of Anti-Brucella antibodies.

	Hypeco	Avian	Shaver
FBD (g) Mean±SD	79.7ª±0.76	72.2ᵇ± 0.75	75.7ᶜ±0.82
CFB (Kg) Mean±SD	3.90ª±0.15	3.54ᵇ± 0.07	3.71ᶜ± 0.06
Weight Mean±SD	1569ª±19.8	1501ᵇ±18.7	1533ᶜ± 20.9
FCR	2.48ª±0.007	2.36ᵇ±0.003	2.42ᶜ±0.002
Mortality %	2.84ª	3.0ᵇ	4.38 ᶜ
N	1345	1352	1384

Means within the same row with different letters are significantly different (P ≤ 0.001)

DISCUSSION

There were significant interactions between breeds at seven weeks of body weight and seasons (figure 2). For seven weeks body weight of Avian and Hypeco breeds had more significant nonlinear interaction with seasons, while the Shaver breed had linear interaction.
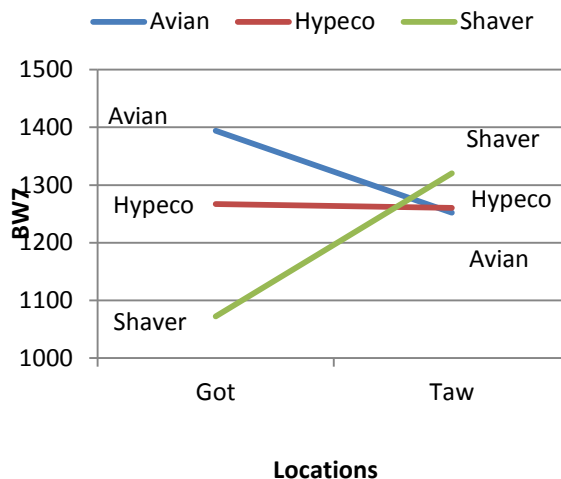

Figure (1): Breed X locations interactions for body weight at seven weeks of age.
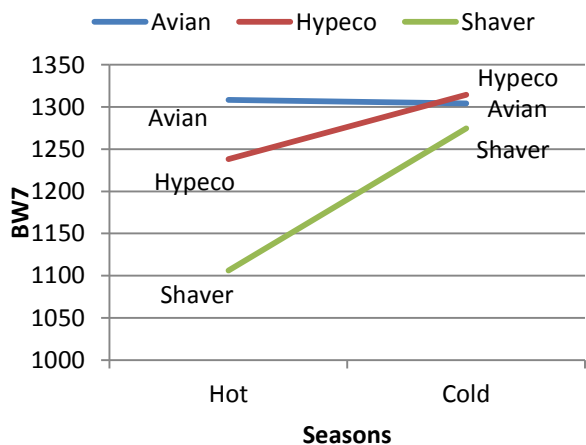
Figure (2): Breed X seasons interaction for body weight at seven weeks of age.

Avian and Shaver breeds had linear interaction. Hypeco and Shave breeds had lower body weight in hot seasons than in cold seasons. This result was in agreement with the finding of (Yalcin et al., 1997b), who concluded that a reduced body weight and body weight gain of
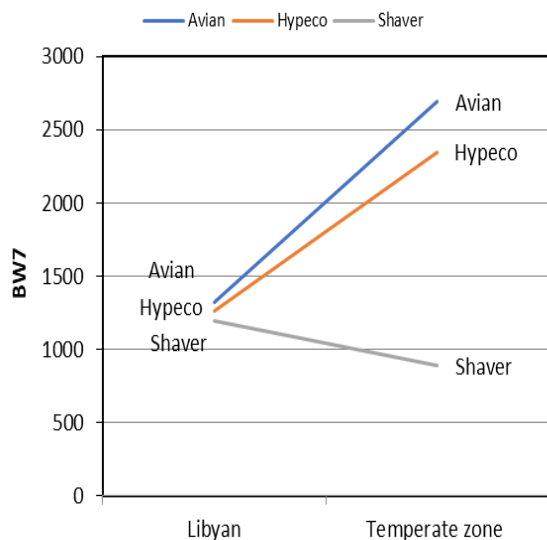
Figure (3): Evaluation of the performance of breeds under Libyan Conditions and temperate zone for seven week body weight.

about 23% and 33.5%, respectively at seven weeks of commercial broilers might be due to the natural heat stress in summer. Also, (Liu et al., 2020) concluded the negative impacts of high temperature on broiler chickens regarding

BWG, FI, FCR, and mortality. The findings by (Aksit et al., 2006) also demonstrated a significantly reduced body weight in broiler chickens at 4 to 7 weeks of age at 34^0C. (Plavnik and Yahav, 1998) concluded that the bodyweight of chickens declined progressively with an increase in temperature. Hypeco breed had a constant body weight during all seasons.

In contrast, the body weight of Shaver recorded had a little bit increased during hot seasons. This result was in agreement with the finding of (Molapo, 2011) who reported that the final body weights of chickens raised in summer were heavier than those in winter despite the feeding level. Also, (Akyuz, 2009) showed that rearing birds in summer could result in higher body weight gains than birds kept in winter though the differences were insignificant. The reduced weight of birds in summer because had a low feed intake due to heat stress. The reason for variation can be attributed to the behaviour of chickens when responding to low temperature. The rationale would be that birds took more time huddled at an early age to generate heat instead of eating. Also, (Yalçin et al., 1997a) reported that three broiler chicken lines, having similar performance in the fall (average temperature 18ºC), showed a significant difference in feed intake, BW gain, and FE in summer (average temperature 28ºC). Similarly, the genotypes of commercial broilers that gain more weight in the spring tend to gain less weight under the hot conditions of summer (Settar et al., 1999). Hence, the broilers' genotype should be considered in broiler production in subtropical and temperate regions, especially given the increasing proportion of world broiler production in these regions.

CONCLUSION

The Libyan government has established many projects like (Ghot Al sultan and Tawergha) to improve the productivity of the local breed and cover the increasing demand for broiler meat. These projects used standard broiler parents (Hypeco, Avian, and Shaver breeds), which were selected from temperate climates. Where-

as the Libyan climate is hot, dry, and humid during summer, moderate- warm in winter with variations between different locations. Therefore, when importing breeds, it needs to consider the interaction between breeds and the location. Furthermore, it essential to evaluate and compare the performance of Hypeco, Avian, and Shaver breeds during the rearing period determining the most suitable breed under local conditions. Finally, the main conclusions of these experimental work should be presented.

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التفاعل الوراثي البيئي للوزن سبعة أسابيع لسلالات الدواجن تحت الظروف الليبية

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المستخلص: هدفت الدراسة لبحث التفاعل بين سلالات الدواجن والبيئة لوزن الجسم عند عمر سبعة أسابيع تحت الظروف الليبية، ومقارنتها بالمنطقة المعتدلة. تم جمع البيانات من السجلات الأسبوعية خلال فترة التربية لثلاث سلالات أجداد Hypeco و Avian و Shaver في مشروع غوط السلطان، وتاورغاء من 1986 إلى 2001. وكانت الصفات المدروسة هي استهلاك العلف اليومي، استهلاك العلف التراكمي، وزن الجسم، نسبة استهلاك العلف، ومعدل النفوق. تم استخدام النموذج الرياضي لتحليل بيانات فترة التربية: المتوسط العام، وتأثيرات الموقع، والسلالات خلال العام، والقطيع، والمواسم، والعمر. بينت النتائج التفاعل في الوزن بين السلالات × المواسم والسلالات × مواقع عند عمر سبعة أسابيع. أظهرت سلالة Avian أداءً ثابتًا في كل من الموسمين الحار، والبارد لمدة سبعة أسابيع من وزن الجسم (1308.3–1304.4 جم)، بينما أداء Hypeco وShaver كان جيدًا في المواسم الباردة، ولكن لم يكن كذلك في المواسم الحارة في ليبيا. أداء Hypeco وAvian أقل في ظل الظروف الليبية مقارنةً بتلك الظروف في المناطق المعتدلة، بينما تعمل Shaver بشكل أفضل قليلاً في الظروف الليبية. بالإضافة إلى ذلك، سجلت Hypeco معدل نفوق أقل بنسبة 2.84% مقارنة مع Avian 3.0% وShaver 4.38% خلال فترة التربية. يمكن استنتاج أن أداء سلالات الأجداد في ظل الظروف الليبية تتأثر بالطقس، والإدارة.

الكلمات المفتاحية: النمط الوراثي، سلالات الدواجن، التفاعل، وزن الجسم، الظروف الليبية

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The Influence of Code Retrieval from the Web on Programmer's Skills, Methodologies, and Coding Behaviors

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Abstract: The development of software projects consists of several stages, such as analysis and design. It also requires a set of skills that the software developer can use to work on the project, such as specifying the requirements and writing code. Developers usually search for source code on the internet for remix and reuse in software production. This paper aims to investigate the influence and effect of code retrieved from the web on programmers' views, decisions, and skills. A questionnaire instrument was designed and distributed to programmers for their feedback. As a result, we were able to address some points and achieved a better understanding of the interaction between programmers and the code from the web, especially the code from programming forums such as Stack Over Flow.

Keywords: Code Reuse; Source Code Search; Code Influence; Code Remix

INTRODUCTION

With a rising number of code forums and open source websites, the internet is considered to be a large repository of information regarding the source code of different programming languages available for every software developer. The amount of source code on the internet has increased dramatically because of the distribution of open-source repositories. Similarly, the term open source has become very commonplace in the past few years (Gabriel, 2005). The most important benefit of having open-source repositories, is that they form a rich source for software projects on the web that can be reused and redeveloped (Szyperski, 2004). For instance, GitHub.com, forge.net, and freshmeat.net host many software projects that support programmer participation and sharing knowledge. An alternative way of finding source code is through the use of general- purpose search engines such as Google and Yahoo as programmers are accustomed to such tools and their ability to get the required documents from the web (Gallardo-Valencia, 2013).

In the past, searching for code has been investigated comprehensively, and researchers suggested many approaches to refine the performance of code search (Haiduc, 2013; Lee Martie, 2017; Haoran Niu, 2017; Mukund Raghothaman, 2016). According to research in software engineering (Janice Singer, 1997), searching the web is the most common activity for software engineers, and program understanding, software reuse, debugging was mentioned as the top prompting for source code searching in that research. In another related study on searching for source code (S. E. Sim, 1998), The search purposes mentioned frequently by the software developers were; code reuse, debugging, program understanding, feature addition, and effect analysis. Software developers also use code search engines (e.g., Krugle, Github native search) to get their code snippets from open source repositories using general natural language queries (Lopes, 2012),

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but many of them are now obsolete, and programmers prefer to use a general-purpose search engine to look for code (Graham, 2016; Susan Elliott Sim, 2011; Stolee, 2014).

Another study investigated the performance of searching with Google and whether it varies for code versus non-code connected search (Md. Masudur Rahman, 2018). Another research has proposed a new technique which could help developers in their search for code by identifying related and particular API classes from a programming task site, written as a natural language query, and then reformulated for better source code search (Roy, 2018). According to Szyperski (2004), software reuse is a prevalent motivation for source code searching on the internet for programmers. In 2018, a study proposed a neural code searching technique for source code retrieval that uses natural language to search for code (Sachdev, 2018). Other research presented a new code retrieval framework that combines both ordering and semantic relationships and demonstrates significantly improved retrieval precision between two known datasets (Akbar, 2019).

Although code retrieval has been an active research topic in recent years, less focus was on the impact of retrieved code on programmers' skills and coding behavior. In this study, the focus is on studying and analyzing the effect and influence of code retrieval from the web on programmers' views, decisions, and abilities. Through reviewing previous studies, it was noted that they were mainly investigating the methods and techniques of searching for code and the effectiveness of online discussion forums. We believe that there are always factors that influence the success or failure of software development projects. We also believe that retrieving code from the web to be deployed in software projects has an effect on the developers.

MATERIALS AND METHODS

Code searching on the internet (CSOI) is an important method for sharing best coding practices. In recent years, software properties have led to programming complexity. Programmers' goal is to implement software design by investigating on similar coding practices which best fit their needs. The aim of the current research is to investigate the issues behind implementing CSOI; the goal is to inspect the factors reflecting on CSOI as a measurable searching process from the viewpoint of the programmer.

We assume that CSOI is a measurable process. When performing CSOI, programmers' top priority is to gain similar code or better coding techniques. If the required source code from CSOI is obtained, we can say that the CSOI results are successful. However, if the programmer was unable to attain the required results from CSOI, then the CSOI results are unsuccessful, and CSOI must be repeated with different factors and priorities.

In recent years, the increased usage of the internet has been accompanied by an equal increase in online surveys that have been used to investigate human behavior over the internet (Ritter., 2011). To achieve the goal of this study, an online survey with 26 ended questions was designed to be distributed to a wide range of programmers who often search for source code on the internet. The population was all programmers with at least six years of programming experience and conducted code searching on the internet (CSOI). The survey was available for about two months in 2019 to collect the required feedbacks. Invitations for participants were shared and sent via email addresses to university academic programmers and private business sectors programmers. This category of candidates was selected because they represent the programming communities in the country and have common interests regarding searching for source code. Thus, potentially better responses could be obtained.

The study sample consisted of 37 respondents. The study first started by cleaning the data set and preparing data for analysis. The

preprocessing step produced 37 cases ready for analysis. The collected data indicated that most of the respondents are C Sharp and Visual Basic programmers with more than seven years of experience.

Analysis: The collected data was analyzed by both quantitative and qualitative techniques. he questionnaire was tested for reliability using alpha Cronbach method (Taber, 2018). The test showed good reliability of 82.8%. The questions were based on a five-point Likert scale with the following possible choices: 1 – strongly disagree, 2- disagree, 3- neutral, 4- agree, and 5- strongly agree. The mean and standard deviation of each question were computed to determine the outcome of each question.

Examining the data individually, we discovered a high level of agreement in our analysis. Then, we merged our analysis and refined the results for clarity of presentation.

THREATS TO VALIDITY

The fundamental deficiency of this study is generalizability, i.e., the number of respondents is insufficiently representative of the population. This is a major problem with empirical research in software engineering as there is an unreliable pattern of population characteristics so that the representativeness of a sample can be estimated.

This research is no exception. Moreover, participants were selected from mailing lists and programming groups. That is why there is no attempt to quantify the propagation of some types of behavior.
Instead, we are searching for a diversity of search behaviors and patterns, which is suitable for a discovery study. The objectives of this study are to find answers to the following four research questions:

Q1. To what extent "Code retrieval from the web" improves programmer's knowledge and skills?

Q2. What is the impact of "Code retrieval from the web" on limiting programmer's views when trying to solve coding problems and bottlenecks?

Q3. What is the best criterion by which to measure the quality of "Code retrieval from the web"?

Q4. What are the advantages and disadvantages of using "Code retrieval from the web"?

RESULTS AND DISCUSSION

Most respondents agreed that they memorize most of the functions in the language they use. They also agreed that they use online programming forums more frequently than the help page files provided with a certain language when trying to solve programming problems. This is similar to the findings of the study of Janice Singer (1997), which reported that searching the web is the most common activity for software engineers. They also reported that code retrieval neither made them change the programming language they use nor forced them to change the version of the programming language itself. It also did not make them adapt to new programming concepts.

Respondents think that retrieved code is scalable and the majority are attempting to understand the retrieved code before using it. Most of them make libraries from the retrieved code for later use. They neither agree nor disagree about having difficulties embedding retrieved code into their projects. They also were neutral about the fact that retrieved code satisfies verification, security, and control procedures of their systems.

Most of them stated that they only use the first solution they find online to their problems without further search for other solutions that could be more flexible and more scalable. Moreover, they claim that code retrieval did not make them dependent on others to solve their

programming problems. The majority of the respondents to this study stated that they do not participate in solving problems posted on online programming forums and they usually find solutions to their problems without posting them. Some respondents claim that there are some projects in which they did not use retrieved code and sometimes they did not find solutions to their problems or even solutions similar to them.

Regarding the first question of the study, the majority of respondents stated that they use retrieved code to understand how a certain

function works rather than directly copy and paste the code into their projects. This is similar to the study of (S. E. Sim, 1998) that showed that program understanding is among the search purposes mentioned frequently by software developers. They also believe that they acquire more knowledge and learn from the code they retrieve. For the questions set in the questionnaire related to the first question of the study as shown in table 1, the average was 3.99, which means there is an agreement that retrieved code has some degree of improvement on the programmer's skills, coding practice, and problem-solving ability.

Table (1): Result of question one of the study

	Strongly disagree	disagree	neutral	agree	Strongly agree	Mean	STDev	Outcome
Q 1	0	0	3	14	20	4.46	0.65	Strongly agree
Q 2	2	4	4	10	17	3.97	1.24	Agree
Q 3	4	5	3	17	8	3.54	1.28	Agree

The second question of the study investigates the impact of retrieved code on limiting programmers' views when solving programming problems. The average of this dimension of the study, as shown in table 2, was 2.89, which indicates that there is no significant impact of retrieved code on limiting programmers' views in programming. Interestingly, the majority of respondents seemed to use the first solution they found online, which may indicate the programmer's reliance on that particular solution with the

possibility of a better solution not being explored.

This on the one hand, could lead to producing less quality code and may be a sign of the limitation of the programmer's skills. On the other hand, considering the advancement in code search engines, the first solution produced by those engines, to some degree, is always a good solution as many users had given it a higher rank. Hence, these first-hit solutions are considered to contribute to better code and improve programmer's skills.

Table (2): Result of question two of the study

	Strongly disagree	disagree	neutral	agree	Strongly agree	Mean	STDev	Outcome
Q 1	15	6	5	8	3	2.41	1.42	Disagree
Q 2	7	4	4	15	7	3.30	1.41	Neutral
Q 3	9	3	6	10	9	3.19	1.52	Neutral
Q 4	10	10	2	13	2	2.65	1.36	Neutral

The third question of the study inspects the best criterion by which to measure the quality of code retrieval from the web. The study revealed that clarity, scalability, security, and ease of

implementation are the most used factors by programmers for the assessment of retrieved code quality.

The last dimension of the study investigates the advantages and disadvantages of code retrieving practice. Here, the study showed the following advantages of retrieving code from the web. Code retrieval from the web provides an easy way to obtain code that can be embedded in various projects and solve numerous problems without the need to read long pages of help files. It prompts the programmer towards learning, understanding, and sometimes adopting new software development concepts and programming methodologies. Code retrieval makes programmers familiar with programming sites and online programming forums and increases their participation in finding solutions to programming problems.

The study also pointed out the following disadvantages of code retrieval from the web: Sometimes solutions found online do not fit directly into one's project and need to be customized, rewritten, or even changed from one language to another to fit into the project.

In some cases, programmers may find themselves frequently adopting new different programming concepts just because they can easily find code that follows those concepts, which may take some time and reduce productivity.

CONCLUSION

This paper has discussed the influence of code retrieval on programmers' skills from a programmer's point of view. The results obtained after careful analysis of the responses that were collected via the designated questionnaire suggested that code retrieval may improve programmers' skills. The study also revealed that there is no notable impact of code retrieval practice on limiting programmers' views when solving programming problems and developing software projects. The research has pointed out some factors, namely: clarity, scalability, security, and ease of implementation, that programmers think are the best criterions to measure the quality of retrieved code. Finally, the paper has shown

some advantages and disadvantages of code retrieval from the web.

Further future work can be done on this topic, including studying exactly which particular programming skills can be improved by code retrieval from the web. Also, future work may focus on investigating which methods and practices programmers use when dealing with code that does not fit directly into their projects.

Furthermore, further investigation can be done on the reasons why respondents consider retrieved code to be scalable and the majority of them are attempting to well understand the retrieved code before using it. Moreover, future studies could focus on investigating the reasons that make programmers less proactive in sharing solutions to problems on programming forums.

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تأثير استخلاص الشفرة البرمجية من الإنترنت على مهارات وطرق وسلوك المبرمج

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المستخلص: في تطوير مشاريع البرمجيات، يتكون المشروع البرمجي من عدة مراحل على سبيل المثال التحليل، والتصميم. وهو يتطلب أيضًا مجموعة من المهارات التي يمكن لمطور البرامج استخدامها للعمل في المشروع على سبيل المثال، تحديد المتطلبات، وكتابة التعليمات البرمجية. يبحث المطورون عادةً عن التعليمات البرمجية على الإنترنت لإعادة المزج، وإعادة الاستخدام في مشاريع البرامج الخاصة بهم. تهدف هذه الورقة إلى التحقق من تأثير الشفرة المستخلصة من الويب على آراء المبرمجين، وقراراتهم، ومهاراتهم. ندرس هذا التأثير من وجهة نظر المبرمج باستخدام استبانة تم إعدادها وتوزيعها على مجموعة من المبرمجين للحصول على آرائهم، وتعليقاتهم .نتيجة لذلك تم إلقاء الضوء على بعض النقاط، وتم تحقيق فهم أفضل للتفاعل بين المبرمجين، والكود المستخلص من الويب، وخاصة الكود المستخلص من منتديات البرمجة مثل: (Stack Overflow)

الكلمات المفتاحية: إعادة استخدام الشفرة البرمجية، استخلاص الشفرة البرمجية من الإنترنت، تأثير الشفرة البرمجية، مزج الشفرة البرمجية.

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Hormonal Physiological Changes of Testis Resulting From Exposure to Vinyl Cyanide and the Possible Protective Role of *β*-cryptoxanthin in Male Rat

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Abstract: Vinyl cyanide (VCN) is an aliphatic nitrile product which is extensively used in various synthetic chemical industries. VCN is known to exert toxic actions to human beings as well as experimental animals. The present study was designed to examine the ability of β-cryptoxanthin, a naturally occurring antioxidant, to attenuate VCN-induced testicular toxicity in adult albino rats. Daily oral administration of VCN at a dose level of 30 mg/kg b.w. (7.2mg/ animal) to male rats for a period of 5 days significantly reduced the levels of serum testosterone (T), androsterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) which indicates injury to the testis function. Compared to VCN-treated animals, pretreatment with β-cryptoxanthin and its co-administration with VCN once daily at a dose of 40 mg/kg b.w. (9.6mg/ animal) for 30 days induced a remarkable degree of improvement in the levels of endocrine parameters including T, androsterone, FSH and LH. In conclusion, the present results clearly demonstrate the protective role of β-cryptoxanthin against VCN-induced physiological changes in the testis of rats.

Keywords: Vinyl cyanide; β-cryptoxanthin; Hormones; Testes; Rats.

INTRODUCTION

Vinyl cyanide (C_3H_3N, VCN), also known as acrylonitrile, a highly reactive compound having an active vinyle and cyanide groups, which has been widely used in industry for production of plastics, elastomers, and synthetic fibres and as an intermediate in the synthesis of industrial chemicals and pharmaceuticals (Humans, 1979). It is also used in the manufacture of soft prosthesis material (Parker & Braden, 1990), coating membranes for Langerhans islets implants (Kessler et al., 1992) and high permeable dialysis tubing (Ward et al., 1993).

Human exposure to VCN could potentially occur during the manufacturing process, end product usage and transportation. Further, such exposure can also be possible in the general population through cigarette smoke and via contamination of drinking water (Byrd et al., 1990). VCN demonstrated acute toxicity in testes of rats, mice, rabbits and guinea pigs having a high acute toxicity result from inhalation, and a high to extreme acute toxicity result from oral or dermal exposure (Mathieu-Denoncourt et al., 2015; Thier et al., 2000). VCN is teratogenic in laboratory animals (rat and hamster) at high doses when maternal toxicity has been already manifested. VCN has been demonstrated to induce embryotoxic effects in rat (Saillenfait & Sabate, 2000).

VCN-induced embryotoxic and teratogenic effects have also been found in VCN-exposed workers (Wu et al., 1995). According to environmental teratologic epidemiological study in inhabitants living in the surrounding region of an acrylonitrile factory, three congenital abnormalities (pectus excavatum, undescended testis and clubfoot) in 46,326 infants showed significant time-space clusters in the

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study region. There was a decrease in risk of undescended testis with increasing distance from the acrylonitrile factory (Czeizel et al., 1999). Therefore, women not professionally exposed would appear to be at risk of terato-genic effects due to VCN toxicity.

VCN is rapidly absorbed and distributed to all major tissues in animals. Previous studies with 14C have shown that VCN covalently binds to thiol group of proteins (Ahmed et al., 1982) and tissue macromolecules and nu-cleic acids (Pilon et al., 1988). Therefore, es-timation of free radical generation and anti-oxidant defence has become an important as-pect of investigation in mammals. Carote-noids (β-cryptoxanthin or what is known as β-carotene) are naturally occurring antioxi-dants that play an important role in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors. The antioxidant func-tion of these micro-nutrients could, at least in part, enhance the immunity by maintaining the functional and structural integrity of im-portant immune cells (Chew, 1995; El-Demerdash et al., 2004).

β-cryptoxanthin, aside from being a major source of vitamin A (retinol), an essential vitamin for spermatogenesis to proceed, has been reported to be a potent free radical quencher, singlet oxygen scavenger and lipid antioxidant (Burton, 1989; El‐Missiry & Shalaby, 2000) focused on the ability of β-cryptoxanthin to function as a chain-breaking antioxidant in a lipid environment at physio-logical oxygen partial pressures that are con-sidered most likely in mammalian cells. Therefore, the aim of the current study was to investigate the efficacy of β-cryptoxanthine on VCN-induced functional and structural alterations related to oxidative stress in the testes of rats.

MATERIALS AND METHODS

Chemicals: Vinyl cyanide (VCN) and β-cryptoxanthin were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) and given by oral gavage at dose of 30 mg/kg b.w. (Takano et al., 2010) and 40mg/kg b.w. (Sadir et al., 2007), respectively. All other chemicals and solvent used were of highest available commercial grade.

Experimental animals: Fourty male Sprague–Dawely rats, each weighing 240 ± 10 g. The animals were housed in stainless steel cages after grouping in batches of five animals under standard animal house conditions of relative humidity (55 ± 5%), temperature (25 ± 2 ˚C) and a 12 hr light/12 hr dark cycle. Rats were allowed free access to standard commercial feed and tap water and were acclimatized to laboratory conditions for a period of one week before the onset of experimentation.

Experimental protocol: Animals were allo-cated to four groups each of ten rats as follows: Group I: (Control) pre-treated with corn oil (2 ml/kg b.w.) once daily for 25 days and treat-ment continued with distilled water (2 ml/kg b.w.) once daily for additional 5 days i.e. from day 26 to day 30 of the experimental period of 30 days.

Group II: (VCN group) pre-treated with corn oil (2 ml/kg b.w.) once daily for 25 days and treatment continued with VCN in a dose of 30 mg in 2ml distilled water per kg b.w. (7.2 mg/ animal) once daily for additional 5 days.

Group III: (β-cryptoxanthin group) pre-treated with β-cryptoxanthin in dose of 40 mg in 2 ml corn oil per kg b.w. (9.6mg/ animal) once daily for 25 days and treatment continued with dis-tilled water (2 ml/kg b.w.) for additional 5 days.

Group IV: (β-cryptoxanthin and VCN group) pre-treated with β-cryptoxanthin (40 mg/kg b.w.) for 25 days and treatment continued with VCN (30 mg/kg b.w) for additional 5 days.

At the end of the experimental period, the test-ed animal groups were sacrificed after 24 h of the last dose of different administrations and

their blood were collected by carotid bleeding in centrifuge tubes, serum was obtained from the blood after centrifugation at 3000 rpm for 10 min.

Methods of analysis: Determination of luteinizing (LH), testosterone (T) and androsterone hormones in serum were carried out according to the method of (Jaffe & Behrman, 1974) and follicle stimulating hormone (FSH) was measured by radioimunoassay (RIA) using the method of (Rose, 1998).

Statistical analysis: Statistical analyses of the resulted data were done using In-Stat version 2.0 (Graph Pad, ISI, Philadelphia, PA, USA, 1993) computer software. The results were expressed as means (±SE). Multiple comparisons were done using one-way ANOVA followed by Tukey-Kramer as a post-ANOVA test. Statistical significance was accepted at $P< 0.001$,

$P< 0.01$, $P< 0.05$.

RESULTS AND DISCUSSION

Analysis studies: Data listed in Table 1 show that treatment with VCN caused a significant ($P<0.001$) decrease in the levels of T, androsterone, FSH and LH, respectively as compared to the corresponding control group. Pre-and co-administration of β-cryptoxanthin to VCN-challenged rats significantly improved ($P<0.001$) the levels of these hormones as compared to VCN-treated group.

From these results it is clear that vinyl cyanide has been demonstrated to induce male reproductive toxicity in laboratory animals (Ahmed et al., 1992; Liu et al., 2004) and also in VCN-exposed workers (Xu et al., 2003).

Table:(1). The effect of vinyl cyanide (30 mg/kg b.w.) and/or β-cryptoxanthin (40 mg/kg b.w.) on serum testosterone, androsterone, FSH and LH of male albino rats.

Groupe Hormone	Control	VCN	βcryptoxanthin	βcryptoxanthin +VCN
Testosterone ng /ml	7.05± 0.04	3.00± 0.03 (- 57.45%) a**	7.63± 0.09 (8.23%) a**b**	4.62± 0.16 (-34.47%) a**b** c**
Androsterone pg/ml	49.41± 0.29	30.41± 0.22 (-38.45%) a**	52.40± 0.27 (6.05%) a**b**	41.61±0.83 (-15.79%) a**b** c**
FSH pg/ml	10.64± 0.12	4.09± 0.26 (-61.65%) a**	12.61±0.16 (18.52%) a**b**	8.32± 0.22 (-21.80%) a**b** c**
LH mIU/ml	8.96± 0.10	4.51± 0.06 (-49.67%) a**	9.26± 0.15 (3.35%) b**	7.48±0.12 (-16.52%) a**b**c**

- Data are expressed as means ± SE (n = 10 in each group).
- Values between parentheses are the difference % of each parameter with respect to control value.
a: Significant change at *P*< 0.05 with respect to control group.
b: Significant change at *P*< 0.05 with respect to VCN-group.
c: Significant change at *P*< 0.05 with respect to β-cryptoxanthin -group.
**Very high significant change exists at *P*< 0.001.

Whole body autoradiography and toxicokinetic studies showed that the brain is a target organ for VCN toxicity (Ahmed et al., 1982). (McLachlan et al., 2002)reported that endocrine support is essential for normal spermato

genesis and disturbance can lead to altered spermatogenesis in both humans and rodents. Therefore, the decrease in the levels of serum T, androsterone, FSH and LH could be explained in the current study. The present results are in accordance with the study of (Ivănescu et

al., 1990) who reported that VCN decreases testosterone synthesis and/or secretion in humans. In this study, the observed decrease in the levels of T and androsterone following VCN administration may be attributed to the increase in oxidative stress. This finding is consistent with that of (Diemer et al., 2003; Mathieu-Denoncourt et al., 2015) who showed that H_2O_2 is a potent oxidant that could inhibit steroidogenesis (reduce testosterone synthesis) in Leydig cells. Similarly, (Yang et al., 2005) reported that serum T level and Leydig cell viability were decreased in rats treated with the structurally similar vinyl monomer, Vinyl cyanide (VCA). Their interpretation is that the decreased viability of Leydig cells caused by VCA treatment lowered testestrone level, which in turn, reduced spermatogenesis in the rat testes. Furthermore, the results of (El-Yamany, 2009) showed a significant decline in serum levels of T, FSH, LH and prolacting (PRL) of rats fallowing VCA administration.

The author attributed the decline of T, FSH, LH and PRL levels to the dysfunction of pituitary gland and also demonstrated that VCA affects the testes directly and/or indirectly through its effect on pituitary gland and decreases the secretion of FSH and LH. Also, the present results are in accordance with the study of (Gunnarsson et al., 2003) who found that cadmium caused a decrease in T production through the decrease in LH receptor messenger ribonucleic acid (mRNA) levels as well as cyclic adenosine monophosphate (cAMP) levesl in rats.

To protect spermatogenesis from toxicant exposure, many clinical and experimental trials of antioxidant agents have been attempted. Carotenoids as potential antioxidant are well known as highly efficient scavengers of singlet molecular oxygen (1O2), and other excited species. The present study indicates the beneficial effects of β-cryptoxanthin against vinyl cyanide induced testicular toxicity. β-cryptoxanthin treatment improved the levels of endocrine parameters including T, androsterone, FSH and LH. These results are in agreement with those obtained by (Livera et al., 2002) who found that in adult rats, retinoids increased basal testosterone secretion in Leydig cell primary cultures. Also, (Hanukoglu, 2006) reported that the antioxidant enzyme activities superoxide dismutase, catalase, and glutathione peroxidase are parallel steroidogenesis and the antioxidant β-cryptoxanthin exerted a protective role on Leydig cell steroidogenesis to produce testosterone; thus it stimulates the development of reproductive organs through the growth of Leydig and Sertoli cells and the promotion of spermatogenesis. Also, (Silva et al., 2001) suggested that pretreatment with another carotenoid, bixin reduced the total number of chromosome aberrations and inhibited the increase in lipid peroxidation induced by cisplatin. Furthermore, (Gupta & Kumar, 2002) elucidated that the effect of oral lycopene, a naturally occurring carotenoid in tomatoes, therapy in men with idiopathic infertility and found improvement in male infertility and especially in sperm characteristics.

A rational mechanism for the protective effects of β-cryptoxanthin could be the potential antioxidant activity. Because β-cryptoxanthin is a lipophilic substance, it exerts its action in hydrophobic environment such as the lipid core of membranes. Thus, it is anticipated that natural β-cryptoxanthin, a chain breaking antioxidants, can contribute to protecting cell membranes from lipid peroxidation (Krinsky, 1998). β-cryptoxanthin can function as an effective antioxidant not only against singlet oxygen but also against lipid peroxidation and the highly destructive, hydroxyl radical OH• that is implicated in many diseases such as cancer and heart disease (O'neill & Thurnham, 1998).

CONCLUSION

In conclusion, this study clearly demonstrated the potential antioxidant benefit of β-cryptoxanthin in managing VCN-induced physiological changes in the testes of rats.

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التغيرات الفسيولوجية الهرمونية للخصية الناتجة عن التعرض لسيانيد الفينيل والدور الوقائي المحتمل للبيتاكريبتوزانثين في ذكور الجرذان

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المستخلص: سيانيد الفينيل منتج أليفاتي يستخدم على نطاق واسع في مختلف الصناعات الكيميائية الاصطناعية، ومن المعروف أن سيانيد الفينيل له تأثيرات سامة على البشر، وكذلك حيوانات التجارب. وقد صُممت هذه الدراسة لفحص قدرة بيتاكريبتوزانثين، وهو أحد مضادات الأكسدة الطبيعية، على تخفيف التغيرات الهرمونية للخصية التي يسببها سيانيد الفينيل في الجرذان البيضاء البالغة. وقد أجريت هذه الدراسة على أربعين من ذكور الجرذان البيضاء البالغة، تزن 240±10 جراما، تم تقسيمها إلى أربع مجموعات (10 جرذان لكل مجموعة). تمثل المجموعة الأولى (التي أعطيت زيت ذرة، وماء مقطر) المجموعة الضابطة. وأعطيت المجموعة الثانية عن طريق الفم جرعات من مركب سيانيد الفينيل تعادل 30 مجم/ كجم من وزن الجسم، وذلك على مدار الأيام الخمسة الأخيرة من نهاية التجربة. وأعطيت المجموعة الثالثة يومياً عن طريق الفم جرعات من البيتاكريبتوزانثين تعادل 40 مجم/كجم من وزن الجسم لمدة 30 يوماً، أما المجموعة الرابعة فقد أعطيت البيتاكريبتوزانثين وسيانيد الفينيل مثل المجموعتين الثانية، والثالثة، وبالجرعات نفسها. وتم جمع عينات الدم من مجموعات الجرذان بعد أربعة وعشرين ساعة من إعطاء الجرعات الأخيرة.وقد أظهرت النتائج أن إعطاء سيانيد الفينيل فى المجموعة الثانية قد تسبب في نقص ملحوظ، وذي دلالة إحصائية في مستويات كل من هرمون التستوستيرون، والأندروستيرون، والهرمون المنشط للحويصلات، وهرمون الجسم الأصفر في مصل الدم، مما يدل على أن لسيانيد الفينيل تأثيراً ضاراً على هرمونات التكاثر في ذكور الجرذان البيضاء، أما المجموعة الرابعة التي أعطيت البيتاكريبتوزانثين قبل وفي وقت متزامن مع سيانيد الفينيل فقد حدث تحسن ذي دلالة إحصائية في مستوي هذه المعايير. وبذلك تكون النتائج قد أوضحت أن للبيتاكريبتوزانثين دور وقائي في تقليل التغيرات الهرمونية لخصى ذكور الجرذان البيضاء الناتجة عن المعاملة بسيانيد الفينيل.

الكلمات المفتاحية: سيانيد الفينيل، بيتاكربتوزانثين، هرمونات، خصى، جرذان.

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Effect of Different Dietary Protein Levels on Survival Rate and Growth Performance of Guppy (*Poecilia reticulata*)

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Abstract: This study was carried out to evaluate the growth response of guppy *Poecilia reticulata* to different dietary protein levels. A total of 72 fry of guppy fish of an initial body weight 0.365 ± 0.01g were distributed randomly into 60 L aquaria at the density rate of 0.05 g/L. Fish were fed to satiation twice daily for eight weeks with diets containing different dietary protein levels (20%, 30%, or 40%.). The survival rate of guppy was unaffected by increasing dietary protein levels. At the end of the experiment, weight gains (g) ranging from 1.93 to 3.55 were obtained corresponding to 20% and 40% crude protein respectively. The growth performance and feeding efficiencies were significantly improved ($P < 0.05$) by increasing dietary protein levels. However, no significant difference ($P > 0.05$) was observed in the specific growth rate of fish that were fed 20% and 30% dietary protein levels. The value of the condition factor was significantly high in fish fed at 40% dietary protein. It can be concluded that a 40% dietary protein level is recommended for guppy fish.

Keywords: Guppy; Protein Level; Survival Rate; Growth Performance

INTRODUCTION

The ornamental fish trade plays an important role in the global economy, as millions of recreational fish are sold annually around the world (AOAC, 1997; Kumaratunga & Radampola, 2019). The major countries participating actively in this trade are the USA, Australia, Europe, Singapore, Japan, the Philippines, Sri Lanka, and Indonesia. The ornamental fish business has been estimated at more than 8 billion USD (Jayalal et al., 2016). Despite the economic significance of this sector, studies of the nutritional requirements of ornamental fish are rarely reported (Lovell, 2000). Most of the nutrition information used for ornamental fish species is based on results about edible fish. It is important to know the nutritional requirements of ornamental fish species, that one may provide a diet rich in essential nutrients to ensure the efficient growth of ornamental fish

from the stage of growth to the stage of hatching and reproduction (Hoseini et al., 2017; Masrizal et al., 2015). In general, protein is considered the most important and most expensive component of fish feed, which often accounts for about 60% of the cost of fish feeds (Khattab et al., 2001). Protein affects the fish's growth and production cycle. Maximum feed proteins provide the maximum growth rate, thus maximizing the benefits to fish farmers (Hoseini et al., 2018). The dietary protein requirements for freshwater ornamental fish are from 30 to 50% in omnivores and carnivores, respectively (Hardy (2003)).

Dietary protein requirements for many ornamental species are still unknown. The guppy fish (*Poecilia reticulata*) is one of the common ornamental fishes that is widely produced in many countries around the world due to its low prices and adaptability. This

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study aimed to investigate the effect of increasing dietary protein on the survival rate, growth performance, and feed utilization of the guppy.

MATERIAL AND METHODS

Experimental diets preparation: Three experimental diets were formulated based on a basal diet formulated from practical ingredients to satisfy all known nutrient requirements *Poecilia reticulata* according to (NRC, 1993).

The formula and analyzed proximate composition of the three experimental diets are shown in Table (1).

The ingredients were hammer milled to a fine powder and then sieved through a mesh size of 0.5 mm diameter. The dry ingredients were mixed for 15 min, and then the lipid was added slowly and further mixed for another 15 min. After which, the distilled water was added at 25% of the total ingredient weight. Three different experimental diets were formulated with an increased dietary protein content to make 20%, 30%, and 40% crude protein concentrations. The proximate composition of diets was determined according to standard AOAC (1997) methods.

Table (1): Ingredient and proximate composition of the experimental diets.

Ingredients	%		
Dietary protein level (% dry weight)	20	30	40
Fishmeal	17	27	36
Soybean meal	26	37	38
Ground corn	48	27	17
Soy oil	7	7	7
Vitamins	1	1	1
Mineral premix	1	1	1
Proximate composition (% dry weight)			
Dry matter	93.12	93.91	94.63
Crude protein	20.81	30.56	40.11
Crude lipid	6.14	6.88	6.65
Ash	6.65	7.1	7.82

Experimental setup: A total number of 72 Guppy (*P. reticulata*) fry were purchased from local suppliers of aquarium fish shops, Libya, and placed in a 500 L fiberglass tank. Fish were randomly and equally stocked into nine rectangular glass aquaria (80×35×50cm) of 60 L dechlorinated fresh tap water for a 20 days acclimation period and fed with a commercial diet designed for ornamental fishes. All guppy in the aquaria were weighed individually at the start of the experiment. At the start of the experiment, each guppy fry was weighed individually, and had an average body of 0.365 ± 0.01 g. The aquaria were then randomly designated to each dietary protein treatment in triplicates. All fish were fed the experimental diets twice daily (9:00 a.m. and 4:00 p.m) until apparent satiation (Belal et al., 2015; McGoogan & Reigh, 1996). Each aquarium received gentle aeration with natural photoperiod (13 hours light 11 hours dark) and an ambient temperature of (26–28°C). The water parameters were measured daily. The pH was measured using a digital pH probe (Jenway 3305), water temperature and dissolved oxygen were measured using OXI 92. pH and the dissolved oxygen were 7.8–8.1 and 4.8–5.6 mg/*l* respectively. At every 72 hours, aquarium water exchange was performed, and the aquaria were thoroughly cleaned. The fish was fasted one day before ending the feeding trial.

Measurement of growth parameters : During the study, length (cm) and weight (g) of individual guppy fry were measured and recorded on the first day and then every 72 hours. After 8 weeks, the fish were mildly anesthetized with clove oil, and then the length and weight of individual guppies were recorded. The following parameters were estimated:

Growth performance: The percent weight gain (WG), average daily gain (ADG), and specific growth rate (SGR) (Han *et al*. 2015) were calculated by the following equations:

WG (%) = [(BWf _ BWi) /BWi] × 100
ADG (g /day) = (BWf _ BWi) / T
SGR (%/day) = [ln (BWf _ lnBWi) / T] ×100
Where, BWf, BWi, and T are the final body weight, the initial body weight and time in days, respectively, and all weights are in grams (g).
Condition Factor (CF) (Rad *et al*., 2012) was calculated by the following equation:
 CF = [TW (g) / L (cm3)] × 100
Where, TW and L are the total body weight (g) and total length (cm), respectively.

Daily feed intake (FI) and Feed conversion ratio (FCR) were calculated according to (Rad *et al*. 2012) by the following equations:
(FI, g /d/ fish) = Diet consumed × 100 / duration in days /fish number per tank
(FCR, g) = [dry weight of feed (g)/ wet weight gain (g)].

The percent of survival was calculated according to (Han *et al*. 2015) by the following equation:
 Survival (%) = (N_f/N_i) × 100
Where, N_s and N_i are the number of surviving fish and the initial number of fish, respectively

Statistical analysis: All data were presented as means (± standard deviation). A one-way analysis of variance (ANOVA) was utilized for the data analyses, after prior confirmation of homogeneity and normality. Statistical significance at $P < 0.05$ was used and means were separated using Duncan's multiple range tests to determine differences between treatments. The SPSS version 22.0 was used for all statistical analyses.

RESULTS AND DISCUSSION

The proximate composition of experimental diets (Table 1) showed that the dry matter, lipid, and ash were not significantly different ($P < 0.05$) between the experimental diets used, while the protein content was significantly different depending on the formulation.

Guppies readily accepted all experimental diets and were observed to feed voraciously throughout the experiment. The fish growth performance, feed conversion ratio (FCR), and weight gain significantly increased ($P < 0.05$) by the increase in protein content in experimental diets (Table 2). However, no significant differences were observed in SGR among fish fed diets containing 20% or 30% dietary protein levels compared to fish fed diets containing a 40% dietary protein level. The fish survival was very high for all groups with no significant differences among them (Figure 1).

Table (2): Growth performance and feed utilization of guppy, fed diets with different protein levels for 8 weeks.

Parameter	Dietary protein level (%)		
	20	30	40
Initial weight(g)	0.364±0.00	0.365±0.00	0.365±0.00
Final weight (g)	2.28±0.20[c]	2.70±0.22[b]	3.91±0.15[a]
ADG (g)	1.93±0.21[c]	2.33±0.22[b]	3.55±0.15[a]
Weight gain (%)	527.91±66.3[c]	639.480±57.3[b]	972.47±35.7[a]
Feed conversion ratio	2.83±0.29[a]	2.21±0.21[b]	1.20±0.00[c]
Specific growth rate	0.57±0.18[b]	0.65±0.07[b]	0.98±0.03[a]
Feed intake	7.31±0.52[a]	6.04±0.43[b]	3.58±0.04[c]

Mean ± SE values within the same row and followed by different superscripts are significantly different ($p < .05$).

Dietary protein plays an important role in fish nutrition and it immediately affects fish growth performance and reproduction (Oliva‑Teles, 2012). When fish are fed diets containing surplus levels of dietary protein, it might cause decreased growth and health obstruction because of raised amino acid
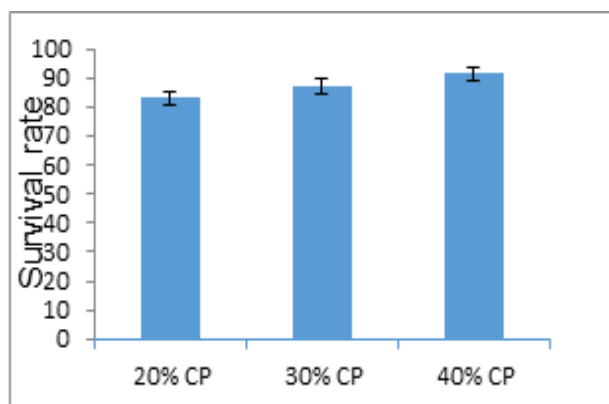
Figure 1: Survival rate (%) of guppy fed diets with different protein levels.

Catabolism. Also, the production of high levels of ammonia and higher energy expenditure to deaminize excessive amino acids (Jiang et al., 2016; Melo et al., 2006). However, sub-optimal protein levels are unsuccessful in meeting the fish requirements for growth, and thus this clarifies the lower growth rate and feed efficiency in diets containing low levels of dietary protein (Bowyer et al., 2013).

In the present study weight gain and FCR had significantly varied among all experimental diets. The best weight gain and FCR were obtained from the fish fed a diet containing a 40% dietary protein level. Which had better growth and the best FCR. These results agreed with the results of (AOAC, 1997; Baishya et al., 2012). Also, (Dahlgren (1980)) found that the better total body weight of guppy *Poecilia reticulafa* was obtained at high dietary protein levels (31% and 47%) rather than 15 % protein. In this current study, The SGR value of the fish fed with the diet containing 40% protein content was found significantly higher than that of the other two groups, which indicates that the nutrients available in the diet containing 40% protein content fulfilled the dietary requirement of guppy to grow faster than the other two groups. These findings are in agreement with Kithsiri *et al*. (2010) who found that SGR increased with increasing dietary pro-

tein content from 18% to 43% in the female guppy, *Poecilia reticulata*. In this study, feed intake was significantly influenced by dietary protein levels. Fish needs diets with a high level of protein content because of their bad utilization of carbohydrates as an energy source, thus the adequate supply of dietary protein is needed for fast growth (Lovell, 2000).

The condition factor (CF) that shows the level of wellbeing of the fish is based on a relationship between length and weight (Nehemia et al., 2012). The CF of fish is influenced by several circumstances such as stress, sex, season, availability of feed, and other water quality parameters (Khallaf et al., 2003). If the CF value is high, this means that the fish has achieved a better health condition. In this study, the condition factor was significantly increased with increasing dietary protein levels (Figure 2). This result agrees with (Khattab et al. (2001)) who reported that the condition factor in Nile tilapia significantly increased with increased dietary protein levels.
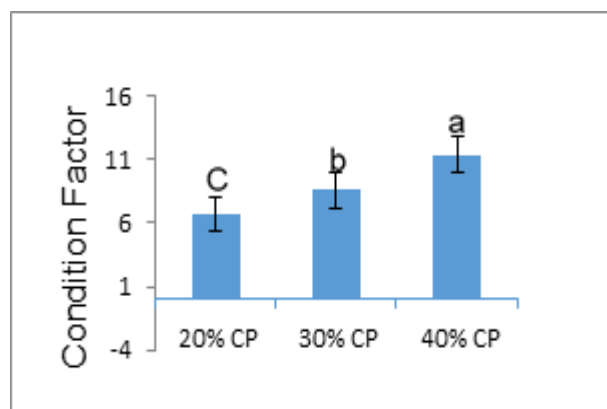

Figure 2: Condition factor of guppy fed different levels of crude protein (CP) diet.

They also added that, a high level condition factor indicated the healthy growth of fish, which is desirable for ornamental fish farms. In conclusion, a dietary protein level of 40% is necessary for maximum growth and a better condition factor for guppy fish.

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تأثير المستويات المختلفة للبروتين الغذائي على معدل البقاء، وأداء النمو لأسماك الجوبي
Poecilia reticulata

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المستخلص : أجريت هذه الدراسة لتقييم استجابة نمو أسماك الجوبي لمستويات مختلفة من البروتين الغذائي، وزعت اثنان وسبعون من زريعة أسماك الجوبي بوزن إبتدائي 0.365 جم بشكل عشوائي في كل حوض (حجم 60 لتر)، بمعدل كثافة 0.05 جم/لتر، غذيت الأسماك إلى مستوى الشبع مرتين يوميًا لمدة ثمانية أسابيع على وجبات تحتوي على مستويات مختلفة من البروتين الغذائي بنسبة 20٪، و 30٪، و 40٪، لم يتأثر معدل البقاء لأسماك الجوبي بزيادة مستويات البروتين في الغذاء، في نهاية التجربة، تم الحصول على زيادة في الوزن (جم) بمعدل يتراوح من 1.93 إلى 3.55 عند مستويات بروتينات 20٪، و 40% بروتين على التوالي، لوحظ تحسن أداء النمو، وكفاءة التغذية معنوياً ($P < 0.05$) عن طريق زيادة مستويات البروتين في الغذاء، ومع ذلك لم يُلاحظ أي فرق معنوي ($P > 0.05$) في معدل النمو النوعي للأسماك التي تمت تغذيتها بنسبة 20٪، و 30٪ من مستويات البروتين في الغذاء، وكانت قيمة معامل الحالة عالية بشكل ملحوظ في الأسماك التي تمت تغذيتها بنسبة 40٪ بروتين غذائي، نستنتج أن مستوى البروتين الغذائي بنسبة 40٪ يوصى به لأسماك الجوبي.

الكلمات المفتاحية: أسماك الجوبي، مستوى البروتين، معدل البقاء، أداء النمو.

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عزل وتعريف مسبب مرض التبقع البني الشكولاتي (*Botrytis faba*) على أوراق الفول البلدي في منطقتي المرج والوسيطة بالجبل الأخضر

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المستخلص: استهدفت هذه الدراسة تعريف الفطر المسبب لمرض التبقع البني على أوراق الفول، حيث جمعت عشوائياً أوراق الفول من حقول المرج، وحقول الوسيطة خلال فبراير ومارس 2017 في مراحل: الإزهار، والإثمار المتأخرة، تظهر عليها أعراض المرض عدد 50 نباتا/ حقلا. وقدرت نسبة الإصابة في العينات وشدتها، عزل مسبب مرض التبقع البني من الأوراق المصابة طبيعيا، وبعد اختبار قدرته الإمراضية، نمي الفطر النقي على الأوساط الغذائية صلبة شملت كل من زابكس (Cz)، وبطاطس دكستروز أجار (PDA)، وبيئة مالت أجار(MA)، وحضنت عند 22 درجة مئوية، بينت نتائج الدراسة إصابة أوراق الفول المزروعة بحقول كل من المرج والوسيطة بمرض التبقع البني، حيث ظهرت أعراضه على شكل بقع بنية محمرة صغيرة على الأوراق، ومتوسط قطرها 0.52 سم، وسجلت كمية المرض بحساب نسبة الإصابة، ووصلت في حقول المرج إلى 87.6%، في حين كانت شدة الإصابة أعلى في حقول الوسيطة، حيث بلغت 49.7%. كما أكدت الدراسة على قدرة الفطر النقي المعزول من أوراق الفول على إحداث الإصابة وتسجيل البقعة على أوراق نبات الفول صنف المصري بعد 48 ساعة من الحقن. واتضح من خلال القياسات أن المسبب المسؤول عن المرض هو الفطر *Botrytis fabae*، المميز بميسليوم مقسم داكن، متوسط سمكه (11.75 ميكرومتر)، وينتج الجراثيم الكونيدية في عناقيد عند أطراف متسلسلة متفرعة، وهي أحادية الخلية، مستديرة لونها بني خفيف، متوسط الطول 14.5 ميكروميتر والعرض 10.8 ميكروميتر، كما أعطت بيئة زابكس أعلى نمو ميسليومي، وجراثيم وأجسام حجرية مقارنة بالأوساط الغذائية الأخرى.

الكلمات المفتاحية: مرض التبقع البني، أوراق الفول، تعريف فطر *Botrytis fabae*

المقدمة

يعد نبات الفول *Viciafaba*L. التابع للعائلة البقولية (Fabaceae) (Cubero،2011) محصولا اقتصاديا عالميا وهو ثالث أهم البقوليات الغذائية، ومن أقدم المحاصيل التي عرفها الانسان منذ 8000 سنة قبل الميلاد، حيث سجل كغذاء لكل من قدماء المصريين، والإغريق، والرومان واليهود؛ والموطن الأصلي له إثيوبيا (Sahile وآخرون، 2009). يتميز بقيمته الغذائية العالية لاحتوائه على البروتين، والمواد الكربوهيدراتية، والكالسيوم، والفوسفور، والحديد، والفيتامينات

(Noorka و El-Bramawy، 2011). ويستخدم مجموعه الخضري محصولاً علفياً، إلى جانب الاستفادة من هذا المحصول في رفع خصوبة التربة من خلال تثبيت النتروجين، وتكوينه للعقد الجذرية. وتشير الدراسات الحديثة إلى ارتفاع إنتاجية هذا المحصول حيث بلغ 1.8 طن/ هكتار، رغم انخفاض مساحة زراعته من 1.8 مليون هكتار سنة 1961 إلى 0.3 مليون هكتار سنة 2007 وذلك بسبب تطور الأصناف التي أصبحت عالية الإنتاج وبلغ الإنتاج العالمي لسنة 2007 1.2 مليون طن، أما في ليبيا 899هكتار ، 1565 طن (FOA، 2018).

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المواد وطرق البحث

جمـع العينـات: أجريت زيارة ميدانيـة لكل من حقول المرج، وحقول الوسـيطة خـلال فبرايـر ومـارس 2017 فـي مراحـل الإزهار والإثمار المتأخرة. من كل حقل، تم اختيار 50 نبتة بشكل عشوائي، وفحصها عن كثب. الأمراض السـائدة تـم تسـجيلها بنـاءً علـى الأعـراض البصـرية. لتأكيـد التشـخيص الميداني بناءً على الأعراض .

تقدير نسبة وشدة الإصابة بمرض التبقع على أوراق الفول: قدرت نسبة الإصابة في العينات التي جلبت إلى المعمل وفقا لمعادلـة (James 1971): (نسبة الإصابة =عـدد الأوراق المصـابة/العدد الكلـي لأوراق100X)، أمـا شـدة الإصابة باستخدام مقياس(2005, ICARDA) الموزعـة درجـات الإصابة مـن 0–5.(Elwakil وآخـرون، 2016) وللتقيـيم أُجريـت العمليــة الحسـابية وفقـاً لمعادلـة (Horsfall وHeuberger،1942):100x(DS=(ab/rN

DS = شـدة الإصابة، a = درجـة المقياس، b = عـدد النباتات في كل درجة، r = عدد درجات المقياس، N= عدد النباتات الكلية) الشكل 1.

تقييم المرض:
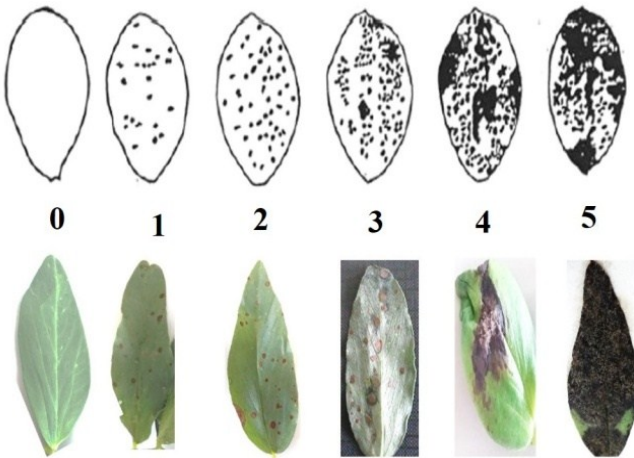
الشــكل (1). مقيـاس مـرض التبقـع الشـوكلاتي علـى أوراق الفول (مصـدر: ICARDA، 2005) 0= نبات سليمة، 1 = بقـع صـغيرة، 2= زيـادة في عدد البقع،3= التحام البقع، 4= البقع تحتل نصف الورقة، 5= موت الورقة

تعد الأمراض الفطرية من أهم العوامل الحيوية المتسببة في الحد من غلة الفول (Ahmed وآخرون 2010). من بينها بقعة الشوكولاتة، والصدأ الذي يعد أهم الأمراض في منطقة البحـر الأبـيض المتوسـط (Bisri وHonounik، 1991). يصاب محصول الفول بأمراض نباتية عديدة تنتقل غالبا عن طريق البذور، من أشهر تلك الأمراض الفطرية المسجلة على المجموع الخضري بمنطقة بنغازي (ليبيا)، مرض التبقع البني النـاتج عـن الفطـر Botrytis fabae (El-Ammari 2019) هو مرض متخصص، ومدمر لمحصول الفول، ويقلل من إنتاجه(Terefe وآخرون، 2015). تظهر أعداد هائلة من جراثيم الفطر على الأوراق ثم تنتقل بسهوله بواسطة الهواء خـلال الطقس الرطب ، كما سجل Elad وآخرون، 2004 الفطر في كل من: أوروبا، والشرق الأوسط، وإفريقيا، وآسيا، وأمريكـا الجنوبيـة، وكوريـا، والهنـد، وأسـتراليا، وكنـدا، وعـزل المسبب المرضي من نباتات الفول على وسط اجار دكستروز، تميز بسرعة النمو، وكان معدل النمو اليومي (1.058 ملم يوم 1-) عند 22 درجة مئوية، حيث وصل إلى (84.0) ملم بعد 5 أيام (Terefeوآخـرون2015) مسـتديرة أو بيضـاوية الشـكل مـع عنـق قصـير علـى حامـل (Pont و Pezet ، 1990). بلـغ متوسـط حجـم الجرثومـة الكونيـدي (24.86 ×16.32ميكرومتـر) (Terefe وآخـرون 2015). الأجسـام الحجريـة صـلبة متحجـرة فـي البيئـة، متكلسـة بعـض الأحيـان غالبـا 1,7 mm-1 قطر ، نادرا أعلى من mm.3 الجراثيم عادة كبيرة مقارنة بالناتجة عن -13 (11-) x (29-) 16-25 (1-) [16 (- 20) µm [I/b ratio 1:25-1:45. في بيئة مستخلص أوراق الفول ينتج كميات كبيرة من الجراثيم الكونيدية بسـبب زيـادة تركيـز الأمـلاح غيـر العضـوية مثـل نتـرات الصوديوم.

استهدفت هذه الدراسـة تعريف الفطر المسبب لمرض التبقع البني على أوراق الفول في منطقتي المرج، والوسيطة بالجبل الاخضر .

(MA) وحضنت عند 22 م°، وقيس النمو الطولي خلال فترات التحضين المختلفة 3، 5 و 7 أيام.

النتائج

أوضحت نتائج الدراسة المسحية في الحقول المبينة بالشكل (2) وجود مرض التبقع البني على أوراق الفول المزروعة بحقول كل من المرج والوسيطة، وسجل ارتفاع في نسبة الإصابة على أوراق الفول المزروعة بحقول المرج حيث وصلت النسبة إلى 87.6%، ولوحظ ارتفاع شدة المرض في حقول الفول بمنطقة الوسيطة مقارنة بحقول المرج حيث بلغت 49.7%
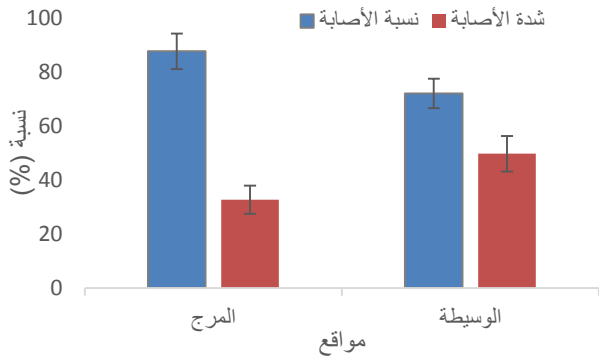
شكل:(2).نسبة الإصابة بمرض التبقع البني على أوراق الفول وشدتها المزروعة بحقول الوسيطة والمرج

وفي هذه الدراسة تم وصف أعراض المرض كما هو مبين بالشكل (3 –أ) حيث ظهرت أعراض بقع بنية محمرة صغيرة على أوراق الفول تتراوح بين 0.1– 1.05سم ومتوسط قطرها 0.52.سم، كما يتضح من الشكل (3 –ب) المبين لنتائج اختبارات القدرة الإمراضية للفطر المعزول من الأوراق المصابة طبيعيا، حيث يظهر بالشكل قدرة الفطر النقي المعزول من أوراق الفول على إحداث الإصابة، وتسجيل البقعة على أوراق نبات الفول صنف المصري بعد 48 ساعة من الحقن.

عزل الفطر *B. fabae* من أوراق الفول المصابة طبيعياً: عزل مسبب مرض التبقع البني من الأوراق وفقا لما ذكره (Gossen وآخرون، 1997). حيث أخذت الأوراق وقُطعت إلى قطع مساحتها 1 سم 2 بحيث يشمل الجزء المصاب والسليم بعد تعقميها سطحيا بواسطة 1% من محلول هيبوكلوريت الصوديوم 3 دقائق تغسل بالماء المقطر المعقم ثلاث مرات متوالية، وتجفف بورق نشاف معقم، ثم وضعت 4 قطع ورقية معقمة وجافة تماما مساحتها 1سم^2 في أطباق بتري تحتوي على الوسط الغذائي (الاجار المائي).درجة تحضين الفطر 19م° بعد 7 أيام من التحضين، نقيت المستعمرات الفطرية بنقلها إلى أطباق جديدة بها بيئة البطاطس دكستروز أجار (PDA). حضنت في درجة حرارة (22م°)، لمدة أسبوعين للحصول على جراثيم.(Harrison, 1988) حتى الحصول على الفطر منفردا بصورة نقية، وأخذ القياسات ودونت المواصفات الشكلية تحت المجهر الضوئي، أما التعريف فقد تم اعتمادا على التراكيب الخضرية والتكاثرية للفطر المعزول وفق المراجع المتخصصة (Ellis وWaller، 1974؛ Barnett و Hunter 1998 ؛ Malone وآخرون، 1997).

القدرة الإمراضية : للتوضيح أكثر زرعت بذور الفول الصنف المصري في أصص بلاستيكية قطرها20 سم، تحتوى على خليط من الطين والرمل والبتموس (8: 1 :1) زرعت 5 بذور في الأصيص، بعد 45 يوم من الزراعة، اختبرت قدرة الفطر *Botrytis fabae* المعزول بالحقن الصناعي بجراثيم الفطر على الأوراق تحت الكيس البلاستيكي لمدة 24 ساعة لرفع مستوى الرطوبة حول النباتات، تم تقييم المرض بعد 48 ساعة.

تنمية الفطر على أوساط غذائية مختلفة للحصول على تراكيب الفطر المختلفة ولحساب سرعة نموه: نمي الفطر النقي في الأوساط الغذائية الصلبة، شملت كل من زابكس (Cz) وبطاطس دكستروز أجار (PDA) وبيئة مالت أجار

ويتميز الحامـل الكونيـدى بلونـه البنـي الخفيف عنـدما يكون حديثا، أمـا الحامـل المسـن لونـه داكـن سـمكه (9.6- 10.9 ميكروميتر) بمتوسط 10.2 ± 0.91، كما يتميـز هـذا الفطـر بوجود حامل العناقيد لون الحديث منه بني خفيف، وأما المسن لونـه داكـن سـمكه (5.8- 6.4 ميكروميتر) بمتوسط 6.1 ± 0.4، ممـا يؤكـد علـى أن عزلـة لــ *Botrytis fabae* Sardina المتحصـل عليهـا مـن أوراق الفـول هـو المسـبب المرضي المسؤول على أعراض مرض التبقع البني.

شــكل :(3) التعـرف علـى مـرض التبقـع البنـي علـى أوراق الفـول. (أ) أعراض مـرض التبقـع البنـي لأوراق مصـابة طبيعيـا و(ب)اختبـار القـدرة الإمراضية

شــكل: (4) التراكيب الخضـرية، والتكاثريـة للفطـر *Botrytis fabae* المعزول من أوراق الفول المزروع بحقول المرج. (أ): ميسليوم الفطر، (ب): جراثيم الفطر بيضاوية الشكل، (ج) جسم حجري، د: جراثيم الفطر على حامل كونيدى عند قوة تكبير X10، هـ: جراثيم الفطر على حامل كونيدى عند قوة تكبير X20، و: جراثيم الفطر على حامل كونيدى عند قوة تكبير X40

ويتضـح من الشكل (4) وصف المسبب *Botrytis fabae*، المميز بميسليوم لون الحديث منه بني خفيف، أما الميسليوم المسـن لونـه داكـن شـفاف، يتحـول كلمـا تقـدم بالعمـر إلى اللـون الزيتوني الفاتح، ثم إلى اللون الرمادي الفاتح (4 –أ) سـمكه (9.0- 15.8 ميكروميتر) بمتوسط 11.75 ± 2.3 (جدول 1) بعد إنتاجه للجراثيم الكونيدية أحادية الخلية، مستدير لونـه بني إعـادة صـياغة خفيـف، متوسـط .الطـول 14.5 ميكروميتـر والعـرض 10.8 ميكروميتـر، بينمـا متوسـط قطـر الجرثومـة 12.6 ميكروميتـر (الشـكل4- ب) (الجـدول 1) يظهـر فـي عناقيد عنـد أطـراف متسلسلة متفرعـة (4 – د، هـ، و). كمـا سـجل وجود الأجسام الحجرية السوداء، وهي صـلبة متحجـرة، قطرهــا بمتوسـط 1.03 ± 0.15 ميكروميتـر، عنـد حسـاب الطـول/ العـرض كـان متوسـط (1.4 ± 0.17) ميكروميتـر،

جدول (1) يوضح قياسات الجراثيم والميسليوم على بيئة زابكس عمر المستعمرة

تراكيب الفطر	الوصف		القياسات بالميكرومیتر	
	اللون	الصفة	المدى	المتوسط ± الانحراف المعياري
الأجسام الحرية	صلبة متحجرة اللون أسود	قطرها	(0.8 – 1.4)	1.03 ± 0.15
		مساحتها	(0.5– 1.43)	0.85 ± 0.25
		الطول/ العرض	(1.1 –1.7)	1.4 ± 0.17
الميسليوم	الحديث بني خفيف المسن لونه داكن	سمكه	(9.0 –15.8)	11.75 ± 2.3
الحامل الكونيدى	الحديث بني خفيف المسن لونه داكن	سمكه	(9.6 –10.9)	10.2 ± 0.91
حامل العناقيد	الحديث بني خفيف المسن لونه داكن	سمكه	(5.8– 6.4)	6.1 ± 0.4
		الطول	(11.9 – 16.2)	14.5 ± 1.1
		العرض	(8.9 – 12.4)	10.8 ± 1.0
الجراثيم	بني خفيف	الطول/ العرض	(1.2 –1.6)	1.4 ± 0.13
		قطر	(10.8 – 14.2)	12.6 ± 0.9
		مساحة	(91.2 – 157.2)	126.2 ± 17.8
اختبار القدرة الإمراضية (نسبة الإصابة)* بعد 7 أيام			43.99%	

• نسبة الإصابة =[(المساحة المصابة/ المساحة الكلية)*100]

شكل (6) نمو الفطر *Botrytis fabae* على أوساط غذائية مختلفة. (Cz: زابكس، PDA : بطاطس دكستروز أجار، MA: مالت أجار)
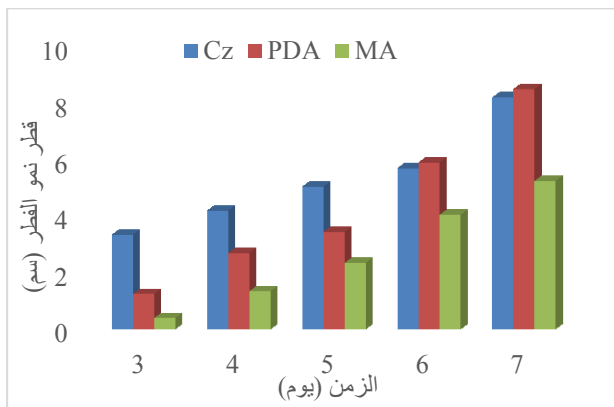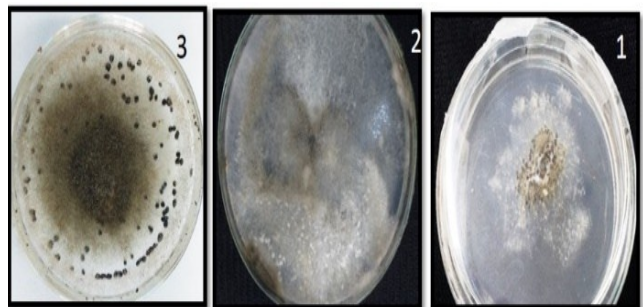
عند تنمية الفطر على أوساط غذائية مختلفة يتضح من الشكل (5) أن البيئة زابكس أجار هي أفضل البيئات المختبرة حيث أعطت نمو ميسليومي وجراثيم وأجساما حجرية مقارنة بالأوساط الغذائية المختبرة، يشير الشكل (6) إلى متوسطات أقطار نمو الفطر النامى على أوساط غذائية مختلفة، حيث يتضح من الشكل أن بيئة زابكس أعطت نموا عاليا يليه، بيئة بطاطس دكستروز أجار، بينما كانت بيئة المالت أجار هى الأقل نموا مقارنة بباقي الأوساط.

شكل (5): الأوساط الغذائية النامي عليها الفطر (1: بيئة مالت أجار، 2: بيئة بطاطس دكستروز اجار و3: بيئة زابكس أجار)

المناقشة

في هذه الدراسة أوضحت النتائج وجود مرض التبقع البني على أوراق الفول المزروعة بحقول كل من المرج والوسيطة، وسجل ارتفاع في كمية المرض بالمواقع المدروسة، وذلك لملائمة الظروف المناخية للمسبب المرضي من حرارة، ورطوبة نسبية، والتي تلعب دورا هاما في تطور المرض

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(Elad وآخرون، 2004). ووصفت أعراض الإصابة على الأوراق المتمثلة في بقع بنية محمرة أو شوكولاتية في الشتاء، وبداية الربيع ، بعد إجراء اختبار القدرة الإمراضية بالفطر المعزول كـان المسبب أكثر عدوانية علـى أوراق صـنف المصري حيث التحمت البقع وتسبب لطخات سوداء عند توفر ظروف الرطوبة العالية مؤدياً إلى موت مساحة كبيرة من الورقة حيث تم التصنيف اعتماداً على الأعراض والصفات الشكلية للفطر المعزول (Ateş. وآخرون، 2017)، كما أوضـحت النتـائج ارتفـاع نسـب الإصابة بمـرض التبقـع الشـوكلاتى ويرجـع ذلـك إلى الظروف المناخيـة مـن حـرارة معتدلة، ورطوبة عالية، وتتفق هذه النتائج مع ما ذكره كل من (Bernierr وآخرون 1993) أما El-Ammari، 2017 فقد سجل إصابة عالية بحقول بنغازي خلال شهري فبراير ومارس 2017. حيث تتحكم درجة الحرارة والرطوبة النسبية في زيادة معدل تكاثر الفطريات، والظروف الفسيولوجية للعائل النباتي، وبالتالي ظهور أعراض المرض وتطوره، ومن جهة أخرى فإن درجة الحرارة تؤثر على مقاومة النبات ضد المرض وفقا لهذه المتغيـرات (Terefe وآخـرون، 2015)، تعـود خطـورة هـذا الفطر اخترق العائل النباتي بتعدد وسائل تكاثره من خـلال امتلاكـه قـدرة كبيرة لإنتاج الجراثيم الكونيديـة علـى الأنابيب الجرثوميـة القصيرة، والتي تتنقل عبر تيارات الهواء الجـاف، والعدوى ناتجة عن التصـاق الكونيديا في قطرات المـاء، كما يتميـز بالتحول مـن النمـو الخضـري السـريع إلى الإنتـاج مـن sclerotia مهمة في بقاء الفطر عندما تصبح الظروف البيئية غيـر ملائمـة (Jarvis وآخـرون،1980)، وتعـد بيئـة زابكس وبيئـة بطـاطس دكسـتروز أجـار ملائمـة لنمـو الفطر وتكـاثره (Yetayew،2017).

الاستنتاج

سجلت هذه الدراسـة مرض التبقع البني بحقول بعض مناطق الجبل الأخضر، وتحديد أعراضه المميزة ، وتم تعريف المسبب المرضي بناء على الصفات الشكلية، وقياس نموه على بعض الأوساط الغذائية.

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Identifying the Cause of Chocolate Spot (*Botrytis fabae*) Disease on Faba Bean Leaves in Al-Marj and Al-Wasitah regions in Al-Jabel Al-Akhdar

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Abstract: This study aimed to identify the fungus that causes chocolate spot disease on bean leaves. Bean leaves were collected randomly from fields in Al-Marj and Al-Wasitah regions during February and March 2017 in late flowering and fruiting stages, showing symptoms of disease 50 plants/field. The infection rate and severity of the samples were estimated. The pathogen of chocolate spot disease was isolated from naturally infected leaves. After testing its pathogenicity, the pure fungus was grown on solid food media that included both Zapex (Cz) and potato dextrose agar (PDA) and environment malt agar (MA) and incubated at 22 Celsius.The results of the study showed that the bean leaves grown in the fields of both Al-Marj and Al-Wasitah were infected with chocolate spot disease, as its symptoms appeared in the form of small reddish-brown spots on the leaves with an average diameter of 0.52 cm. The severity of the disease was recorded by calculating the infection rate, and it reached 87.6% in Al-Marj fields, while the severity of infection was higher in Al-Wasila fields, reaching 49.7%. The study also confirmed the ability of the pure fungus isolated from bean leaves to infect and record the stain on the leaves of the Egyptian bean plant 48 hours after injection. It became clear through the measurements that the pathogen responsible for the disease is the fungus *Botrytis fabae*, characterized by dark divided mycelium, its thickness (average of 11.75 micrometers), and its production of conidia bacteria in clusters at the ends of a branched-chain, which are single-celled, round, light brown in color, average length 14.5 micrometers and width 10.8 micrometers. Also, the Zapex environment gave the highest growth of mycelium, germs, and microsclerotia compared to other nutritional media.

Keywords: Chocolate Spot Disease; Fabae Bean Leaves; *Botrytis fabae*.

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