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Effect of Hormone Types and Concentrations on *In Vitro* Multiplication and Growth of Pineapple (*Ananas Comosus* (L.) Merr.) Cv Moris on Liquid MS Medium

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Abstract: The effect of two cytokinins, 6-benzyleaminopurine (BAP) and kinetin (KN) and two auxins, naphthalene acetic acid (NAA) and indole acetic acid (IAA) each applied at 10 concentrations with an increment of 0.5 mg/l in the *in vitro* shoot formation of Moris pineapple was evaluated in static liquid MS medium. The highest shoot formation (9 shoots per explant) was obtained in medium enriched with NAA but at high concentration (4.5 mg/l). The second best was 8 shoots per explant obtained in medium enriched with BAP at 2.0 mg/l. Out of 10 concentrations, NAA was better than BAP at the highest 5 and less at lowest 5 concentrations and at all concentrations both hormones resulted in production of more shoots per explant than hormone free medium. Except at two concentrations, NAA and BAP were better than KN. Either one of these three hormones, BAP, NAA, KN could be claimed, depending on the tested range of concentrations, better than the others for *in vitro* shoot formation of Moris pineapple. All of the IAA concentrations, on the other hand, failed to produce more shoots than hormone free medium. But, the longest shoot (56 mm) obtained in medium enriched with IAA at 2.5 mg/l and the shortest shoots (6 mm) on medium enriched with KN at 4.0 mg/l. Seven of the IAA concentrations resulted in shoots longer than hormone free medium (27 mm) while the shoots length on the BAP, NAA and KN contained media was two times shorter than hormone free medium. BAP at 2.0 mg/l was the best treatment for multiplication and IAA at 2.5 mg/l was the best for elongation of Moris pineapple.

Key words: Pineapple, *Ananas comosus*, liquid culture, *in vitro* multiplication.

INTRODUCTION

Tissue culture of pineapple were reported using solid medium(Bhatia and Ashwath 2002, Sripaoraya *et al.,* 2003, Hamad and Taha 2008a, Hamad and Taha 2008c, b, Dutta *et al.,* 2013, Nelson *et al.,* 2015) Hamad,2017), semi liquid (Akin-Idowu *et al.,* 2014), double phase system (Pérez *et al.,* 2012), filter paper bridge (Mathews and Rangan 1979, 1981), direct placement in a stationary liquid (Almeida *et al.,* 2002, Hamad and Taha 2003, Be and Debergh 2006, Teixeira *et al.,* 2006, Pérez *et al.,* 2012) and agitated liquid culture at 50 (Kofi and Adachi 1993), 70(Fernando 1986), 100 (Aydieh *et al.,* 2000, Soneji *et al.,* 2002, Zuraida *et al.,* 2011) and 120 rpm (Zepeda and Sagawa, 1981). In cases in which medium states effect was compared, liquid was better than solid medium and agitated culture was better than the stationary one. Bioreactor system was also used to reduce the cost of pineapple *in vitro* culture (Escalona, *et al*., 1999; Firoozabady and Gutterson, 2003; Scherer, *et al*., 2013). The most commonly used hormone for multiplication was BAP. It was used singly (Zepeda and Sagawa, 1981; Fernando, 1986; Aydieh *et al*., 2000; Almeida

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et al., 2002; Be and Debergh, 2006; Hamad and Taha, 2008c; Akin-Idowu, *et al*., 2014; Nelson, *et al*., 2015) as well as in combination with auxins. Combinations of three hormones, BAP, NAA, IBA (Khatun, *et al*., 1997), KN, NAA and IBA (Mathew and Rangan, 1976; Rahman, *et al*., 2001; Soneji, *et al*., 2002), KN, NAA, IAA (Mathew and Rangan, 1981), BAP, IAA and IBA (Teixeira, *et al*., 2006), two hormones BAP and IAA (Gangopadhyay, *et al*., 2005; Hamad and Taha, 2008 a and b; Dutta *et al*., 2013;) , BAP and NAA (Kofi and Adachi, 1993; Escalona *et al*. 1999; Vesco, *et al*., 2001; Firoozabady and Gutterson, 2003; Perez, *et al*., 2012; Scherer *et al*., 2013), and KN and NAA (Fitchet, 1990) were used for *in vitro* multiplication of pineapple.

Comparison of KN and ZN (Hamad and Taha, 2003), and BAP and KN (Omokoio, *et al*., 2001) and BAP and NAA (Zuriada, *et al*., 2011; Usman, *et al*., 2013) were made but at narrow concentration range. The effect of singly applied IAA, on the other hand, were neither reported at one fixed nor at different concentrations. Using of stationary liquid culture and application of single hormone is cheaper and simpler than solid medium with combined application of two and three hormones. In addition, it is well known that singly applied hormone could, depending on the applied concentrations, promote or suppress the *in vitro* shoot formation. Hence, when few concentrations are used for investigation of hormone effect, it is very likely that the best concentration may left out of comparison. The objective of this study is to compare the effect of two cytokinins (BAP, KN) and two auxins (NAA, IAA) applied singly each at 10 different concentrations on the *in vitro* shoot formation and elongation of Moris pineapple on stationary liquid MS medium for two months of incubation.

MATERIALS AND METHODS

Full strength MS medium (Murashige and Skoog, 1962) were prepared, enriched with sucrose at 20 g/l and divided into 41 glass jars (15 x 5 cm.) each received 30 ml of the medium. The jars were divided into five groups. The first group consisted of one jar, the second, third, fourth and fifth group each consisted of 10 jars. No hormone were added to the first group of jars and BAP at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mg/l were added to the second group, KN, NAA and IAA each at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mg/l were added to the third, fourth and the fifth group of jars respectively. The medium of each jar was adjusted to pH 5.0. The jars were closed with plastic lid and the media were autoclaved at 121 $^{\circ}$C and 1.5 kg/cm^2 for 25 minutes. The content of each glass jar (30 ml) was dispensed under laminar cabinet into 3 sterilized culture tubes (10 ml per culture) using sterilized syringe. Shoots obtained from Moris stock cultures were cultured at density of two shoots per culture tube. After two months of incubation under constant temperature of 25 $^{\circ}$C and 16 hours of light provided by cool white fluorescent lamp, the multiple shoots complex was picked out of the culture tubes, placed over squared paper and separated into individual shoots for counting the shoots and measuring their length. The total shoots per culture were divided by two (explants density) to get the shoot formation per explant and the sum of the shoots length divided by the total shoots to get the average length of shoot. The data were subjected to two ways analysis of variance (hormones and concentration) and the significance of the mean of the different treatments were screened by Duncan Multiple Range Test at $p \leq 0.05$ using SPSS statistical package No. 11.

RESULTS

Analysis of variance showed significant differences between hormone types ($p \leq 0.000$), concentrations ($p \leq 0.003$) and significant interaction between the two factors ($p \leq 0.005$). Average overall concentrations showed that BAP and NAA resulted in highest shoot formation (5.7 and 5.9 shoots) and KN in intermediate shoot formation (4.6 shoots) and each of these hormones resulted in shorter shoots (16 mm).

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Explants treated with IAA produced the fewest (3.8 shoots per explant) but longest (34 mm) shoots (Table, 1). Of all combinations, the highest shoot formation (9 shoots) was obtained in medium enriched with NAA at 4.5 mg/l and the lowest (2 shoots) was in medium enriched with KN at 4.0 mg/l while the tallest shoots (56 mm) was obtained in medium enriched with IAA at 2.5 mg/l and the shortest shoots (6 mm) in medium enriched with KN at 4.0 mg/l. The shoot formation of the BAP treated explants increased as the BAP concentration increased up to 2.0 mg/l and declined afterwards. None of the BAP concentrations resulted in shoot formation less than that of hormone free medium and only one concentration was as effective as hormone free medium (Table, 1).

BAP concentrations could be divided into five different groups. The first and second groups included only one concentration each (2.0 and 3.5 mg/l) and resulted in higher shoot formation (8 and 7 shoots respectively) than the other BAP concentrations. The third group consisted of 3 (BAP at 1.0, 3.0 and 5 mg/l) and the fourth group consisted of 4 (0.5, 2.5, 4.0 and 4.5 mg/l) concentrations and each group resulted in formation of 5 and 6 shoots per explant respectively. The fifth group consisted of one concentration (1.5 mg/l) and the shoot formation was not different from that of hormone free medium (4 shoots per explant). The shoot formation of KN treated explants increased as the KN concentrations increased up to 3.0 mg/l and decreased afterwards. KN concentrations could be divided into three groups. Five concentrations (0.5, 1.0, 2.0, 2.5, and 3.5 mg/l) resulted in more (5 to 7 shoots) shoots than hormone free medium. Three concentrations of KN (1.5, 3.0 and 5.0 mg/l) was as effective as hormone free medium (4 shoots per explant) and two concentrations (4.0 and 4.5 mg/l) produced less shoots (2 and 3 shoots) than hormone free medium. Compared to other KN concentrations, KN at 2.5 mg/l was the best resulting in highest shoot formation (7 shoots) among the KN treated explants.

The shoot formation of NAA treated explants increased as the NAA concentrations increased up to 4.5 mg/l. In fact, the highest shoot formation of all combinations of hormone and concentration (9 shoots) was obtained in medium enriched with NAA at 4.5 mg/l. The NAA concentrations could be divided into 6 groups. NAA at 3.5 and at 4.5 mg/l was the best resulting in 8 and 9 shoots respectively. NAA at 4.0 and 5.0 mg/l resulted in 7 and NAA at 2.0 mg/l resulted in 6 shoots while NAA at 0.5, 1.5 and 2.5 mg/l resulted in formation of 5 shoots. NAA at 1.0 and 3.0 mg/l each resulted in formation of 4 shoots and was not different from hormone free medium. None of the NAA concentrations resulted in shoot rate less than hormone free medium and only 2 concentrations were as effective as hormone free while 8 resulted in more shoots than hormone free medium. The highest shoot formation of the IAA treated explants not only did not exceed 5 shoots, but also required application of highest IAA concentrations (4.5 and 5.0 mg/l). The other concentrations (0.5, 2.5, 3.0, 3.5 and 4.0 mg/l) and (1.0, 1.5 and 2.0 mg/l) resulted respectively in formation of less and equal shoots as that of hormone free medium (3 and 4 shoots). On the other hand, the tallest shoot (56 mm) of all combinations was obtained in medium enriched with IAA at 2.5 mg/l and the shortest (6 mm) was obtained in medium enriched with KN at 4.0 mg/l (Table, 1). All concentrations of BAP and all of the NAA except at 3.0 mg/l and KN except 1.0 mg/l resulted in shoot shorter (two time less) than hormone free medium. On the contrary, IAA at 4 concentrations (low and high) had no effect and at the other 6 concentrations (intermediate) promoted the shoot elongation. The shoot length increased as the IAA concentration increased to a peak of 56 mm long at 2.5 mg/l (two times the shoot length in hormone free medium) and then declined afterward.

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Table (1). Effect of hormone types and concentrations on the *in vitro* proliferation and growth of Moris pineapple in liquid full strength MS medium

Conc. (mg/l)	Hormones types				
	BAP	KN	NAA	IAA	Average
Shoots per explants					
0	4 cde	4 cde	4 cde	4 cde	4 C
0.5	6 abcd	5 bcde	5 bcde	3 de	4.75 ABC
1	5 bcde	6 abcd	4 cde	4 cde	4.75 ABC
1.5	4 cde	4 cde	5 bcde	4 cde	4.25 C
2	8 ab	5 bcde	6 abcd	4 cde	5.75 AB
2.5	6 abcd	7 abc	5 bcde	3 de	5.25 ABC
3	5 bcde	4 cde	4 cde	3 de	4 C
3.5	7 abc	6 abcd	8 ab	3 de	6A
4	6 abcd	2 e	7 abc	3 de	4.5 BC
4.5	6 abcd	3 de	9a	5 bcde	5.75 AB
5	5 bcde	4 cde	7 abc	5 bcde	5.25 ABC
Average	5.7 A	4.6 B	5.9 A	3.8 C	
Shoot length (mm)					
0	27 cdefgh	27 cdefgh	27 cdefgh	27 cdefgh	27 A
0.5	13 ghi	17 fghi	14 fghi	22 cdefghi	16.5 CD
1	11 ghi	32 bcdef	20 defghi	25 cdefgh	22 ABCD
1.5	17 fghi	23 cdefghi	17 fghi	35 bcde	23 ABC
2	11 ghi	17 fghi	12 ghi	29 cdefg	17.2 BCD
2.5	14 fghi	10 hi	14 fghi	56 a	23.5 ABC
3	14 fghi	18 efghi	23 cdefghi	46 ab	25.25 AB
3.5	9 hi	12 ghi	11 ghi	29 cdefg	15.25 CD
4	12 ghi	6 i	12 ghi	25 cdefgh	13.75 D
4.5	11 ghi	9 hi	12 ghi	36 bcd	17 BCD
5	18 efghi	11 ghi	14 fghi	39 bc	20.5 ABCD
Average	14.3 B	16.5 B	15.9 B	33.5 A	

Data were means of 6 explants cultured at density of two explants on culture tubes containing 10 ml of liquid full strength MS medium supplemented with sucrose at 20 g/l and incubated for 60 days under constant temperature and 16 hours of light provided by cool white fluorescent lamps. Mean followed by same letters were not significantly different according to Duncan Multiple Range Test at $p \leq 0.05$.

DISCUSSION

Indole acetic acid (IAA) is definitely out of choice for *in vitro* shoot formation but was the best of the tested hormones for shoot elongation of Moris pineapple in stationary liquid full strength MS medium (Table, 1). None of IAA concentrations resulted in more shoots than in any of the other three tested hormones (BAP, KN, NAA). In addition, out of ten concentrations only two of IAA concentrations resulted in more (promoted) while three in equal (no effect) and 5 in less shoot formation (inhibited) than hormone free medium. The possibility that IAA may promote *in vitro* shoot formation not only was very low (20 %) but also high percentage (50%) of the concentrations inhibited the process of shoot formation. Any of the other hormones (BAP, NAA and KN) could be claimed better than the other for *in vitro s*hoot formation of Moris pineapple depending on how wide the range of tested concentrations was and which concentrations included in that range.

Comparing the three hormones each at equal concentrations (Table, 1) showed that if the tested concentrations were limited to two (1.0 and 2.5 mg/l), KN would be the best hormone while if limited to three (0.5, 2.0 and 3.0 mg/l), BAP would be the best hormone and if limited to five (1.5, 3.5, 4.0, 4.5 and 5.0 mg/l), NAA would be

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the best. Limiting the comparison to each two of the three hormones showed that BAP was better than KN at seven, equal at one and less at two, better than NAA at five and less at five concentrations. Similar, NAA better than BAP at five and less at five, better than KN at six, equal at two and less at two. KN, on the other hand, was better than BAP at two, equal at one and less at seven and better than NAA at two, equal at two and less at six concentrations. (Usman, *et al*. 2013; Zuraida, *et al*. 2011) reported that either BAP or NAA could be better than the other for *in vitro* multiplication of pineapple depending on which concentrations was compared to. (Omokoio *et al.* 2001) found that KN at 4 mg/l was better than BAP while (Fitchet, 1990) reported that KN at 2 mg/l was better than BAP and ZN for Smooth cayenne. On the contrary, Table (1) showed that KN at 4 mg/l resulted in the lowest rate (2 shoots) and at 2.0 mg/l BAP was better than KN. (Aydieh *et al.* 2000; Almeida *et al.* 2002; Fernando, 1986) tested BAP for Queen, Perola and Mauritius pineapple and recommended different concentrations of BAP, 2.0, 1.5 and 1.0 mg/l respectively. This study showed that BAP at 2.0 mg/l was the best for Moris while at 1.5 mg/l had no effect and at 1.0 mg/l induced little effect on shoot formation. The highest shoot formation (9 shoots) obtained using NAA at 4.5 mg/l (Table,1). BAP at 2.0 mg/l was the second best treatment with 8 shoots per explant and would be favored over using NAA at high concentrations (4.5 mg/l). IAA at 2.5 mg/l resulted in the tallest shoots (56 mm). (Firoozabady and Gutterson, 2003) suggested two-stage system for *in vitro* multiplication of Smooth cayenne pineapple. One for shoot formation using BAP at 3.0 mg/l and the other for shoot elongation using combination of BAP at 1.0 and GA_3 at 1.0 mg/l. According to this study two-stage system would also be recommended for Moris pineapple but using different hormones and concentrations. BAP at 2.0 mg/l would be recommended for shoot formation and IAA at 2.5 mg/l for shoot elongation. The contradicting reports about which hormone type or concentration was the best may be due to the incorrect selection of the concentrations for comparison of different hormones. It is well known that hormone could induce three different kinds of effect on organ growth (promote, inhibit and no effect) depending on hormone concentration and type of organ. Nevertheless, the comparison of different hormones was usually based on equal concentrations rather than the kind of effect that concentrations could induce on the growth. Out of ten, nine of BAP and eight of NAA concentrations resulted in more shoots than hormone free (promoted).

That is 90 % and 80 % of the used concentrations of BAP and NAA promoted shoot formation process. For KN, 5 concentrations resulted in more (promoted), three in equal (no effect) and two in less (inhibited) shoot formation than hormone free. That means the chance of KN for promoting shoot formation is only 50 % while 30 % had no effect and 20 % of KN inhibited the shoot formation process. We called these concentrations which resulted in more, equal and less shoots than hormone free medium "promoting", "no effect" and "inhibiting" concentrations respectively and divided each concentration range of similar effect into most and least promoting, and most and least inhibiting. Most of the concentrations that make one hormone better than the other are related to whether the so called most promoting or inhibiting range of that hormone were used. If the concentrations were within the most promoting concentrations of one hormone but within the least promoting or inhibiting concentrations of the other, the comparison from the beginning is in favor of one over the other. For instance, at 5 concentrations (0.5, 1.0, 2.0, 2.5 and 3.0 mg/l) BAP resulted in more shoots than NAA while at the other 5 concentrations (3.5, 4.0, 4.5 and 5.0 mg/l) NAA resulted in more shoots than BAP. That means the possibility of which one of these two hormones is better than the other is 50%. Similar, if the comparison of KN and IAA were limited to two concentrations (4.0 and 5.0 mg/l), IAA would be better than KN. In other words, for valid assessment of hormone suitability for *in vitro* shoot formation, the promoting and inhibiting concentration range of each hormone should be first determined and the comparison limited to the

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most promoting concentration range of each hormone.

The results (Table, 1) showed also that there are some cases in which different hormones could result in equal shoot formation at different and at equal concentrations, and different concentrations of the same hormone could result in equal rate. This indicated that the process of shoot formation could be run using different alternatives. That is the increase in concentration of one hormone and the decrease in concentration of other could compensate for lower or higher shoot formation obtained when equal concentration of the different hormones were used.

Obtaining of equal rate of shoot formation at equal concentration of different hormones did not mean that the different hormones have equal effect. Instead, one of the medium components have reacted differently with different hormones in such way that lowered the effect of one hormone and increased the effect of the other to the point that the two hormones resulted in equal shoot formation. According to this study we suggested that the range of concentration used for testing hormone effectiveness for *in vitro* multiplication should be more than six concentrations. If fewer concentrations were used, it is very likely that the best concentration might be left out of comparison. In addition, for comparison of hormones, the range of promoting, inhibiting and those with no effect should first be determined and then the comparison is limited to concentration ranges of similar effect. However, elucidation of the hormone role could not be made unless concentrations which induced equal and those which induced contrading effect were first identified. Selection of equal concentrations of different hormones that induced promoting effect of one hormone but inhibiting effect of other and different concentrations of same hormone that induced equal effect and testing of their effect in medium of different pH adjustments, medium strength, volume and type accompanied with histological and biochemical analysis would be usefull for elucidation the mechanism of hormone effect.

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تأثير نوع وتركيز الهرمون علي تضاعف ونمو عزلة أناناس (*Ananas comosus* (L.) Merr.) صنف موريس في بيئة موراشيج وسكوق السائلة

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المستخلص : اختبر تأثير عشرة تركيزات (0.5 ، 1.0 ، 1.5 ، 2.0 ، 2.5 ، 3.0 ، 3.5 ، 4.0 ، 4.5 ، 5.0) لنوعين من السيتوكينين (BAP و KN) ونوعين من الأوكسين (NAA و IAA) على نموات عزلة لنبات الأناناس صنف موريس في وسط موريشيج وسكوق (MS) السائل المحتوي على 20 جرام في اللتر وحموضته (pH) 5.0 ، استعمال هرمون (Naphthalene Acetic Acid (NAA أعطى أعلى عدد نموات للعزلة الواحدة (9 نموات) ، العدد الأعلى الثاني (8 نموات) تم الحصول عليها في وسط يحتوي على (BAP) بتركيز 2.0 مليجرام في اللتر ، من بين عشرة تركيزات اختبرت في هذه الدراسة فإن (NAA) كان أفضل من (BAP) في التركيزات الخمسة الأعلى وأقل في حالة التركيزات الخمسة الأدنى لكن في كل التركيزات العشرة كلا الهرمونين أدى إلى نموات أكثر مما لو استعمل في وسط خالٍ من الهرمون ، ما عدا تركيزين فأن هرمون BAP و NAA كان أفضل من هرمون KN، لهذا يمكن القول أن أي من هذه الهرمونات الثلاثة KN و NAA و BAPمن الممكن أن يعتبر أفضل من الهرمونين الاخرين اعتماداً على التركيزات التي تستعمل في المقارنة بينها، من الناحية الأخرى فإن هرمون IAA لا يصلح لزراعة عزلات الأناناس إذا كان الوسط في حالة سائلة، حيث إن كل التركيزات العشرة من هرمون IAA أعطت نموات أقل مما تم الحصول عليه من استعمال الوسط الخالي من الهرمون ، لكن استعمال هرمون IAA بتركيز 2.5 مليجرام أدى للحصول على أطول نمو (56 مليمتر) وسبعة تركيزات أدت إلى نموات أطول من تلك في الوسط الخالي من الهرمون (27 مليمتر) في حين كانت أطوال النموات في الأوساط المحتوية على أي من الهرمونات الثلاثة الأخرى أقل من نصف الطول في الوسط الخالي من الهرمون، بهذا فأن هرمون BAP بتركيز 2.0 مليجرام كان أفضل معاملة لمضاعفة عدد النموات من العزلة الواحدة وبينما هرمون IAA بتركيز 2.5 مليجرام كان أفضل معاملة للحصول على أطول نمو .

الكلمات المفتاحية: الأناناس، المزرعة السائلة، مضاعفة نموات العزلة.

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Antioxidant Activity of Lipoic Acid on Cyclosporine A-Induced Physiological Changes to the Kidneys in Male Albino Rats

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Abstract: Cyclosporine A (CsA) is the most widely used immunosuppressive drug for preventing graft rejection and autoimmune disease. However, the therapeutic treatment induces several side effects such as nephrotoxicity, cardiotoxicity and hepatotoxicity. This study aimed to assess the protective role of lipoic acid (LA) on kidney toxicity of male albino rats induced by cyclosporine (CsA). Forty adult male rats were allocated into four groups: Group (I) served as a control group. Group (II); received treatments orally with CsA (25 mg/kg b.w.), daily for 3 weeks. Group III: (Recovery CsA group): treated orally with CsA (25 mg/kg b.w.), daily for 3 weeks, then recovered for another 3 weeks. Group IV (LA and CsA group): received LA (100 mg/kg b. w.) orally 1 h before treatment by CsA (25 mg/kg b. w.) daily for 3 weeks. The results indicated that treatment of CsA caused a significant elevation in the concentrations of serum urea, creatinine, and uric acid which indicate injury to the kidney function. Renal malondialdehyde (MDA) concentration was markedly increased reflecting increased lipid peroxidation, whereas, reduced glutathione (GSH) and superoxide dismutase (SOD) were significantly decreased. On the other hand, LA plus CsA dose-dependently inhibited activities of serum urea, creatinine, and uric acid. The administration of LA plus CsA exhibited significant reduction in lipid peroxidation while GSH content and SOD activity were enhanced significantly which reflect an improvement in renal toxicity. In conclusion, the results indicated a negative role of CsA on kidney function and oxidative stress in induction toxicity, suggested Thus, Lipoic acid play a positive role on toxicity of kidney induced by cyclosporine A.

Key Words: Lipoic acid, cyclosporine A, oxidative stress, renal toxicity.

INTRODUCTION

As a highly potent immunosuppressive drug, cyclosporine (CsA) remains largely used for the prevention of acute rejection in solid organ transplantation, and for the treatment of various auto-immune diseases. However, CsA can lead to a chronic form of renal damage characterized by a progressive and irreversible deterioration of renal function associated with interstitial fibrosis, tubular atrophy, arteriolar hyalinosis and glomerulosclerosis (Nankivell et al., 2004, Chapman and Nankivell 2006).

Alpha-lipoic acid (LA), or 1,2-dithiolane-3-pentanoic acid, is a naturally occurring dithiol compound synthesized enzymatically in the mitochondrion from octanoic acid. LA is a necessary cofactor for mitochondrial α-ketoacid dehydrogenases, and thus serves a critical role in mitochondrial energy metabolism. In addition to synthesis, LA is also absorbed intact from dietary sources, and it transiently accumulates in many tissues. There is growing evidence that orally supplied LA may not be used as a metabolic cofactor but instead, elicits a unique set of biochemical activities with potential pharmacotherapeutic value against a host of pathophysiologic insults. LA has a potent antioxidant, a detoxification agent and improve age-associated cardiovascular, cognitive, and

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neuromuscular deficits (Scott et al., 1994, Smith et al., 2004, Koh et al., 2005). This impressive array of cellular and molecular functions has piqued considerable interest among the lay public and the research community for the use of LA both as a nutritive supplement and as a pharmacotherapy. In light of this growing interest, we will attempt to provide an update on the biochemical, toxicological, and pharmacological mechanisms of LA. As many excellent reviews already exist that outline the metabolic role of LA as a covalently bound enzyme cofactor, only a brief summary of this particular aspect of LA function will be presented herein. Instead, a focus mainly on the cellular actions of orally supplied, nonprotein-bound LA will be presented. Pertinent clinical benefits of LA will also be discussed in light of this molecular mechanism(Liu et al., 2002, Shay et al., 2009).Therefore, this study investigated the modulating and antioxidant activity of lipoic acid on renal toxicity induced by cyclosporine A in male albino rats.

MATERIALS AND METHODS

Chemicals

Cyclosporine A (CsA) is presented in the form of ampoules under traditional name Sandimmune and provided by Novartis Pharma (Basel, Switzerland). It is presented as a clear, yellow liquid supplied in 1ml ampoules containing 50 mg/ml and was further diluted with olive oil. Alpha-Lipoic acid (LA) was purchased in the form of a yellow powder from Sigma chemical company (St Louis, Missouri, USA) and was suspended in sterile normal saline, before use.

Experimental animals

Male Wistar albino rats, each weighing 180 ± 20 g, were obtained from an animal house in Medical Research Center (MRC), Faculty of Medicine, Ain Shams University. The animals were acclimatized to the laboratory conditions for a period of 14 days. They were maintained at an ambient temperature of 25 ± 3°C, 50 ± 20% relative humidity and 12/12 h of light–dark cycle and were given a standard rat feed and water ad libitum. All experimental procedures were conducted according to the ethical standards approved by the Institutional Animal Ethics Committee guidelines for animal care and use, Ain Shams University, Cairo, Egypt.

Experimental protocol

The rats were randomly divided into four groups, each of eight rats as follows:

Group I (Control): received saline (2 ml/kg b. w.) and olive oil (2 ml/kg b. w.) orally for 21 days.

Group II (CsA-treated group): was treated orally by gastric gavage with CsA (25 mg/kg b.w.), daily for 21 days.

Group III (Recovery CsA-treated group): was treated orally by gastric gavage with CsA (25 mg/kg b.w.), daily for 21 days and recover for another 21 days.

Group IV (LA and CsA-treated group): received LA (100 mg/kg b. w.) orally (Jalali-Nadoushan and Roghani 2013) , 1 h before treatment by CsA (25 mg/kg b. w.) daily and concurrently for 21 days.

At the end of the experimental period, the animal groups were sacrificed after 24 hrs. of the last dose of different administrations and their blood were collected, by carotid bleeding, in centrifuge tubes and serum was obtained from the blood after centrifugation at 3000 rpm for 10 min. The kidney tissue was immediately excised, cleaned of adhering connective tissue, rinsed in physiological saline, weighed and stored at -20°C until analysis studies.

Methods of analysis

Serum urea, creatinine and uric acid were estimated by using the method of (Fawcett and Scott 1960, Seeling and Wust 1969, Barham and Trinder 1972, Scott et al., 1994) respectively. Renal glutathione (GSH) was spectrophotometrically assayed by the method of (Sedlak and Lindsay 1968). The activity of renal SOD was determined by assessing the inhibition of pyrogallol autoxidation (Marklund 1985). Malondialdehyde (MDA) was determined in kidney by using the method of (Uchiyama and Mihara 1978).

Statistical analysis

Statistical analyses of the resulted data were done using InStat 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

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followed by Tukey-Kramer as a post-ANOVA test. Statistical significance was accepted at P< 0.001, P< 0.01, P< 0.05.

RESULTS

CsA administration caused a significant increase in serum urea, creatinine and uric acid concentrations (P<0.001) in the CsA treated groups compared to the control group I. These concentrations tended to be highly significant compared to the values of the control group I (Table 1).

Table (1): The effect of LA on CsA - induced changes on serum urea, creatinine and uric acid concentrations.

Groups	Parameters		
	Urea mg/dl	creatinine mg/dl	Uric acid mg/dl
G I (Control)	15.81± 0.22	0.73 ± 0.020	5.57 ± 0.025
G II: (CsA)	44.45 ±0.43a'	1.90 ±0.02a**	8.45 ±0.04a**
G III: (Recov.)	33.76 ±0.291 ab**	1.51 ±0.035 ab**	6.69 ±0.045 ab**
GIV (LA &CsA)	21.47 ±0.153 ab**	0.87 ±0.015 ab**	5.86 ±0.19 ab**

Data are expressed as means ± S.E. (n = 6 in each group).
a: Significant change at p< 0.05 with respect to control group I.
b: Significant change at p< 0.05 with respect to group II.
*Highly significant change at p < 0.01. **Very highly significant change at p < 0.001.N.S: Non-significant change.

The present data showed a significant elevation in the level of renal MDA (P<0.001), while a significant reduction in renal GSH and SOD activity (P<0.001) was observed in CsA treated group compared to the control group I (Table 2).On the other hand, treatment with LA plus CsA caused a highly significant decrease in serum urea, creatinine and uric acid concentrations (P<0.001) in the LA plus CsA treated group IV compared to the CsA group II (Table 1).Renal MDA was restored significantly (P<0.001), also renal GSH content and SOD activity were attenuated in the LA plus CsA treated group IV as compared to the CsA group II (Table 2).

Table (2): The effect of LA on CsA – induced changes on renal GSH, SOD and MDA levels.

Groups	Parameters		
	Renal		
	MDA U/g wet tissue	GSH U/g wet tissue	SOD U/g wet tissue
G I (Control)	30.34 ± 0.071	28.45 ± 0.115	81.31 ± 0.083
G II: (CsA)	56.20 ± 0.049a**	56.14 ± 0.044a**	63.28±0.057 a**
G III: (Recov.)	30.32 ± 0.062	28.41± 0.093	81.38 ± 0.0859
GIV(LA and CsA)	38.28 ± 0.058 ab**	76.39 ± 0.076 ab**	40.20 ± 0.049 ab**

Data are expressed as means ± S.E. (n = 6 in each group).
a: Significant change at p< 0.05 with respect to control group I.
b: Significant change at p< 0.05 with respect to group II.
*Highly significant change at p < 0.01.
**Very highly significant change at p < 0.001.
N.S: Non-significant change.

DISCUSSION

Deciphering new biological pathways that contribute to CsA renal toxicity is of great importance because they may lead to the development of early biomarkers of kidney injury. A significant elevation in serum urea, uric acid and creatinine concentrations were observed in CsA treated rats as compared with control (group I). These results are in agreement with(Tirkey et al., 2005) who showed that chronic administration of CsA caused a marked impairment of renal function along with significant oxidative stress in the kidneys. Oxidative stress promote the formation of a variety of vasoactive mediators that can affect on renal function directly by causing renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient and thus reducing glomerular filtration rate (Garcia-Cohen et al., 2000, Burdmann et al., 2003). CsA inhibits mitochondrial mediated apoptosis but also induces mitochondrial apoptotic cell death in the kidney (Pallet et al., 2008) caused ischemia induced up-regulation of endothelin receptors, support the potential for an important role for up-regulation of endothelin receptors in pathophysiologic mechanisms of CsA-induced glomerular dysfunction(Fogo et al., 1992).In this study, CsA treated rats (group II) showed a

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significant increase in the levels of renal MDA with excess production of hydrogen peroxide in living cells, accompanied with a significant decrease in GSH and SOD that leads to decline in the activity of the antioxidant enzymes depletion of both the GSH and protein thiols. Similar biochemical changes were previously reported in other studies(Khan et al., 2006, Ajala et al., 2008) which then give rise to increased hydroxyl radical formation. The effects of reactive oxygen species (ROS) on cellular and extracellular components of organisms have been investigated extensively in recent years. CsA promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides. These ROS enhance the peroxides and reactive hydroxyl radicals. These lipid peroxides and hydroxyl radical may cause cell membrane damage and thus destroy the cell. It also inhibits the activities of free radical quenching enzymes such as catalase, superoxide dismutase and glutathione peroxidase. The role of CsA in ROS production was observed in the present study by increased amount of renal and hepatic lipid peroxides (LPO). The intracellular generation of hydrogen peroxides (H2O2) could be involved in the initiation of CsA toxicity in rats, caused cell membrane damage like lipid peroxidation which leads to the imbalance between synthesis and degradation of enzyme protein.

The excess production of ROS may be due to its ability to produce alteration in mitochondria by blocking the permeability transition pore .Reactive oxygen metabolites are generated by specialized phagocytic cells (neutrophils) as cytotoxic agents to fight invading micro-organism, a process known as the respiratory or oxidative burst. Therefore, phagocytes use the membrane bound NADPH oxidase complex which catalyzes one electron, reduction of O2 into O-2. The ROMs are generated in a biological system via several enzymatic and non-enzymatic pathways(Morel et al., 1991, Agar et al., 2011).On the other hand, the present results illustrated that the antioxidant LA administration had an ameliorating effect on the changes of the biochemical parameters associated with CsA challenge. This effect was indicated by improvement of serum urea, uric acid and creatinine concentrations. These results are in agreement with (Sivaprasad et al., 2004) who found that administration of LA one hour after CsA offered marked protection against nephrotoxicity. This protection was manifested as a significant reduction in serum levels of urea, uric acid, creatinine and amelioration of apoptotic markers (Forbes et al., 2008) Voltage-dependent anion channels (VDAC), known as mitochondrial porins, are membrane proteins encoded by nuclear gene and synthesized in ribosome.

VDAC plays crucial roles in the physiological and pathologic processes, including energy metabolism and cell apoptosis. VDAC was actually more sensitive to oxidative stress-induced cell death (Wang et al., 2013). In addition, free radical scavengers may also be helpful in prolonging survival time of dopaminergic neurons (Chen and Le 2006). In this respect, LA could attenuate neuronal damage and loss through counteracting oxidative stress, possibly via regulating antioxidant defense system as well as inhibition of free radical generation(Connell and Saleh 2012). LA and its reduced form dihydrolipoic acid are present in all prokaryotic and eukaryotic cells and considered a vitamin, and can be synthesized in human cells. LA is involved in the regulation of carbohydrate and lipid metabolism in converting blood glucose into energy(Malinska and Winiarska 2005), improving glycemic control.In the present study, administration of LA prior to CsA treatment markedly ameliorated LPO in the rat kidney as manifested by decreased MDA level accompanied by increased GSH content and SOD activity. In agreement with the present findings, (Wollin and Jones 2003) mentioned that LA is a naturally occurring cofactor within pyruvate dehydrogenase and α-keto-glutarate dehydrogenase. Also, Free LA has the ability to scavenge superoxide, hydrogen peroxide, hydroxyl radicals, and peroxynitrite, and can also recycle glutathione (GSH), α-tocopherol and ascorbic acid. In vitro, α-lipoic acid decreased plasma susceptibility to oxidation(Marangon et al., 1999), and were protective against haemolysis of human erythrocytes induced by peroxyl radicals (Constantinescu et al., 1994) and increased GSH synthesis in isolated human erythrocytes (Han et al., 1997). Also, α-lipoic acid attenuated superoxide generation and kidney

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expression of NADPH oxidase in diabetic rats, and it was concluded that α-lipoic acid improves pathology in diabetes by reducing oxidative stress (Lexis et al., 2006). Thus, LA has been shown to reduce oxidative stress both in vivo and in vitro studies. Reduction of renal GSH, SOD activity in CsA-treated rats was observed in this study, which was similar to the previous studies (Mohamadin et al., 2005). LA has proved to possess lipid lowering, anti-lipo peroxidative and antioxidant properties (Amudha et al., 2006).

It has been demonstrated to play an important role in regulating antioxidative capacity by increasing SOD, GSH and catalase activities by upregulating the gene expression of SOD, GSH and catalase (Hagar *et al.,* 2006). Reduced glutathione together with GPx is important in maintaining the structure of mitochondrial and cell membranes. In addition, (Huong and Ide 2008) reported that a-lipoic acid reduced the activities and mRNA levels of various lipogenic enzymes together with the mRNA levels of various proteins. As that Alpha-lipoic acid (α-LA), a naturally occurring dithiol compound, has long been known as an essential cofactor for mitochondrial bioenergetic enzymes. As an antioxidant, α-LA directly terminates free radicals, chelates transition metal ions, increases cytosolic glutathione, vitamin C, E levels and prevents toxicities associated with their loss (Singh and Jialal 2008).In conclusion, LA is a main active component for immuno-modulating and antioxidant activities, differ greatly in the chemical composition and physical properties, show the same basic multivitamins and protect the immune cells from oxidative damage. Thus, LA has a potential protective effect against CsA toxicity and is improving the renal function by decreasing the kidney tissue damage of CsA-induced nephrotoxicity in rats.

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النشاط المضاد للأكسدة لحمض الليبويك على التغيرات الفسيولوجية للكلى الناجمة عن التعرض للسيكلوسبورين-أ في ذكور الجرذان البيضاء

نورا إبراهيم الزاعل

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المستخلص : استخدم البحث 40 من ذكور الجرذان البالغة والتي قسمت إلى 4 مجاميع، المجموعة الأولى: تمثل المجموعة الضابطة. وتم تجريع المجموعة الثانية بعقار السيكلوسبورين-أ (25 ملجم/كجم) عن طريق الفم يومياً لمدة 3 أسابيع. وتم تجريع المجموعة الثالثة السيكلوسبورين- أ (25 ملجم/كجم) عن طريق الفم يومياً لمدة 3 أسابيع ثم تركت فترة تعافٍ لمدة 3 أسابيع أخرى. والمجموعة الرابعة تم تجريعها بحمض الليبويك (100 ملجم/كجم) عن طريق الفم قبل ساعة من المعاملة بعقار السيكلوسبورين-أ (25 ملجم/كجم) يومياً لمدة 3 أسابيع، وتشير النتائج إلى أن المعاملة بعقار السيكلوسبورين-أ قد تسببت في ارتفاع ذي دلالة معنوية لتركيز كل من اليوريا والكرياتينين وحمض البوليك في الدم، مما أدى إلى ضعف في وظائف الكلىK وقد لوحظ أيضاً ارتفاع مالونداي الدهيد (MDA) الكلوي بشكل ملحوظ مما يعكس زيادة الدهون البيروكسيدية، في حين انخفض كل من الجلوتاثيون، (GSH) وفوق أكسيد الدسموتيز (SOD). من ناحية أخرى فأن المعاملة بحمض الليبويك مع السيكلوسبورين-أ أدت إلى خفض مستوى اليوريا والكرياتينين وحمض البوليك في الدم لتصبح أقرب للمجموعة الضابطة، فضلاً عن انخفاض كبير في الدهون البيروكسيدية (MDA) وتحسن في كلٍّ من محتوى GSH. ونشاط SOD بشكل ملحوظ يعكس مدى تحسن تسمم الكلى، من هذه الدراسة نستنتج الدور السلبي لعقار السيكلوسبورين-أ على وظائف الكلى من خلال رفع مستوى الإجهاد التأكسدي واسحثاث التسمم الكلوي، في حين يقوم حمض الليبويك بدور إيجابي في تقليل تسمم الكلى الناجم عن عقار السيكلوسبورين-أ.

الكلمات المفتاحية: حمض الليبويك، سيكلوسبورين-أ، الإجهاد التأكسدي، التسمم الكلوي.

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Effect of sucrose, pH and medium states on in vitro shoot formation and growth of Moris pineapple (*Ananas comosus* L. Merr.)

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Abstract: The effect of medium states (solid, semi solid and liquid) of full strength MS medium enriched with 6-benzylaminopurine (BAP) at 2.0 mg/l on *in vitro* shoots formation and shoot length of Moris pineapple were tested at 16 combinations of sucrose (10, 20, 30 and 40 g/l) and pH (5.0, 5.7, 6.0 and 6.5). The highest shoot formations (7 shoots/ explant) were obtained in liquid and solid media each adjusted to pH 5.0 but enriched with different sucrose concentrations, sucrose at 20 g/l for liquid and at 30 g/l for solid medium. Increasing the medium sucrose to 40 g/l or adjusting the medium to pH 6.0 caused 50 % decline in the shoot formation capacity in both medium states. However, while that decline could be reversed in liquid medium by adjusting the pH to 6.5, such pH adjustment failed to overcome the inhibitory effect of the sucrose at 40 g/l in the solid medium. Out of 16 combinations of sucrose and pH, liquid medium (no agar added) was better than solid (7.0 grams of agar /l) and semi solid (3.5 grams of agar /l) at 8 combinations, equal to solid at 4 and to semi solid at 5 combinations and less than solid at 3 and than semi solid at 2 combinations. Adopting of the commonly used combination of sucrose at 30 g/l and pH 5.7 not only did not fit all medium states but also resulted in lower shoot formation (4 shoots) than the possibly obtainable (7 shoots). Simple modification of the medium pH (pH 5.0 instead of 5.7) doubled the rate of shoot formation.

Key words: Sucrose concentration, pH; Pineapple, *Ananas comosus*, Media states.

INTRODUCTION

Solid MS medium was recommended by several researchers for pineapple in vitro culture and proven to have high potential for production of thousands of propagules (Sripaoraya *et al.,* 2003, Hamad and Taha 2008, Pérez *et al.,* 2009). At the same time, pineapple shoot on semi liquid(Akin-Idowu *et al.,* 2014), static (Almeida *et al.,* 2002, Pérez *et al.,* 2012) and agitated liquid cultures (Soneji *et al.,* 2002a), filter paper bridge (Mathews and Rangan 1979, Fernando 1986) and temporary immersion system (Escalona *et al.,* 1999, Firoozabady and Gutterson 2003) were found to be much better than solid medium for *in vitro* multiplication of pineapple. However, these comparisons were made at fixed sucrose (30 g/l) and pH (5.7) in both medium states. For multiplication on solid MT medium, (Fitchet 1990) adjusted the pH to 5.0 while (Teixeira *et al.,* 2006) used pH 6.5 during multiplication in liquid MS medium. At fixed sucrose(20 g/l), comparison of different pH adjustments during multiplication stage showed that liquid was better than solid medium and adjusting to pH 5.0 was better than adjusting to pH 5.7, 6.0 and 6.5 (Hamad, 2017a). Sucrose at 20 (Soneji *et al.,* 2002b, a) Smith, *et al.,* 2002,), 35(Kofi and Adachi 1993) and 40 g/l (Almeida, *etal,* 1997) were used for establishment and multiplication.

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(Sripaoraya *et al.,* 2003) used sucrose at 50 g/l during establishment but decreased the sucrose to 30 g/l during multiplication stage. Comparisons of sucrose effect on pineapple culture were made at range of 10, 20, 30 and 40 g/l for shoot formation (Hamad, 2017b), callus induction (Benega, *et al.,* 1997) and at range of 0.0, 30, 60, 90 and 120 g/l for total fresh weight per bioreactor(Pérez *et al.,* 2004). Highest shoot formation and highest fresh weight were obtained at 30 g/l while either sucrose levels were equally effective for callus induction. Since sucrose is an indispensable component and both of sucrose and agar comprise the largest part of the medium components, determination of optimum level is not only important for *in vitro* shoot formation and growth but also as a cost factor. Application of extra amount above that required for the optimal shoot formation is just an avoidable added cost. The objective of this study is to compare the effect of combinations of four concentrations of sucrose (10, 20, 30 and 40 g/l) and four pH adjustments (5.0, 5.7, 6.0 and 6.5) on shoot formation and growth of Moris pineapple on three states (solid, semi solid and liquid) of full strength MS medium enriched with BAP at 2.0 g/l.

MATERIALS AND METHODS

Full strength MS medium were prepared from stock solutions and enriched with BAP at 2.0 mg/l. The medium was divided into 4 beakers (750 ml each) marked A, B, C and D and 7.5, 15.0, 22.5 and 30.0 grams of sucrose were added to each beaker respectively to give a sucrose enrichment of 10, 20, 30 and 40 g/l. The content of each beaker was divided into another 4 beakers marked with same marks (A, B, C and D) plus numbers from 1 to 4 and the medium pH of the beakers marked with 1, 2, 3 and 4 was adjusted to pH 5.0, 5.7, 6.0 and 6.5 respectively. The content of each beaker of the same sucrose and pH combination was divided into 9 glass jars **(**20 x 5 cm**)** and each three jars marked with the same marks on the beaker plus S, E and L. Agar at 0.14 and 0.7 grams was respectively added to each jar marked with S and E letter to give medium solidification of 7.0 and 3.5 g/l and no agar was added to jars with L letter. The jars were closed by autoclavable plastic lids and the medium was autoclaved at 121 ^{o}C and 1.5 kg / cm^2 for 25 minutes and kept in a culture room. One shoot from Moris stock cultures was cultured per each jar under laminar cabinet and the cultures were incubated under constant temperature of 25 ^{o}C and 16 hours of light provided by cool white fluorescent lamps. After two months of incubation, the multiple shoot buds complex of each culture were picked out of the jars and separated into individual shoots for counting the shoots and measuring their length. Each jar was considered as a replicate and the data were subjected to ANOVA analysis and means separation by Duncan Multiple Range Test at $p \leq 0.05$ using SPSS statistical package No. 11.

RESULTS

Analysis of variance (Table, 1) showed that the shoot formation and the shoot length were under direct effect of medium states ($p \leq 0.0166$ and $p \leq 0.0001$ respectively) and sucrose concentrations ($p \leq 0.0002$ and ≤ 0.00003 respectively). The sucrose effect on shoot formation and shoot length was influenced by a significant interaction with pH ($p \leq 0.0039$ and ≤ 0.00001). On the contrary, the medium states effect on shoot formation was independent of pH ($p \leq 0.3936$) and sucrose ($p \leq 0.0764$) while the medium state effect on shoot length was influenced by the medium sucrose content ($p \leq 0.0141$) but independent of pH ($p \leq 0.0764$). Medium pH on the other hand had no direct independent effect on both of shoot formation (p 0.3031) and shoot length ($p \leq 0.7794$), but influenced both of shoot formation ($p \leq 0.0039$) and shoot length ($p \leq 0.00001$) via interaction with sucrose content of the medium. Furthermore, the three factors together showed no significant collective interaction on shoot formation ($p \leq 0.1520$) and shoot length ($p \leq 0.2460$). The highest shoot formation (7 shoots) were obtained in liquid medium enriched with sucrose at 20 g/l and also in solid medium enriched with sucrose at 30 g/l both adjusted to

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pH 5.0. The lowest shoot formation (3 shoots per explant) as well as equal shoot formation (6, 5 and 4 shoots) was also obtained in liquid and solid medium, but at different combinations of sucrose and pH (Table, 2). At fixed pH of 5.0, equal shoot formation (3 shoots) were obtained in both solid and liquid media enriched with sucrose at 10 and 40 g/l while in media enriched with sucrose at 20 g/l, liquid produced more shoots (7 shoots) than solid (5 shoots) medium. In media enriched with sucrose at 30 g/l, solid on the contrary produced more shoots (7 shoots) than liquid (5 shoots). At fixed pH of 5.7, solid medium enriched with sucrose at 10 and 40 g/l produced more shoots (4 and 5 shoots) than liquid medium (3 and 4 shoots) while in media enriched with sucrose at 20 and 30 g/l, liquid medium on the contrary produced more (6 and 5 shoots respectively) shoots than solid medium (3 and 4 shoots). At fixed pH of 6.0, equal shoot formation (4 shoots)were obtained in solid and liquid media enriched with sucrose at 10 and 20 g/l (low sucrose enrichment) while in media enriched with sucrose at 30 and 40 g/l (high enrichment) liquid medium produced more (6 and 6 shoots) shoots than solid medium (5 and 3 shoots). At pH 6.5, liquid medium enriched with sucrose at 20, 30 and 40 g/l produced more (6, 5 and 6 shoots respectively) shoots than solid medium (3, 4 and 3 shoots) and in media enriched with sucrose at 10 g/l equal shoot formation (4 shoots) were obtained in both medium states.

Table (1). Main and interaction effect of medium states, sucrose and pH on *in vitro* shoot formation and shoot length of Moris pineapple

Factors	Df	Parameters	
		Shoot/ explant.	Shoot length(mm)
		p values	
Medium states	2	0.0166 *	0.0001 **
Sucrose	3	0.0002 **	3.9E-08 **
pH	3	0.3031	0.7994
States*Sucrose	6	0.0764	0.0141 *
States*pH	6	0.3935	0.6044
Sucrose*pH	9	0.0039 **	1.0E-05 **
States*Sucrose*pH	18	0.1520	0.2460
Error	96		
Total	144		

DISCUSSION

Previous studies of pineapple tissue culture concluded that using of semi liquid(Akin-Idowu *et al.,* 2014), static liquid(Almeida *et al.,* 2002, Pérez *et al.,* 2012), agitated liquid cultures (Soneji *et al.,* 2002a) and filter paper bridge (Mathews and Rangan 1979, Fernando 1986) resulted in higher shoot formation than solid medium. This study demonstrated that superiority of the medium state depended on the sucrose enrichments

Table (2). Effect of medium states, sucrose and pH on *in vitro* shoot formation of Moris pineapple

Sucrose (g/l)	pH	Medium states		
		Solid	Semi	Liquid
		Shoots per explant		
10	5.0	3 c	3 c	3 c
	5.5	4 bc	3 c	3 c
	6.0	4 bc	3 c	4 bc
	6.5	4 bc	4 bc	4 bc
20	5.0	6 ab	5 abc	7a
	5.5	3 c	3 c	6 ab
	6.0	4 bc	4 bc	4 bc
	6.5	3 c	4 bc	6 ab
30	5.0	7a	5 abc	5 abc
	5.5	4 bc	4 bc	5 abc
	6.0	5 abc	4 bc	6 ab
	6.5	4 bc	4 bc	5 abc
40	5.0	3 c	5 abc	3 c
	5.5	5 abc	6 ab	4 bc
	6.5	3 c	3 c	6 ab
	6.0	3 c	3 c	4 bc

Data were means of 3 explants cultured on full strength MS medium enriched with BAP at 2.0 mg/l and incubated for 60 days
Means of the same parameters (shoot per explant and shoot length) followed by the same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test.

and pH adjustments and the solid medium had a very critical requirement of narrow range while liquid medium had a wider range of sucrose-pH combinations (Table, 2). The highest shoot formation (7 shoots) was obtained in solid as well as in liquid medium enriched with sucrose at 30 and 20 g/l respectively and adjusted to pH 5.0.

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The lowest shoot formations (3 shoots) was obtained also in liquid and solid medium enriched with sucrose at 10 and 40 g/l and adjusted to pH 5.0. In both of solid and liquid medium the soot formation ranged from a minimum of 3 to a maximum of 7 shoots. Furthermore, in 8 out of 16 combinations of sucrose and pH, liquid medium resulted in more shoots than both solid and semi solid media (50 % of the cases).

At other 5 combinations (31 %), the shoot formation in liquid was equal to that in solid and at other 3 combinations (19 %) was less than that of solid. At 5 combinations (31 %), the shoot formation in liquid medium was equal and at 2 combinations (13 %) was less than that in semi solid medium. Solid medium was better than liquid in 3 combinations (19 %) and than semi solid at 5 combinations (31 %) and less than semi solid at 3 (19 %) of the sucrose-pH combinations. Hence, any of the medium states could be claimed better than the other depending on which sucrose-pH combination was used. However, being only 25 % of the sucrose-pH combinations (4 of 16) in solid and semi solid media resulted in more than 5 shoots (71 % of the possibly obtainable shoots (7 shoots) while 50 % of the combinations (8 of 16) in liquid medium resulted in more than 5 shoots (71 % of the possibly obtainable shoots (7 shoots) indicated that selection of proper sucrose and pH is critical and very specific in solid than in liquid medium. Generally, liquid state with low sucrose enrichment would be favored for low cost. This study demonstrated that sucrose at 30 g/l and pH at 5.7, which is the most common used combination for *in vitro* culture, is not proper for Moris pineapple. Table 2 showed that in solid medium keeping sucrose at 30 g/l and decreasing the pH to 5.0 and lowering both (sucrose to 20 g/l and pH to 5.0) resulted in 100 % increase in shoot formation. Similar, in liquid medium keeping the sucrose at 30 g/l and increasing the pH to 6.0, keeping pH at 5.7 and decreasing sucrose to 20 g/l, increasing both (the sucrose to 40 g/l and pH to 6.5) and lowering both (sucrose to 20 g/l and pH to 5.0) resulted in 17 to 20 % increase in shoot formation. Using solid medium adjusted to pH 5.7, Hamad (2017b) found that proper sucrose concentration varied among different pineapple cultivars. (Sucrose at 30 g/l for Moris and at 20 g/l for Smooth cayenne). However, at fixed sucrose enrichment (20 g/l), Moris cultured in liquid medium adjusted to pH 5.0 produced more shoots than in solid medium (Hamad, 2017a). It is important to point out that pH adjustment was a simple none cost item and generally ignored factor that could doubled or drastically reduce the shoot formation (Table, 2).

Liquid medium enriched with sucrose at 10 g/l and adjusted to pH 5.0 resulted in low shoot formation but the rate increased when medium pH increased while when enriched with sucrose at 20 g/l resulted in higher shoot formation but the rate decreased when the medium pH increased. If this trend of response to pH adjustments is persisted, using of pH range higher than 6.5 in medium enriched with sucrose at 10 g/l and pH range lower than 5.0 in medium enriched with sucrose at 20 g/l may improve the shoot formation and is suggested for future testing. The different rate of shoot formation at different combinations of sucrose enrichment and pH adjustment in same medium state and to the same combination in different medium states indicated that adopting of one single sucrose-pH treatment as it is commonly done could not lead to a valid comparison of medium states and investigation of the physiology of shoot formation. In fact, one fixed combination of sucrose and pH could drastically reduce the shoot formation in one medium state while doubling the shoot formation in the other one (Table, 2). Medium states, sucrose contents and pH adjustments played important role in the process of shoot formation. However, that role is not clearly understood yet.

It is generally assumed that the promotion effect of liquid medium is due to the accessibility of medium component to the explants. Liquid state was in general better than solid and sucrose at 40 g/l inhibited the shoot formation (Table, 2). However, the superiority of liquid state and the inhibition of high sucrose content could be blocked and even reversed by pH adjustment.

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Liquid medium enriched with sucrose at 20 g/l at all pH except 6.0 and with sucrose at 30 g/l at all pH except pH 5.0 resulted in more shoots than solid medium. Adjusting the medium enriched with sucrose at 20 g/l to pH 6.0 blocked the promotion effect of liquid medium (equal shoots obtained in both medium states) while adjusting the medium enriched with 30 g/l to pH 5.0 reversed the situation and solid promoted more shoots than liquid (Table, 2). That is the pH and sucrose affected the ability of liquid state to promote shoot formation. If the superiority of liquid medium was due to nutrient accessibility, the statistical analysis (Table, 1) should have shown that the medium states had significant interaction with the other factors that reverse the state superiority. Table 1 showed that medium states affect was independent of the sucrose concentrations and pH adjustments. Neither the interaction of medium states with pH and with sucrose each alone nor the collective interaction of the three factors together was significant while a significant interaction was detected between sucrose and pH.

This indicated that the shoot formation was controlled by the interaction of pH and sucrose more than the medium state. The interaction of sucrose and pH presumably resulted in either fast or slow uptake of sucrose or formation of complex that could either promote or inhibit the shoot formation depending on how much sucrose the medium contained and to what pH was adjusted rather than the types of medium state. In conclusion, this study demonstrated that *in vitro* shoot formation is controlled by the sucrose and pH of the medium more than the medium state. The medium pH adjustment which is none cost item and generally ignored factor is essential for obtaining a substantial increase in shoot formation. The contradicting responses to different sucrose-pH combinations in same and different medium states could help in the selection of specific combinations of sucrose concentrations and pH adjustments that would be proper for investigation of the physiology of *in vitro* shoot formation, elucidation of pH and sucrose roles and developing of medium for optimum shoot formation and elongation.

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تأثير حالة الوسط ودرجة حموضته ومحتواه من السكروز علي عدد النموات المتكونة من عزلة نبات أناناس صنف موريس

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المستخلص: اختبر تأثير ثلاث حالات (الحالة السائلة والصلبة والنصف صلبة) من وسط موراشيج و سكوق (MS) المحتوي على هرمون 6-benzylaminopurine (BAP) بتركيز 2 مليجرام في اللتر، والمعدل محتواه من السكروز ودرجة حموضة الوسط (pH) ب 16 توليفة مكونة من أربعة تركيزات من السكروز (10 ، 20 ، 30 و 40 جرام في اللتر) وأربع درجات من الحموضة pH (5.0، 5.7 6.0 و 6.5) على عدد النموات المنتجة من عزلة نبات أناناس صنف موريس . استُعمل الوسط السائل المحتوي على سكروز بتركيز 20 جرام في اللتر والوسط الصلب المحتوي على سكروز بتركيز 30 جرام في اللتر وكل منهما حموضته (pH) 5.0 وهذا أدى إلى الحصول على أعلى متوسط عدد نموات من العزلة (7 نموات)، زيادة تركيز السكروز إلى 40 جرام في اللتر أو تعديل درجة حموضة الوسط (pH) إلى 6.0 أدى إلى أن تفقد العزلة 50 % من قدرتها على إعطاء نموات في كل من الوسطين السائل والصلب . في حين كان من الممكن التغلب على فقدان القدرة على إعطاء النموات وعكسه في حالة الوسط السائل بواسطة تعديل حموضة الوسط إلى 6.5 مثل هذا التعديل فشل في التغلب على التأثير المثبط للتركيز المرتفع للسكروز (40جرام) في حالة الوسط الصلب . في العموم فإن استعمال الوسط السائل أعطى أكثر عدد نموات من تلك المتحصل عليها من استعمال الوسط الصلب والوسط النصف الصلب في ثماني توليفات وعدد نموات متساوية مع الوسط الصلب في 4 توليفات، ومتساوية مع الوسط نصف الصلب في 5 توليفات وعدد نموات أقل من الوسط الصلب في أربعة توليفات، وأقل من نصف الصلب في توليفتين من إجمالي 16 توليفة من السكروز و درجة الحموضة، استعمال التوليفة الأكثر شيوعاً واستعمالاً في دراسات زراعة الأنسجة (سكروز 30 جرام في اللتر ودرجة حموضة 5.7) لم تكن فقط غير ملائمة لكل حالة من حالات الوسط الثلاثة بل أدت إلى إعطاء عدد أقل من النموات (4 نموات) مما هو بمقدور العزلة أن تعطيه (7 نموات) لو استعملت توليفة مثلى من تركيز السكروز ودرجة حموضة الوسط ، النتائج أظهرت أن إجراء تغيير بسيط في الوسط وذلك باستعمال درجة حموضة (pH) 5.0 بدلاً من درجة الحموضة الشائعة الاستعمال (5.7) أدى إلى مضاعفة عدد النموات التي يمكن الحصول عليها من العزلة الواحدة .

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

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Co-Composting of Sewage Sludge with Food Waste Using Bin Composter

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Abstract: Optimization of sludge composting has been analysed by co-composting of sewage sludge with food waste by using a bin composter with regular aeration. Two bin composting experiments were set up to study the effect of different mixing ratios of food waste from the wet market to sewage sludge from Al Hadhbah waste water treatment plant which is located in Tripoli on some physical and chemical characteristics of the resulted compost. The selected ratios were 1:2 (A) and 1:3 (B) of food waste to sewage sludge respectively. Composting was conducted for 100 days and the sampling from the mixtures was performed each five days. Temperature (T), pH, electrical conductivity (EC), total organic carbon contents (TOC %) and total nitrogen contents (TN %) were monitored during the composting. The ratio of C/N, inorganic residual material (ash content) and nutrient changes as represented by potassium (K %) and phosphorus (P %) contents was also determined. The compost produced in this study has a favorable ratio of C/N (13.43 for compost A and 14. 32 for compost B). With significant amount of nutrients (TN %, K%, P%) The results of analysis of variance (ANOVA) revealed a significant effect of elapsed composting time (at the probability level of 0.05) on T, pH, TOC%, K% and P%. The effect of composting ratio was insignificant (at the probability level of 0.05) on T, TOC %, TN %, C/N and P%. The multiple comparison results using Tukey's test showed significance difference between the mean values of EC and K% among the composting ratio at the confidence level of 95%. Generally, the compost ratio of 1:2 was found to be the most suitable for use as organic fertilizer.

Key words: a bin composter, food waste, sewage sludge, fertilizer.

INTRODUCTION

To date, human all over the world have been generating more and more waste at a steadily increasing rate. In most parts of the world, solid wastes are disposed of either in open dumps or sanitary landfills or by incineration. Methods of treatment like incineration and sanitary landfilling are expensive. Solid waste management expresses the main matter where it does not have any organized and efficient system in managing solid wastes. This poor management has definitely brought about negative influences to the natural environment. By way of a result, the growing amount of waste has led to the exhaustion of natural resources that further provides a way to environmental risks (Kathirvale *et al*., 2003) Biodegradable wastes are certainly linked with the humiliating environment, especially as we shed light on the maddening quantity of contaminants being produced; they are leachate and methane gas. Section stores and shops have also augmented popularity as the responses to people's needs. In developing countries, the state of the wet market is unwell constructed. For example fruits and vegetable waste and food waste from restaurants

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inside wet market which are products to be thrown away on a daily basis(Omotara *et al.,* 2004) Tee (2009) stated that waste created by local residents is 16000 tonnes of domestic waste per day and the average waste per capita has escalated to 1.44 kg from 0.45 kg a day, determined by the economic status of the involved zone.

The average percentage of solid waste is 45% for food waste, 24% for plastic waste, 7% for papers, 6% for steel 3% for glass and 15% for others .(Baeyens *et al.* 1997) reported that the cost of sewage sludge treatment is high, as it has roughly 35-50% of the total operating costs of the wastewater treatment. (Chooi, 1984) stated that sewage sludge can be dried and used as compost as it covers high nutrient value. In comparison, the sludge is able to recover soil fertility and products more crops by safeguarding adequate source of nutrients which are important to the soil(Rantala *et al.,* 1999, Kuai *et al.,* 2000). Therefore, cost-effective sustainable technologies are needed by the industries to ensure the safe disposal of industrial sludge. The high moisture content, low carbon content and high nutrient value of the sludge are the reason why it is usually dried and used as a fertilizer. Currently, sewage sludge disposal has been prepared through a number of ways such as incineration, land-filling, and composting(Kim *et al.,* 2000).

The first method, incineration is inept as its moisture content is high, there is need to handle unsafe gas emission appropriately. Land-filling method is found to be very expensive because it is not easy to get unfilled grounds suitable for the purpose and also, once a landfill is opened; there will be a set of stringent rules and regulations that need to be followed (Hackett *et al.,* 1999). Land-filling process also produces unpleasant smell and some by-products. The by-products of these gases have a great propensity to pollute the water surface and similarly the ground(Yun *et al.,* 2000). Composting is a method of solid waste organization whereby the organic component of the solid waste stream is physically decomposed under measured conditions to a state in which it can be handled, kept and or applied to the land without adversely affecting the environment(Xiao *et al.,* 2009). Co-composting of sludge with solid food waste was expected to be one choice for waste use and might offer several environmental and economic welfares. Mixing of two types of materials can provide better moisture content and more stable nutrients for the microorganisms to carry out the composting process(Singh *et al.,* 2010, Yeoh *et al.,* 2011) reported that the co-composting is one of the important bio-waste treatments in the palm oil manufacturing for reaching sustainable process and zero waste.Nonetheless, improper conditions of composting may result in several problems such as gas release, bad odor, low quality product production postponement and high handling cost. The wide uses of microorganisms in composting processes are similarly enclosed under clarifications of many investigators. The maturation process of compost can be quicker by supporting the microorganisms to get finial product. However, the microorganisms have desirable effects on the biological characteristics of the organic material.. This study focused on the investigation of the co-composting process of sewage sludge from waste water treatment plant which was mixed with food waste from the wet market in Tripoli Libya. Two different ratios of mixing 1:2 and 1:3 were adopted for this study. The aims were to determine the physicochemical changes of co-composting of food waste to sewage sludge and to determine the best mixing ratio of food waste to sewage sludge by using bin composters.

MATERIALS AND METHODS

Co-composting was used to produce compost from food waste and sewage sludge. Food waste samples were collected from the wet market and sewage sludge samples were collected from Al hadhbah waste water treatment plant in Tripoli Libya. Both samples were mixed and homogenized manually at different ratio of 1:2 and 1:3 (i.e., waste: sludge) by weight, respectively. The composter has measurements of 90 cm in height and 60 cm in diameter and was painted in black to absorb the sun ray. A mixture of sewage sludge and food wastes was prepared and poured from the top of

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the composter. Two kgs of recycled compost were added to the mixture to further facilitate the composting process. A flow of air was created in the composter that aids the aeration. The final product of the compost was collected from the bottom of the composter using an opening designed at the bottom of the composter. This was achieved through constructing a number of uniformly distributed holes each had a diameter of 2 cm and located at the lower side of the bin.

Compost Analysis

Sampling was performed by taking 100 gms of compost from the bin composter at different places(Brinton *et al.,* 2012). The temperatures were measured by means of thermometer at the core of the reactor at the beginning of each experiment and every 5days for 100 days. Many samples of the compost were taken to determine pH, Total nitrogen, Potassium (K%) and Phosphorus (P%), Total Organic Carbon (TOC%), and Electrical Conductivity(EC). The pH values of the compost were determined using a method defined by (Carnes and Lossin 1970). Total nitrogen was determined using Kjeldahl method. Potassium (K%) and phosphorus (P%) were measured by inductively coupled plasma mass spectrometry. Total organic carbon TOC% was determined by heating the dried sample at 550 ˚C for 4 hours. The organic matter was converted to carbon content using a factor of 54 % (Navarro *et al.,* 1990). Wet density was estimated by filling 500 mL of distilled water in a beaker with the material(Schulze 1962). C/N ratio was analyzed using CHNS-O Analyzer which is an elemental analyzer dedicated to the simultaneous determination of the percentage (%) of carbon, pH, nitrogen, Electrical conductivity values of the compost were determined using a method defined by(Rayment and Higginson 1992). All the measurements which conducted on the samples were performed at the beginning of each experiment and every 5 days for 100 days from June 2016 to September 2016.

Statistical Analysis

Analysis of variance without replication (ANOVA) was performed to statistically assess the effects of the elapsed composting time and the mixing ratio on some physical and chemical properties of the compost using a 95% confidence level. For more convenient purposes, it was suggested to use the intervals of 10 days rather than 5 days for the elapsed composting time factor. Tukey's test was used to determine differences among the composting ratio using a 95% confidence level, as well. All statistical analyses were performed using MINITAB statistical package software (Inc 2003).

RESULTS AND DISCUSSION

Physicochemical analyses were conducted on raw materials of food waste and sewage sludge before mixing which is shown in Table 1. The parameters of initial food waste and sewage sludge as depicted in Table (1) are responsible for compost maturity. The values of these parameters are within their optimum range as mentioned by (Anwar *et al.,* 2015). Results of co-composting of food waste to sewage sludge with mixing ratio of 1:2 and 1:3 are shown in Table 2.

Table (1). Parameters of initial food waste and sewage sludge.

Parameters	Food Waste	Sewage Sludge
pH	8.75	6.32
Nitrogen%	1.35	1.07
C/N	31.97	13.48
Potassium%	1.13	0.75
Moisture%	63.52	68.97
Phosphorus%	0.53	1.05
TOC%	33.27	32.42

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Table (2). Physical and chemical parameters of initial material mixture of food waste to sewage sludge with mixing ratio 1:2 and 1:3.

Parameters	Initial material mixture of food waste to Sewage Sludge	
	Ratio1:2Bin composter A	Ratio1:3 Bin composter B
Color	Black	Black
Odor	Smells like soil	Smells like soil
Size distribution	Friable, fine and uniform	Friable, fine and uniform
Phosphorus%	1.13	0.98
Potassium%	0.38	0.58
pH	7.84	8.32
Nitrogen%	2.40	1.50

Temperature

The initial temperatures of bin composters were 23 and 26.5 0C for A and B, respectively as shown in figure 1. Temperatures of both composters increased significantly (at the probability level of 0.05) and sharply to around 43 and 42 °C, from day 2 until day 20 of the treatment. The rapid rise in temperature might be due to bacterial decomposition activity in the compost. The thermophilic phase in the present stage was continued until day 25 of the treatment before the temperature of bin composter declined gradually after it got the maturity phase on day 40 of the treatment when the curing stage was started. The comparisons between the mean values of temperature among the compost ratio as determined by Tukey's test (at the confidence level of 95%) indicated no significant difference. The final temperature of bin composters reduced gradually to reach 18 and 20 °C for composter A and B, respectively. These were observed at the end of the composting process which shown a good degree of stability (Satisha and Devarajan 2007).

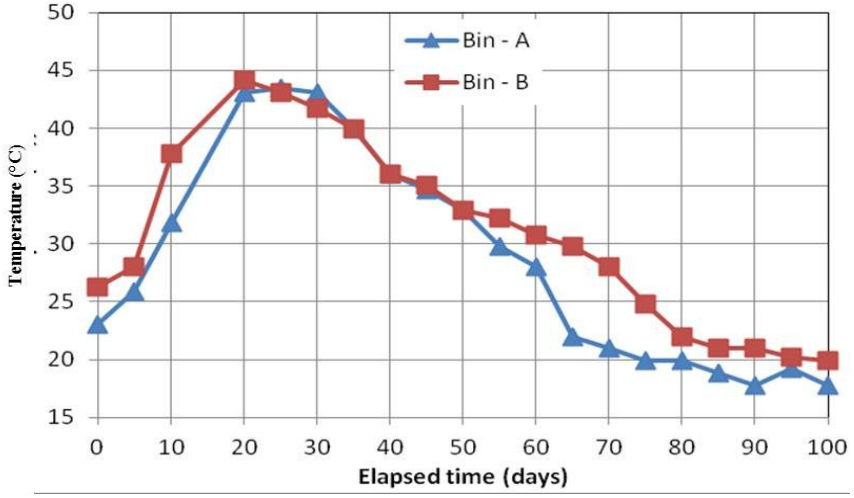

Figure (1). Temperatures profile of composting using bin composters A and B.

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pH

In Figure 2, it is shown that the initial pH of the compost were 8.46 and 8.32 for composters A and B, respectively, then augmented sharply to maximum values of about 8.87 for composter A and 8.53 for composter B in the first month of composting. The results of ANOVA indicated that the effects of the elapsed composting time and the composting ratio were significant at the probability level of 0.05. Growth of pH value at the beginning of the composting process might be due to the protein mineralization which led to the increase in ammonia produced by the biochemical reactions of nitrogen-containing materials and varying of amino acids and peptide to the ammonia (Crawford, 1983; (Liao *et al.,* 1997, Paredes *et al.,* 2002). According to Tukey's test, the comparisons among the compost ratio revealed no significant difference between the mean values of pH at the confidence level of 95%. The final values of pH were reduced to 7.01 and 7.36 for composters A and B respectively, as depicted in figure 4. These values agreed with the study of (Sundberg *et al.,* 2004). They stated that for fully developed composting, the pH frequently ranged from 7 to 9.

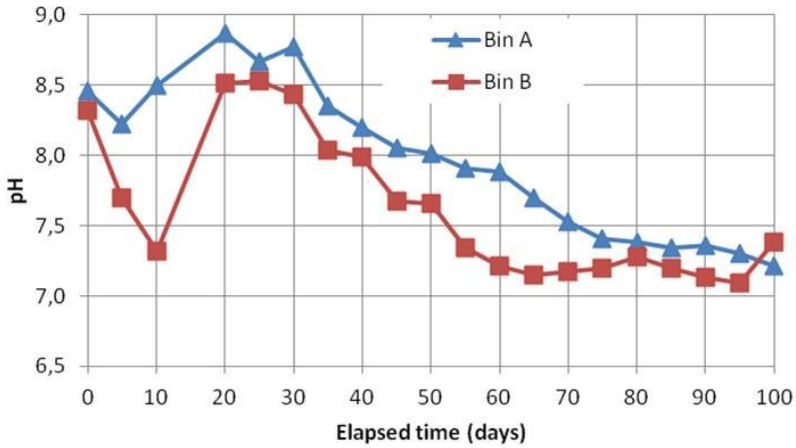

Figure (2). pH profile of composting using bin composters A and B.

Electrical Conductivity (EC)

Electrical conductivity is the ability of a material to transmit an electrical current and electrical conductivity is expressed in the units of dS/m. The initial concentrations of EC were 1.48 dS/m and 1.53 dS/m for composter A and B, respectively. These values augmented with the final concentration of 1.89 dS/m for composter A and 1.53 dS/m for composter B. It can be observed from figure 3 that composter A presented a significant increase (according to Tukey's test at the confidence level of 95%) over composter B. This is a significant application of compost in agriculture since high soil salinity may prevent plant development and growth. The data obtained in this study were in the range of another study by (Helić *et al.,* 2011) on composting of municipal solid waste with different additives and values of electrical conductivity with mixture composting of municipal solid waste, poultry manure and sawdust. They found that EC were 1.50 dS/m and 2.62 dS/m for both reactors, respectively.

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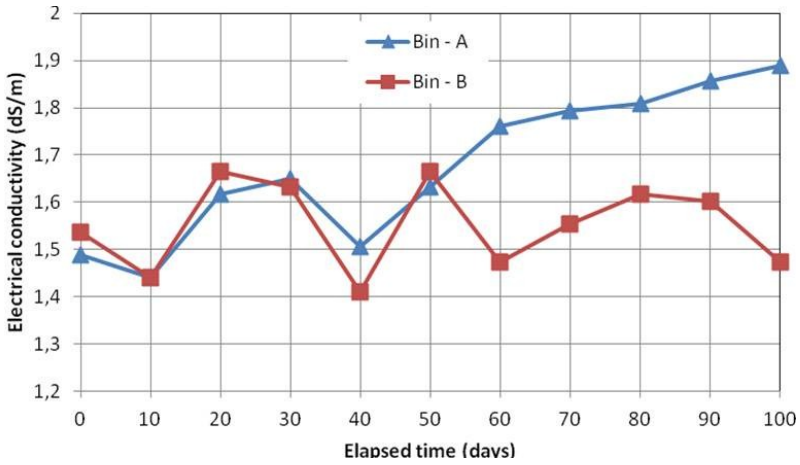

Figure (3). Electrical conductivity profiles of composting using bin composters A and B.

Total Organic Carbon

Unlike the composting ratio, the results of ANOVA showed a significant effect of the elapsed composting time on TOC%. The results also revealed that TOC % decreased in a similar way in both composters A and B. The initial values of TOC% for composters A and B were 46.23 and 48.04% respectively, and dropped quite dramatically to 40.21 and 37.08% at the end of composting as depicted in figure 4. Such decreasing in TOC% during composting time was also noted in many similar studies (Sampedro *et al.,* 2007, Haque and Gholami 2012). However, according to Tukey's test, the comparisons among the compost ratio revealed no significant difference between the mean values of TOC % at the confidence level of 95%.

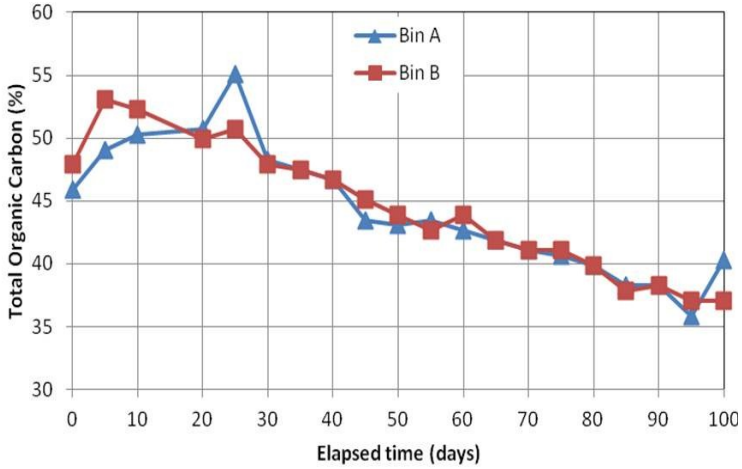

Figure (4). TOC profile of composting using Bin Composters A and B.

Total nitrogen

Total nitrogen content in both composters A and B increased from initial values of 0.89 and 0.86%, respectively. Composter B experienced a series of insignificantly statistical ups and downs (at the probability 0.05 level) in nitrogen concentration during thermophilic stage as shown in figure 5. Moreover, the results of Tukey's test indicated that the comparisons

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among the compost ratio revealed no significant difference between the mean values of total nitrogen at the confidence level of 95%. The final values of total nitrogen observed were 1.187 and 1.30% for composter A and B, respectively. However, (Nutongkaew *et al.,* 2011) reported a similar trend in increasing of the total nitrogen during the composting process.

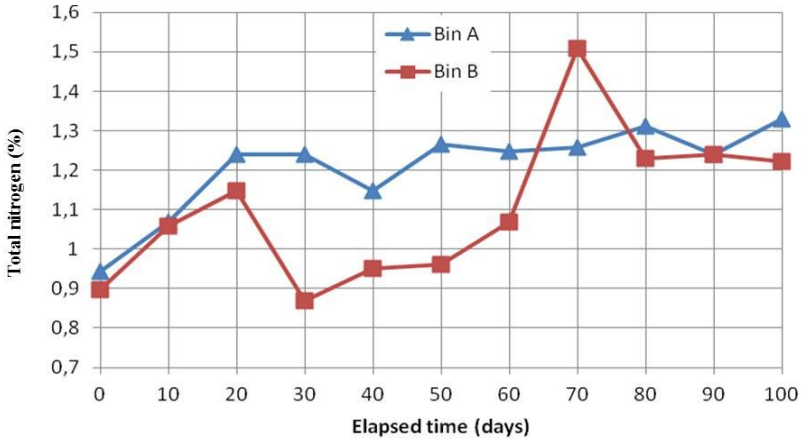

Figure (5). Changes in TN profile of composting using Bin Composters A and B.

C/N ratio

C/N ratio decreased gradually in the first two weeks of composting treatment for both composters A and B. Such typical reductions in C/N ratio were statistically significant according to the analysis of variance (ANOVA), at the probability levels 0.05 during the composting process i.e., the elapsed composting time. According to Tukey's test, the comparisons among the compost ratio revealed no significant difference between the mean values of C/N ratio at the confidence level of 95%. However, the final values for both composters were 13.43 and 14.32%, respectively, after maturation as depicted in figure 6. Such final values of C/N are favorable for composting industries.(Van Heerden *et al.,* 2002) stated that the C/N ratio less than 20 could be considered as a suit maturation level of compost. However, this enhances net mineralization of nitrogen in the soil for plant use if used as fertilizer.

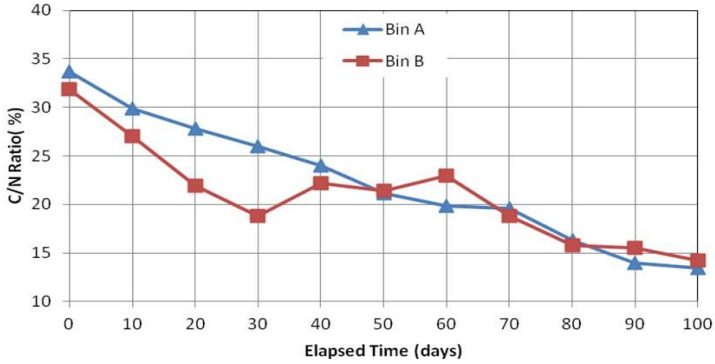

Figure (6). Changes in C/N ratio profile of composting using Bin Composters A and B.

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Nutrient Changes

Unlike P%, the analysis of variance (ANOVA) indicated that the effects of composting ratio on K% were significant at the probability levels of 0.05. On the other hand, it was found that the effects of elapsed composting time on both K% and P% were significant at the probability level of 0.05. Figure 7 shows that the content of K% in composter A increased from 1.5% to 2.97%. The content of P% also increased in composter A from 0.1% to 1.27%. Similarly, in the composter B the content of K% increased from 0.53 to 1.72% and from 0.48 to 1.08% in terms of P%. According to Tukey's test (at the confidence level of 95%), the comparisons between the mean values of K% were found to be in significant difference among the composting ratio. Unlike the mean values of K%, the mean values of P% had relatively no differences among the composting ratio. The increase of K% and P % at the end of composting could be attributed to a decrease in organic matter (Haug 1993). On the other hand, (Fei-Baffoe *et. al.*, 2015) attributed the increase of macro elements such as K% and P% to the organic matter decomposition which is leading to the net loss of dry mass, which in turn, might have concentrated the phosphorus and potassium in the compost.

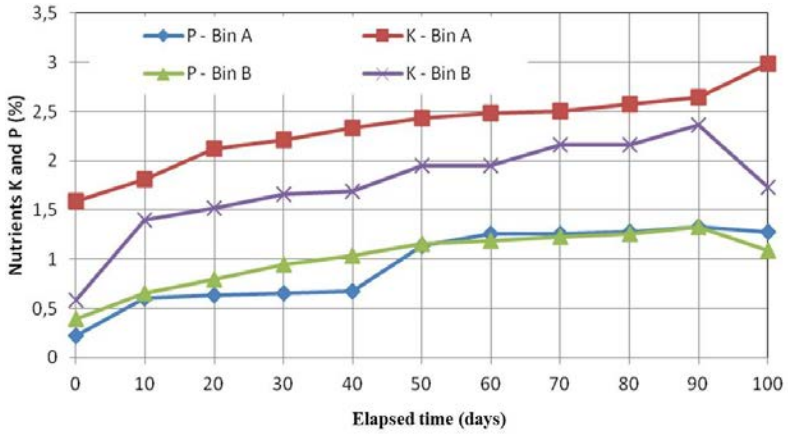

Figure (7). Changes of nutrients profile of composting using bin composters A and B

Ash Content

This test is a measure of the inorganic residual material gone after burning the oven-dried compost sample at 500 ± 50°C. The quantity of ash was depending on the kind of feedstocks composted. Initial ash content of composters A and B were 26.79 and 28.21% respectively. The final values obtained in this study of both composters were 11.32 and 12.23% for A and B respectively as depicted in figure 8. The results of analysis of variance (ANOVA) revealed that unlike composting ratio, the effects of elapsed composting time on ash content were significant at the probability level of 0.05. Similar results were reported by (Helić *et al.,* 2011, Razali *et al.,* 2012).

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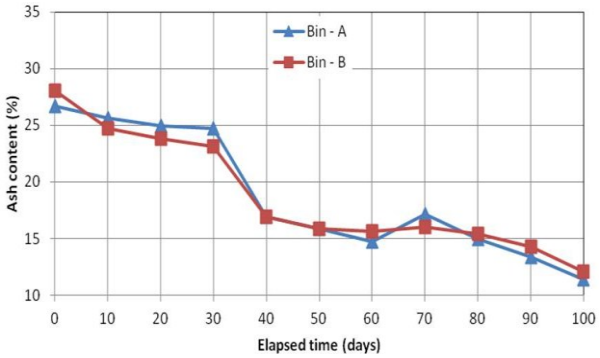

Figure (8). Ash content profile of composting using bin composters A and B.

Table (3). Physicochemical properties of the final compost at two stages

	pH	C/N	K%	P%	N%	T^{0}C
Bin composter A	7.01	13.43	2.97	1.27	1.20	43
Bin composter B	7.36	14.32	1.72	1.08	1.30	42

CONCLUSION

Co-composting of food waste to sewage sludge can be used as another way of converting these materials into a beneficial product using a cheap and easy system, which is the bin composter. Improved biodegradation was achieved by mixing food waste to sewage sludge. In addition, the temperature achieved in composting met the sanitary requirement for pathogen killing. The compost produced in this study has a C/N ratio of 13.43 and 14.32 with significant amounts of phosphorus, potassium and nitrogen. It is noteworthy that the final product of compost had high nutrient content especially P and K. Thus, the compost can be applied safely to the soil as a conditioner to enhance its chemical quality.

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التسميد المشترك من حماة الصرف الصحي مع النفايات الغذائية باستخدام مستوعب التسميد (الكمبوست)

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المستخلص : أجرت الدراسة التحليل الأمثل لسماد الحمأة المصنع من الحمأة مع النفايات الغذائية باستخدام مستوعب الكمبوست مع التهوية العادية. نفذت تجربة الدراسة باستخدام معاملتين ناتجتين من الخلط المتجانس لسماد الحمأة المتحصل عليها من محطة معالجة مياه الصرف الصحي بمحطة الهضبة بطرابلس مع فضلات الطعام من السوق الرطب في طرابلس ليبيا وبنسب مختلفة من 1: 2 والتي تمثل المعاملة (A) و بنسبة1: 3 التي تمثل المعاملة (B). أُخذت عينات من الخليط كل خمسة أيام خلال كامل فترة تحضير الكمبوست. وتم تقدير كل من درجة الحرارة ودرجة التفاعل و التوصيل الكهربي و محتوى الكربون العضوي (%) و محتوى النيتروجين الكلي (%) ونسبة الكربون إلى النيتروجين والرماد المتبقي (%) والتغيرات التي حدثت في نسبة الفسفور(%) والبوتاسيوم (%) في عينات الكمبوست. وأظهرت النتائج المتحصل عليها أن الكمبوست النهائي احتوى قيماً مرغوبة من نسبة الكربون إلى النيتروجين (13.43 للمعاملة A و 14.32 للمعاملة B) مع قيم مرتفعة من محتوى النيتروجين الكلي (%) والبوتاسيوم (%) والفسفور (%). كما بينت نتائج تحليل التباين أن هناك تأثيرا معنوياً لزمن التحضير عند مستوى معنوية 0.05 وذلك على كل من درجة الحرارة و درجة التفاعل و محتوى الكربون العضوي (%) ونسبة الكربون إلى النيتروجين و البوتاسيوم (%) والفسفور (%). وفيما يتعلق بنسبة الخلط فكان تأثيرها غير معنوي عند مستوى معنوية 0.05 على كل من درجة الحرارة ومحتوى الكربون العضوي (%) و محتوى النيتروجين الكلي (%) ونسبة الكربون إلى النيتروجين والفسفور(%). وقد أظهرت نتائج المقارنة المتعددة باستخدام اختبار توكاي (Tukey's test) عند مستوى ثقة 95%، أن هناك اختلافات معنوية بين متوسطات كل من درجة التوصيل الكهربي و كذلك بين متوسطات محتوى البوتاسيوم (%) بين معاملات نسب الخلط المختلفة. وعموماً، فإنه بالإمكان اللجوء إلى استخدام نسبة الخلط 2:1 المكونة من فضلات الطعام: حمأة المجاري للحصول على منتج سمادي أكثر احتواء على العناصر الغذائية الأساسية لنمو النبات موازنة مع نسبة 3:1.

الكلمات المفتاحية: مفاعل التسميد، نفايات غذائية، حمأة الصرف الصحي، السماد.

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Totally Volume Integral of Fluxes for Discontinuous Galerkin Method (TVI-DG) I- Unsteady Scalar One Dimensional Conservation Laws

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Abstract: The volume integral of Riemann flux in the discontinuous Galerkin (DG) method is introduced in this paper. The boundaries integrals of the fluxes (Riemann flux) are transformed into volume integral. The new family of DG method is accomplished by applying divergence theorem to the boundaries integrals of the flux. Therefore, the (DG) method is independent of the boundaries integrals of fluxes (Riemann flux) at the cell (element) boundaries as in classical (DG) methods. The modified streamline upwind Petrov-Galerkin method is used to capture the oscillation of unphysical flow for shocked flow problems. The numerical results of applying totally volume integral discontinuous Galerkin method (TVI-DG) are presented to unsteady scalar hyperbolic equations (linear convection equation, inviscid Burger's equation and Buckley-Leverett equation) for one dimensional case. The numerical finding of this scheme is very accurate as compared with other high order schemes as the weighted compact finite difference method WCOMP.

Key words: Scalar conservation laws; Higher order methods, Discontinuous Galerkin; Divergence theorem

INTRODUCTION

There has been a surge of researches activities in high order methods as spectral volume (SV) method, spectral difference (SD) method, the weighted essentially non-oscillatory (WENO) method and (DG) method. Most of the aforementioned methods have a common feature: they achieve high order accuracy by locally approximating the state variables (numerical solutions) as high order polynomials inside the element. While WENO method achieves a high order accuracy by approximating the state variables as high order polynomials over a stencil (groups of cells or elements). In this work, we concerned ourselves to the compact and weighted scheme which is the DG method.

The DG method is introduced by Reed and (Hill in 1973) for neutron transport problems and then developed for fluid dynamics by Cockburn and Shu in series of papers among them (Cockburn *et al.*, 1989, Cockburn and Shu 1989, Cockburn *et al.*, 1990, Cockburn 2001). (Huynh 2007) introduced a flux reconstruction (FR) approach, in which the formulation is capable of unifying several popular methods including the discontinuous Galerkin method, staggered-grid method, spectral difference method and spectral volume method into a single formula. The final mathematical form of the discretized governing equation is governing equation in the differential form. After that, (Wang and Gao 2009) extended (FR) approach to multidimensional flow and unstructured mesh under the named lifting collocation penalty (LCP) formulation. Therefore, the differences between DG and other methods lie in the definition of degrees of freedom (DOFs)

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and how the DOFs are updated (Wang and Gao 2009).

It is well known that the discontinuous Galerkin method is as an efficient and low error magnitude than the other methods. In the DG formulation, the boundary flux is integrated over the boundary of the cell or element as traditional methods like finite volume (FV) methods. While for its development the weighted function at the boundary of the cell can be transformed into the correction function $g(\xi)$ for (FR) or lifting coefficients $\alpha_{i,j}$ for LCP formulations. Thus $g(\xi)$ and $\alpha_{i,j}$ are dependent on the weighted functions over the boundaries(Gao and Wang 2009, Wang and Gao 2009). Therefore the weighted functions at the boundary play an important rule for boundary flux calculation in the DG method and its development. In general, there are two types of flux integrals, the first one is the volume integral of the physical flux over the entire element domain, and the second type of integral is the boundary integral of the Riemann flux (Godunov flux) over the boundaries of the elements (over the surface areas of the element).
This difficulty motivated us to introduce a new family of DG methods independent of the weighting functions at the boundaries. Therefore no boundary integral is needed for this new formulation.
The paper is organized as follows: Section 2 introduced the new DG method formulations. The verification of the new formulation is introduced in section 3. Finally, conclusion remarked is introduced into section 4.

Totally Volume Integral (TVI) DG Method Formulation

Space Discretization

For the convenience of discussion, a review for DG semi-discretization for partial differential equations (PDE) is introduced. This can be done by firstly considering the conservation laws in divergence form:

$$Q_t + \nabla \cdot \boldsymbol{F} = 0. \quad (1)$$

The numerical solution of Eqn. (1) is sought on the computational domain Ω subject to proper initial and boundary condition. where Q is the conservative variable and $\boldsymbol{F}$ is the conservative flux vector.
In Eqn. (1) Q and $\boldsymbol{F}$ are scalar or column, representing scalar or system of equations. The weighted residual formulation is obtained by multiplying Eqn. (1) by a scalar test function (weighting function) W and integrating by parts over the domain Ω

$$\int_{\Omega}[WQ_t - \nabla W \cdot \boldsymbol{F}(Q)]d\Omega + \int_{\partial\Gamma} W\boldsymbol{F}(Q)\cdot \boldsymbol{n}d\Gamma = 0. \quad (2)$$

A discretization analogue of Eqn. (2) over each element can be obtained by subdividing the computational domain Ω into N non-overlapping elements $\Omega = \bigcup_{k=1}^{N} \Omega_h$. By applying Eqn. (2) to each element Ω_h, the semi-discrete analogue of Eqn. (2.2) over the computational grid yields:

$$\int_{\Omega_h}\left[W_h \frac{\partial Q_h}{\partial t} - \nabla W_h . \boldsymbol{F}(Q_h)\right] d\Omega_h + \int_{\Gamma_h} W_h \boldsymbol{F}(Q_h).\boldsymbol{n}d\Gamma_h = 0, \quad (3)$$

Γ_h denotes the boundary of the element Ω_h and **n** is outward vector normal to the boundary. Let Q_h and W_h represent the finite element approximation to the analytical solution Q and the test function W respectively where Q_h and W_h are approximated by a piecewise polynomial function of degree k, which is continuous within each element and discontinuous between the elements interfaces.

$$Q_h(x,t) = \sum_{j=1}^{j=n} \varphi_j Q_j(t) \text{ and } W_h(x) = \sum_{j=1}^{j=n} \varphi_j W_j, \quad (4)$$

Where n is the dimension of the polynomial space p^k and φ_j is the basis of the polynomial. The expansion coefficients $Q_j(t)$ and W_j denotes the degrees of freedom (DOFs) of the numerical solution and the test function in element Ω_k, respectively. Thus the summation in eqn. (3) is equivalent to the following system of n equations

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$$\int_{\Omega_h}\left[\varphi_j Q_t - \nabla\varphi_j \cdot F(Q_h)\right] d\Omega_h + \int_{\Gamma_h} \varphi_j F(Q_h) \cdot \mathbf{n}\, d\Gamma_h = 0,\ 1 \le \mathrm{j} \ge \mathrm{n}, \quad (5)$$

since the discontinuities are permitted at the interfaces of elements in the DG method. Because the approximated solution is discontinuous at the element boundaries, the interface flux is not uniquely defined. In this stage, the Riemann fluxes used in the Godunov finite volume method are borrowed.

The normal flux function $\boldsymbol{F}(Q_h).\mathbf{n}$ appearing in the last terms of eqn(5) is replaced by a numerical Riemann flux function $F_{up} = \boldsymbol{F}(Q_L,Q_R,\mathbf{n})$ that depends on Q_L and Q_R which are the approximated solutions of the conservative state variables Q_h at the left and right side of the element boundary, respectively. In order to guarantee consistency and conservation, the Riemann flux must satisfy the following

$$F_{up} = \boldsymbol{F}(Q_L,Q_R,\mathbf{n}) = \boldsymbol{F}(Q_h).\mathbf{n} \text{ and } -F_{up} = \boldsymbol{F}(Q_L,Q_R,-\mathbf{n}) = -\boldsymbol{F}(Q_h).\mathbf{n}. \quad (6)$$

In the present work, the Riemann flux is approximated by using Lax and Friedrich (LF) flux for nonlinear flux. This scheme is called discontinuous Galerkin method of degree *k* as given in the classical form, or in short notation DG (k) method. The surface and volume integrals in Eqn. (5) are calculated in case of DG method by using 2k and 2k+1 order accurate Gauss quadrature formulas, respectively.

In order to unify the integrals (surface integral and volume integral), the totally volume integral of the upwind flux scheme for DG method is used for this purpose. The relation between the surface and volume integrals for any vector A is given by the divergence theorem as

$$\oiint_S \mathrm{A} \cdot \mathbf{n}\mathrm{d}\Gamma = \iiint_V \nabla.\,\mathrm{A}\ \mathrm{dV}, \quad (7)$$

where Γ and V are surface and volume of the problem domain. The totally volume integral DG method is accomplished by applying the divergence theorem to the last term of Eqn. (5) and rearranging to give the following form

$$\int_{\Omega_h}\left[\varphi_j \frac{\partial Q_h}{\partial t} - \nabla\varphi_j F(Q_h) + \nabla\varphi_j F_{up} + \varphi_j \nabla.\, F_{up}\right] d\Omega_h = 0, \quad (8.a)$$

for one dimensional case Eqn. (8.a) can be written as:

$$\int_{\Omega_h}\left[\varphi_j \frac{\partial Q_h}{\partial t} - \frac{\partial\varphi_j}{\partial x} F(Q_h) + \frac{\partial\varphi_j}{\partial x} F_{up} + \varphi_j \frac{\partial F_{up}}{\partial x}\right] d\Omega_h = 0. \quad (8.b)$$

The Riemann or upwind flux vectors are approximated by polynomial of order k as done for the state variable in Eqn. (4). $F(Q_h) = \sum_{i=1}^{i=n} \phi_i F_i(Q_h)$ $F_{up} = \sum_{i=1}^{i=n} \phi_i F_{up,i}$, the last two terms of Eqns. (8.b) can be companied into one term as follows

$$\int_{\Omega_h}\left[\varphi_j \frac{\partial Q_h}{\partial t} - \frac{\partial\varphi_j}{\partial x}\varphi_i F_i(Q_h) + \left(\frac{\partial\varphi_j}{\partial x}\varphi_i + \varphi_j \frac{\partial\varphi_i}{\partial x}\right) F_{up,i}\right] d\Omega_h = 0. \quad (9)$$

Equation (9) is the DG method in totally volume integral form.

Coordinate Transformation

In order to achieve an efficient implementation, all elements are transformed from the computational space (x,y,z) into standard space (ζ,η,ξ). Consequently, all partial derivatives with respect to the standard space are related to the partial derivative in the computational space as in the finite element methods. For one dimensional case, the value of the x can be obtained as:

$$x = \sum_{i=1}^{i=N} x_j \varphi_j(\zeta). \quad (10)$$

The derivative of x with respect to ζ is obtained as:

$$\frac{\partial x}{\partial\zeta} = x_\zeta = \sum_{i=1}^{i=N} x_j \frac{\partial\varphi_j(\zeta)}{\partial\zeta}. \quad (11)$$

The derivatives of any function with respect to the standard coordinate can be written as:

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$$\frac{\partial(\ )}{\partial\zeta} = \frac{\partial(\ )}{\partial x}\frac{\partial x}{\partial\zeta} = \frac{\partial(\ )}{\partial x}x_\zeta, \text{with} |J| = |x_\zeta|. \quad (12)$$

Where |J| is the determinant of Jacobian matrix. Also, the derivatives of any function with respect to physical coordinates can be written as:

$$\frac{\partial(\ )}{\partial x} == \frac{\partial(\ )}{\partial\zeta}\zeta_x, \text{with} |J^{-1}| = |\zeta_x|. \quad (13)$$

From equations (11) and (13), $x_\zeta = 1/\zeta_x$, with $n_\zeta = \zeta_x/|\zeta_x| = 1$. Thus no negative values of the Riemann flux F_{up} = F(Q_L,Q_R,$\mathbf{n}$□) at the boundaries. By substituting into Eqn.(9) and rearrangement yields:

$$\int_{\Omega_h}\left[\varphi_j\frac{\partial Q_h}{\partial t} - \frac{\partial\varphi_j}{\partial\zeta}\varphi_i\left(\zeta_x F_i(Q_h)\right) + \left(\frac{\partial\varphi_j}{\partial\zeta}\varphi_i + \varphi_j\frac{\partial\varphi_i}{\partial x}\right)\left(\zeta_x F_{up,i}\right)\right]d\Omega_h = 0. \quad (14)$$

Finally after the spatial discretization is accomplished, equation (14) can be written into the following form

$$M\frac{dQ}{dt} = R(Q), \quad (15)$$

where R(Q) is the residual and M is called the consistent mass matrix

Time Integral

The semi-discrete equation as Eqn. (15) can be integrated in time using explicit methods. The explicit three-stage third-order TVD Runge-Kutta scheme RK(3,3) and five-stage forth order RK(5,4) are the widely used methods given in many references among them(Gao and Wang 2009). The RK(3,3) can be expressed in the following form:

$$Q^{(1)} = Q^n + \Delta t M^{-1} R(Q^n) \quad (16.a)$$

$$Q^{(2)} = 3/4Q(n) + 1/4[Q(1)+\Delta t M -1 R(Q(1))] \quad (16.b)$$

$$Q^{n+1} = 1/3\ Qn + 2/3[Q(2) + \Delta t M-1\ R(Q(2))] \quad (16.c)$$

This method is linearly stable for a Courant number less than or equal to 1.

Numerical Results

All of the computations are performed on a Compaq laptop computer (2.33 GHz Intel (R) Core (TM) 2 CPU T7600 with 4G Bytes memory) using Ubuntu 14.05 Linux operating system. The code was written in C Language and compiled with the default gcc compiler. As a preliminary test we apply the totally volume integral discontinuous Galerkin method to several one-dimensional examples involving linear advection equations, inviscid Burger's equation and Buckley-Leverett equations.

The global error is calculated as the difference between the exact solutions and the numerical solutions. The discretize L_1 norm error is given as

$$L_1 = \sum_{j=1}^{j=N}\sum_{i=1}^{i=edof}\left|Q^{ex} - Q_i^h\right|/tdof,$$

where *N* is the total number of elements, *edof* is the element degree of freedom and tdof=(*N*□□ edof)is the total degree of freedom.

Numerical Tests and Comparison.

Example-1

The first example is linear advection equation considered in many references among them(Zhang *et al.*, 2008).

$$\frac{\partial Q}{\partial t} + \frac{\partial \boldsymbol{F}(Q)}{\partial x} = 0, \quad (1)$$

with $\boldsymbol{F}(Q) = Q$. The initial condition is given as $Q(x,0) = \sin^4(\pi\ x)$ with periodical boundary conditions. The exact solution is $Q(x,t) = \sin^4(\pi(x-t))$. The domain [-1,1] is divided into N equally space elements. The approximated solutions are constructed from polynomials of orders *k* from 1 to 3. The RK (3,3) is used for *k* = 1 and 2, while RK (5,4) is used in case of *k* = 3, where the RK methods are used for evaluating the time integral part. The numerical results are obtained at time t =1.0. Table (1) exhibits the L_1 error and order of accuracy using TVI-DG method. Whereas Table (2) reveals the L_1 error and order of accuracy using weighted compact

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method of forth and sixth order of accuracy from (Zhang *et al.,* 2008). Figure(1) displays the numerical solution at t = 1.0 using polynomial of order k = 2. Figure 2 reveals L_1 error of TVI-DG method with polynomials from 1 to 3 and the L1 error of the weighted compact method of orders 4 and 6 from (Zhang *et al.,* 2008). Figure (2) and Table (2) show that TVI-DG method has lower error magnitude as compared with weighted compact method.

Table (1) L_1 error and the order of accuracy for 1D linear advection eqn. with periodic boundary conditions at t = 1 by using TVI-DG method with polynomials of orders k =1 to 3.

	k=1		*k=2*		*k=3*	
N	L_1 error	Order	L_1 error	order	L_1 error	order
10	2.90933e-01	-	6.005329e-02	-	3.991401e-03	-
20	9.40716e-02	1.628	4.146367e-03	3.856	1.021990e-04	5.287
40	2.811773e-02	1.742	2.284089e-04	4.182	5.570254e-06	4.197
80	4.257114e-03	2.723	2.093349e-05	3.447	3.388487e-07	4.039
160	5.529017e-04	2.944	2.419287e-06	3.113	2.12720e-08	3.993

Table (2) L_1 error and the order of accuracy for 1D linear advection eqn. from (Zhang *et al.,* 2008)

Method	N	L_1 error	L_1 order
	10	3.56e-1	-
	20	1.42e-1	1.33
WCOMP4	40	2.62e-2	2.44
	80	2.21e-3	3.57
	160	1.64e-4	3.76
	10	3.56e-1	-
	20	9.27e-2	1.94
WCOMP6	40	7.22e-3	3.68
	80	3.06e-4	4.56
	160	1.10e-6	8.12

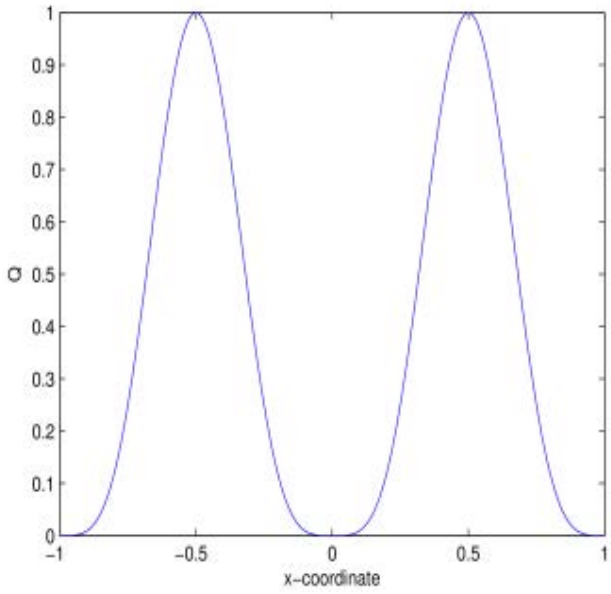

Figure (1). The numerical solution of 1D linear advection eqn. with initial condition $Q=\sin^4(\pi x)$
at t=1.0, N=200 elements by using polynomial of order k = 2

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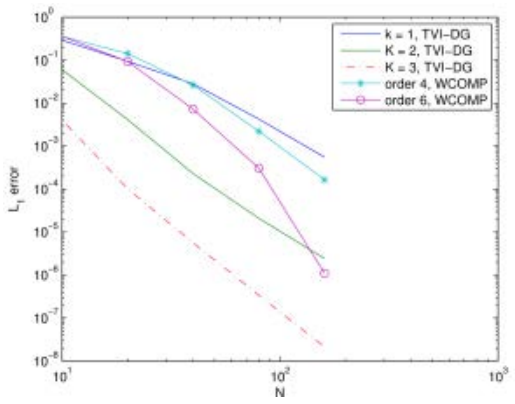

Figure (2). L_1 errors for TVI-DG, with k=1 to 3 and weighted compact method from (Zhang *et al.*, 2008).

Example-2

The second example is the linear advection equation, eqn. (1) with $\boldsymbol{F}(Q) = Q$. The initial condition Q(0,x) = 0.0 and the boundary condition given as $Q(0,t) = sin\left(\frac{1}{2}\pi t\right)$. The problem is considered in many references among them Ref. (Liu *et al.*, 2010). The problem domain x ∈ [0,100] is divided into 200 elements. The approximated solutions are constructed from polynomials of order *k* from 2 to 4.Due to the smooth solution; there is no need for using the stabilization technique. The time part is evaluated using RK(3,3) and RK(5,4). Figures 3 to 5 display the numerical solutions at t = 20, 40 and 60, respectively. The figures demonstrate that the TVI-DG is a very efficient method for solving problems with sine wave propagation from the boundary to the main domain, without losses in the wave amplitude in case of long time intervals.

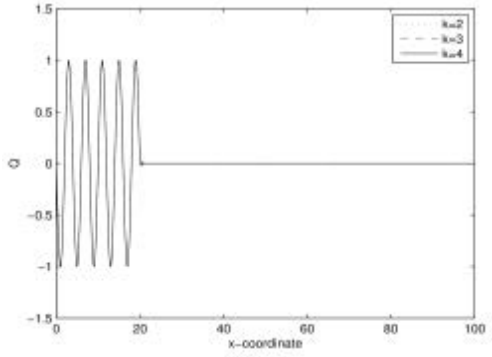

Figure (3). The numerical solution of example 2. by using TVI-DG with k =2 to 4 at time t=20.

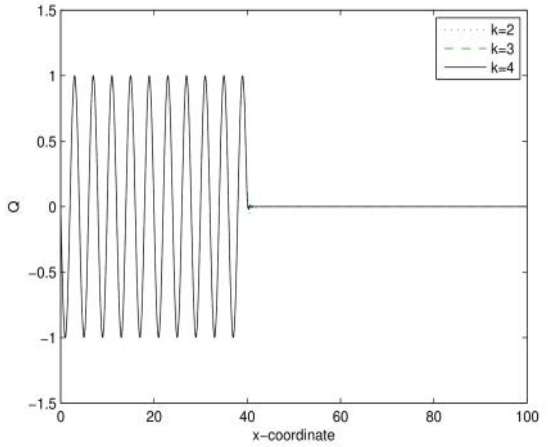

Figure (4). The numerical solution of example 2. by using TVI-DG with k =2 to 4 at time t=40.

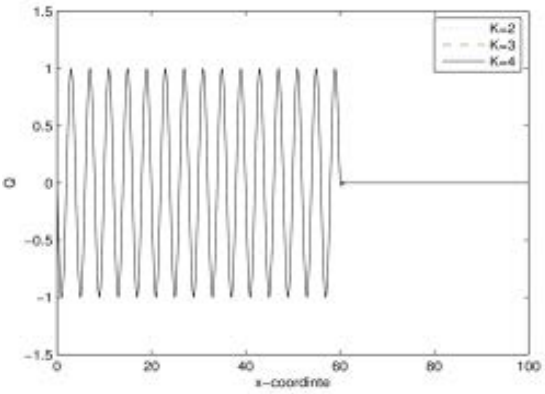

Figure (5). The numerical solution of example 2. by using TVI-DG with k =2 to 4 at time t=60.

Example-3

The third test example is the inviscid Burger's equation, considered in many references among them(Wang *et al.*, 2008).

$$\frac{\partial Q}{\partial t} + \frac{\partial F(Q)}{\partial x} = 0, \text{ with } \boldsymbol{F}(Q) = \tfrac{1}{2}\, Q^2. \qquad (2)$$

The initial condition is u(0, x) = sin(π x) with periodical boundary conditions. The problem domain [0,2] is divided into 150 equally spaced elements. The approximated solutions are constructed from polynomials of orders k from 2 to 4. The RK (5,4) are used for evaluating the time integral part. The numerical results are obtained at time t = 1. Due to discontinuity, the modified streamline - upwind stabilization technique is used to capture the unphysical

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oscillation in the flow problem. Figure (6). display the numerical solutions using TVI-DG method at t =1. The figure reveals that the TVI-DG method is very efficient and there is no significant unphysical flow in case of shock wave problems.

Example-4

The fourth example is Buckley-Leverett equation for two phase flow(Xin and Flaherty 2006).

$$\frac{\partial Q}{\partial t}+\frac{\partial F(Q)}{\partial x}=0,\text{with}F(Q)=\frac{Q^2}{Q^2+\frac{1}{2}(1-Q)^2}. \qquad (3)$$

The initial condition is given as $Q(x,0)=\begin{matrix}1, & x<0\\ 0, & x\geq 0\end{matrix}$. The problem domain [-1,2.5] is divided into 200 equal elements. The approximated solutions are constructed from polynomials of orders *K* from 2 to 4. The RK (5,4) are used for evaluating the time part. The numerical solution is obtained at time t = 1.0. Figure (7) demonstrates that the TVI-DG method with modified streamline upwind stabilization technique is very efficient and there is no nonphysical oscillation of the numerical solution for the shocked flow where the solution involves one moving shock wave followed by expansion wave(Xin and Flaherty 2006). However there is no closed form of the equation (exact solution), thus the exact solution can be obtained by using 1000 element.

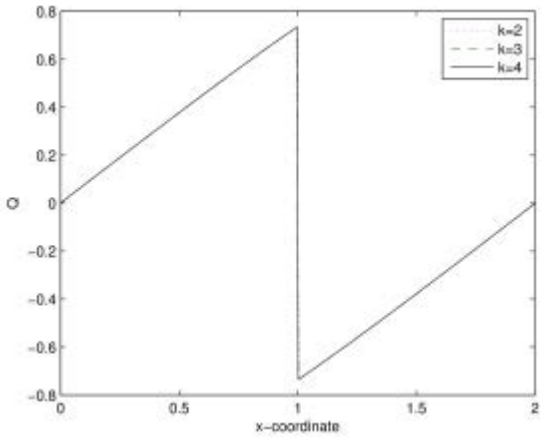

Figure (6.) The numerical solution of example 3. by using TVI-DG with k =2 to 4 at time t = 1.

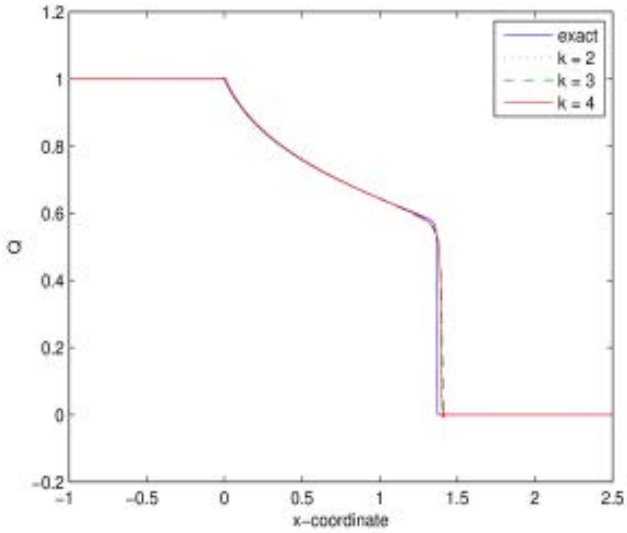

Figure (7). The numerical solution of example 4. by using TVI-DG with k =2 to 4 at time t = 1.

Example-5

The fifth test example is the Buckley-Leverett equation with the standard parameters, considered in (Xin and Flaherty 2006). The standard parameter of the flux is $F(Q)=\frac{Q^2}{Q^2+\frac{1}{4}(1-Q)^2}$. The initial condition is given as:

$$Q(x,0)=\begin{matrix}1, & -.5\leq x\leq 0\\ 0, & x\geq 0\end{matrix}.$$

The problem domain [-1,1] is divided into 200 equal elements. The approximated solutions are constructed from polynomials of orders *k* = 2 and 3. The RK (5,4) are used for evaluating the time integral part. The numerical results are obtained at time t = 0.4.

Figure (8) displays the numerical solutions at time t = 0.4, by using TVI-DG method with k =2 and 3 and the stabilization technique is used to capture the unphysical oscillation in the flow. The solution involves two moving shock waves each followed by an expansion wave (Xin and Flaherty 2006).

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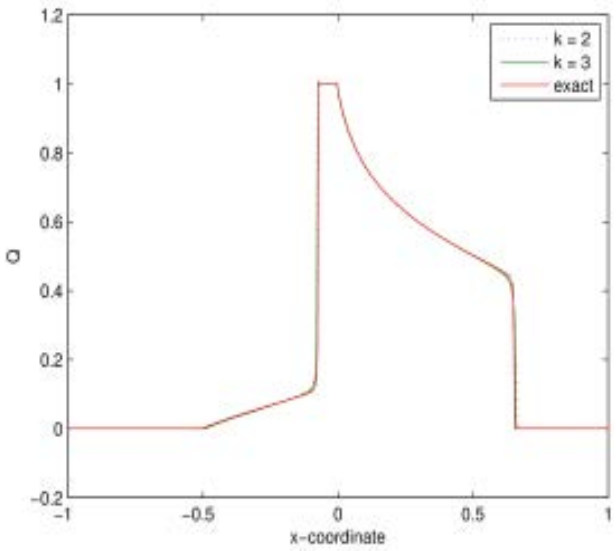

Figure (8). The numerical solution of example 5. by using TVI-DG with k =2 and 3 at time t = 0.4.

DISCUSSION AND CONCLUSIONS

The transformation of the boundaries integrals into the volume integrals is introduced in this paper under the named TVI-DG method. Thus, there is no need for using the integrals of test functions at the boundaries as in the classical DG method. The totally volume integral discontinuous Galerkin method is used to solve hyperbolic conservation laws. The numerical finding presented that the TVI-DG scheme is very efficient and had lower error magnitude than the other high order schemes as weighted compact scheme.

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التكامل الحجمي لكل الفيض لطريقة جلركين غير المتصلة (TVI-DG)
1- قوانين الحفظ اللامتجهة وأحادية البعد

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المستخلص : يقدم الباحثان في هذا البحث طريقه لتكامل الفيض على كامل الخلية "تكامل بالنسبة للحجم" وذلك في نظرية جلركين غير المتصلة الحدود. إن عملية تحويل التكامل على الحدود للفيض إلى تكامل على كامل الخلية يتم باستخدام نظرية داي فرسن وفي بعض المصادر تسمي نظرية جاوس وبذلك تكون نظرية جلركين غير المتصلة الحدود خالية من التكاملات على حدود الخلية كما هو معروف من خلال نظرية جلركين. في حالة انسياب الموائع وبها موجات صادمة نستخدم طريقة بتروف جلركين المحورة لاستخدامها خلال نظرية جلركين غير المتصلة الحدود وذلك لكبح التذبذبات غير الفيزيائية خلال انسياب الموائع. وقام الباحثان بتطبيق طريقة تكامل الفيض على كامل الخلية على عدة معدلات تفاضلية أحادية البعد مثل معادلة بيرجر. إن نتائج هذا البحث كانت دقيقة جداً عند مقارنتها مع أعمال سابقة وكانت النتائج خالية من التذبذبات في الانسياب به موجات صادمة.

الكلمات المفتاحية: قوانين الحفظ العددية؛ طرق النظام العالي، جلركين غير المتصلة الحدود، نظرية التباعد.

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Effect of the Seasons and Salt Concentrations on Microbial Load of Wet-Salted Fermented Product (Fassiekh)

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Abstract: The study aims at investigating the microbial load of wet-salted fermented product (a traditional fermented product in Sudan named locally; fassiekh) and determination of total viable bacterial count, according to salt concentrations (20%, 25% and 30% of the fish weight) and seasons (summer, autumn and winter) variations. Fessiekh processed from popular fessiekh fish species (*Hydrocynus* spp, local name: Kass).The isolation and identification of bacteria and mould were examined for the microbial quality of fassiekh. The total viable count of bacteria in fresh fish used as raw materials in fessiekh preparation (*Hydrocynus froskalii*), ranged from $2x10^3$-$5.5x10^3$cfu/g. The concentrations of salt had direct effect on the microbial quality of salted *Hydrocynus froskalii*. It was observed that the total bacterial count was increased during the first five days in summer and autumn while at winter season it showed increases till the tenth day, followed by remarkable decreasing. The counts began to decrease as salting proceeded. Five *Staphylococcus spp* (*Staph.aureus*, *Staph.rostri*, *Staph.lentus*, *Staph.epidermidis* and *Staph.pyogen*) were isolated from all samples of salted fish and appraises about 46.77% of total isolates. Also three species of *Micrococcus* were isolated (*Micro.leuteus*, *Micro.roseus* and *Micro.lactis*), and *Aerococcus viridans*, and they represented 36.17% & 12.9% of all isolated samples respectively. The viable bacteria counts of commercial fessiekh were significantly higher ($p<0.05$) when compared to the experimentally salted fish at the same salt concentrations. No yeasts or fungi were detected in tested samples.

Key words: Salt concentration, Seasons, Microbiological analysis, Fessiekh.

INTRODUCTION

Food safety is everybody's concern, and it is difficult to find anyone who has not encountered an unpleasant moment of food borne illness at least once in his lifespan. Food borne illnesses may result from the consumption of food contaminated by microbial pathogens, toxic chemicals or radioactive materials (Macachor, 2016). Fish are highly perishable food items as they start to spoil as soon as they are harvested. So, processing and storage methods are vital factors in fish consumption. During transportation from point to markets, there is a great chance for the fish to be contaminated by bacteria (Clucas and Ward 1996). Preservation of fish by salt is an old age technology. This method of preservation still has popularity in many developing countries due to its simplicity and low cost of processing (Takagi *et al,*. 1984).Sudan has a number of large water reservoirs, which contains a huge wealth of fish of several types, and the estimated wealth was about 110 thousand tons. The main sources of fish in Sudan are the Blue Nile, White Nile, River Nile, lake reservoirs behind dams and irrigation

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canals, as well as the Red Sea. In Sudan, nearly 70% of the total fish landings are consumed in forms of fresh fish. The rest is cured either by salting, fermentation or sun-drying. The process of fish salting and fermentation is termed locally as "Fessiekh" making. Fessiekh is a wet salted product, soft in texture with a strong pungent smell and a shiny silvery appearance. It can be stored for more than three months (FAO, 1992).Fessiekh is not a truly indigenous Sudanese food product but it is the major fermented product from fish in Sudan. This product has an immense popularity in Egypt and is also familiar with other regions of the Middle East, (Makie *et al*., 1971). It is consumed after cooking together with additives such as peanut paste and some spices, which made it more acceptable and delicious. According to (Osman *et al.,* 2012), the process of fassiekh making is about a century old, introduced to Sudan from Egypt during the Turko-Egyptian rule (1821-1885), and then transferred and established traditionally within families or through non-formal training(Sulieman and Khamis 2011).

The fermented fassiekh, normally produced in houses or in small-scale sectors by covering fish with salt in alternate layers for up to 7 days or less, depending on temperature, then transferred to be fermented with additional salt and left to fermentation up to 10 days (Dirar 1993, Anihouvi *et al.,* 2012, El Hag *et al.,* 2012) reported that the prevalence of traditional preservation methods employed throughout Sudan are defective and need efforts pertaining to their improvement and development. Therefore, the objective of the present study was to examine the microbial load present in salted fassiekh in order to evaluate the hygienic practices of fassiekh processing.

MATERIALS AND METHODS

Collection of samples

Samples of fresh fish namely Kass (*Hydrocynus forskalii*), were brought from local fish markets and weighed about 71kg. These samples were kept in polyethylene bags with crushed ice and transported to the Fisheries Research Center, where microbiological investigations were immediately carried out.

Processing

Fresh fishes were washed, eviscerated, washed again and transferred to baskets to dry up while covered by a thin cloth to prevent insect's invasions. Fish were weighed to the nearest gram using a dial balance (KRUPS type 875) and for the purpose of salting, divided into three equal groups. The first group was subjected to a total weight of salt amounting to 20% of the fish weight, the second to 25% and the third to 30%. The procedure used is called dry-salting. In this method, salt was applied by hand and brushing off the fish surface, the inner lining of eviscerated abdominal cavity and the gills chambers. This process was conducted by separating the fish layers by coarse salt mattresses inside a plastic container. When the salt penetrates the flesh, it extracts the fluids through plasmolysis. The extracted fluid (pickle) was allowed to drain continuously. Used salt is removed from the fish surfaces and the fish restocked with new dry salt between the layers once during the ripening process. During processing of fessiekh in the laboratory, sampling was carried out every five days for 7 times (about a month), the first sample took place after the treated fish became as a fessiekh product.

The steps applied above were repeated three times according to the seasons of the year: summer, autumn, and winter (average air temperatures 37, 30, 27^{o}C respectively). Commercial fessiekh samples (used for comparison) were obtained from Central Vegetables and Fruit market, south of Khartoum.

Microbiological examination

Appropriate serial dilution was made by using a desired amount of samples (20g) and transferred to a sterile bottle containing 180 ml of Peptone water (0.1% w/v) to give 10^{-1} dilution, then 1ml from the bottle was transferred to a tube containing 9 ml of Peptone water to give 10^{-6} dilutions; then further dilutions were made in a similar manner. A total viable count was enumerated by pouring plate method using Plate

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Count Agar (PCA) at (37 ± 1° C, 48 h) and Mannitol Salt Agar (MSA) at (37 ± 1° C 36 - 48h) was used as a selective and differential characteristic medium for identification of *Staphylococcus* and *Micrococcus* spp. as described in (Harrigan 1998). Pure colonies of staphylococci isolates were differentiated by conducting coagulase test as well as biochemical tests such as Urea test, Voges–Proskauer (VP) test, and Sugar fermentation as described in(Barrow GHandFeltham 1993, Harrigan 1998). Potato dextrose agar was used for counting mold and yeast (22±1°C, 5 days).

Statistical analysis

Data obtained were analyzed as a completely randomized design and the means were compared by T- tests described by SPSS software (Version 13), with 0.05 level of significance.

RESULTS AND DISCUSSION

The total viable count of bacteria in fresh fish used as raw material in Fessiekh preparation (*Hydrocynus froskalii*) was presented in (Table 1). From the results, the total viable count of bacteria in fresh fish (whole) ranged between $3x10^3$ -$5.5x10^3$cfu/g. The number of bacterial counts could be explained on the basis of contamination of fish during catching, handling, transportation, and exposure to the surrounding environment. It could be noticed that the total bacterial plate counts of the samples from gills, viscera and whole fish were slightly different. (Shewan 1977) and Gram, (1989) noted that the bacterial flora on newly caught fish depends on the environment in which it was caught rather than on the fish species. Among fish parts (Table 1), the viscera contained the highest bacterial counts. The numbers of microorganisms in the gastrointestinal tract of fish were far higher than in the surrounding water. This indicated the presence of a favorable ecological niche for the microorganisms (FAO, 1995).

Table (1): Total Viable Bacterial counts (cfu/g) of fresh *Hydrocynus forskalii*

Seasons	Whole	Gill	Viscera
Summer	3.5×10^3	2×10^3	4×10^3
Autumn	5.5×10^3	2×10^3	8.5×10^3
Winter	3.5×10^3	2.5×10^3	4.5×10^3

On the contrary, authors believed that the microflora of the gastrointestinal tract was merely a reflection of the environment and the food intake. Liston, (1980) stated that the total number of organisms fall in the range of 10^2-10^7cfu/cm^2 on the skin surface. Microbial contents of species after salting with different concentrations varied with time (Table 2).

Table (2): Total Viable Bacterial counts (cfu/g) of salted samples during different seasons

Salt concentration	20%			25%			30%		
Seasons	Summer	Autumn	Winter	Summer	Autumn	Winter	Summer	Autumn	Winter
0 day	2×10^3	4×10^3	12.5×10^3	5×10^3	2×10^3	7.5×10^3	3×10^3	1×10^3	8.5×10^3
5	1.5×10^3	4.5×10^3	15×10^3	3.5×10^3	3×10^3	12.5×10^3	2.5×10^3	2×10^3	7.5×10^3
10	1×10^3	4.5×10^3	14.5×10^3	1×10^3	1×10^3	10×10^3	0.5×10^3	1×10^3	7.5×10^3
15	4.5×10^3	2.5×10^3	14×10^3	3.5×10^3	1×10^3	5×10^3	4.5×10^3	0.5×10^3	4.5×10^3
20	3.5×10^3	1×10^3	10×10^3	1.5×10^3	2×10^3	5×10^3	1.5×10^3	0.5×10^3	2.5×10^3
25	1.5×10^3	0.5×10^3	3.5×10^3	0.5×10^3	2×10^3	3.5×10^3	0.5×10^3	0.5×10^3	1.5×10^3
30	0.5×10^3	0.5×10^3	2×10^3	0.5×10^3	1×10^3	1.5×10^3	0.5×10^3	0.5×10^3	1.5×10^3

There was an increase in the case of total viable bacterial counts during the first five days (average temperatures 30°C, and 37°C) and ten days at temperatures 27°C. (Tsai *et al.,* 2005), found that

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total viable bacterial count of mackerel fish salted and sold in Taiwan markets ranged between $4.3x10^2$ and $5x10^5$cfu/g. On the other hand, (Gun *et al*., 1996) reported that bacterial counts were $4.7x10^3$cfu/g and $5x10^5$cfu/g on the beginning and the end of storage period respectively, at storage period of trout preserved by salt for 9 weeks. The findings of this study are confirming the findings of (Gun *et al*., 1996; (Tsai *et al.,* 2005).

The decreasing of the total viable bacterial counts with time could be explained on the basis that in a short processing period as that in the case of Fessiekh, it is hard to believe that the substrate for microbial growth comes from the degradation of the protein. It is more likely the microbial growth occurs as a result of attacking proteinaceous and other soluble nitrogenous compounds contained in the fish juice. This is substantiated by the observation that the growth occurs during the zero and ten days after the salted fish became a product, and then the counts drop steadily, this occurs with salt penetration inside the muscle and that sodium chloride has been used as a preservative for a long time. Also, the early increase occurred while fish were wet and the provision of salt promoted the growth of halotolerant and halophilic bacteria in fish. As the fish became drier, there was a decrease in water activity and this together with the accumulated salt in the flesh resulted in suppression of bacterial growth. Birch *et al*., (1986) reported that when moisture content or water activity was lowered, the amount of water available for supporting microbial growth was reduced. The primary objectives of high levels of salt used in fish fermentation are to select the halophilic organisms, which will affect the degradative process on the organic compounds in the fish muscle to bring about the desired flavors in the product (FAO, 1992). Similarity, (Dirar 1993) mentioned that the salt and the mats used in the fessiekh fermentation process might contribute important halotolerant strains. (Eltom 1989) reported that after the addition of the salt in Fessiekh fermentation, the viable bacterial count rose to 1.8×10^8cell/g on the fourth day followed by decreasing to 8.6×10^5 cell/g on day twelve. This pattern of rising and fall in the microbial count was observed during fish fermentation by different researchers (Hamed *et al*., 1973;(Ahmed *et al.,* 2010, El Hag *et al.,* 2012).The bacterial genera isolated from salted fish (*Hydrocynus froskalii*) were *Micrococcus* spp (38.17) and *Aerococcus* spp (12.9%) while *Staphylococcus* spp was found in all samples of salted fish with a record about (46.77%) and *Staphylococcus aureus* was the most dominant species isolated as shown in (Table 3).

Table (3): Bacterial groups isolated from salted samples

Days	Microorganisms
0	*Staphylococcus aureus+ Staphylococcus pyogenes+ Staphylococcus rostri*
5	*Staphylococcus aureus+Staphylococcus lentus+Micrococcus roseus+ Micrococcusleuteus*
10	*Staphylococcus aureus+ Staphylococcus pyogenes + Micrococcus roseus+ Micrococcus leutus*
15	*Staphylococcus aureus+Micrococcus leutus +Aerococcusviridans Staphylococcus epidermidis*
20	*Staphylococcus aureus + Aerococcusviridasn*
25	*Staphylococcus aureus+ Micrococcus roseus+Micrococcus leutus*
30	*Staphylococcus aureus+Micrococcus leuteus+ Micrococcus lactis*

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The obtained result was in disagreement with (El Hag *et al.,* 2012) who found that *Staphylococcus xylosus* species were the dominant bacteria

isolated from salted Kawara fish (*Alestes* spp) during storage. (Goja 1993) reported that *Staphylococcus saccharolyticus* was predominant species of *Staphylococci* isolated from the salted fassiekh produced in Ed Dueim city. *Staphylococcus* spp can reach high levels ($>10^5$cfu/g) in products prepared with hands under bad conditions and can cause food poisoning (Varnam & Evans, 1991). Also, (Vishwanath *et al.,* 1998) reported that *Staphylococcus aureus* grew well in salted food and in low water activity. Although (Hernandez-Herrero *et al.,* 1999) reported that, *Staphylococcus aureus* was not identified as an indigenous flora of fish culture and in fish hunting from clean water. (Eltom 1989) found that the most commonly encountered bacterial genera in Fessiekh fermentation were *Bacillus*, *Staphylococcus* and *Micrococcus*. *Bacillus* and *Micrococcus* have also been reported to be present in fermented fish in many countries of South East Asia (Saisithi *et al.,* 1966, Goja 1993, Ahmed *et al.,* 2010) Mackie *et al*., 1971; Hassan *et al*., 1972; Goja, 1993; Ahmed *et al.* , 2010; Goja, 2013)

The viable bacteria counts of commercial Fessiekh presented in (Table 4), were significantly higher ($p<0.05$) when compared to the laboratory prepared samples at different salt concentrations (Table 5).

Table (4): Total viable bacterial counts (cfu/g) and commonly isolated bacteria from commercial fessiekh

Days	Viable bacterial counts (cfu/g)	Dominant microorganisms
0	$3x10^4$	*Micrococcus luteus*
5	$21x10^3$	*Micrococcus luteus*
10	$21x10^3$	*Micrococcus luteus*
15	$20x10^3$	*Staphylococcus aureus*
20	$15x10^3$	*Micrococcus luteus*
25	$12x10^3$	*Staphylococcus aureus*
30	$12x10^3$	*Micrococcus luteus*

Table (5): Comparison between viable bacterial counts (cfu/g) of commercial and experimental fessiekh at different salt concentrations during the study.

Salted fish (fessiekh)	Mean of viable count	T- Test	Sign
Hydrocynusspp 20% salt	2714.29	-3.111	
Commercial fessiekh	13285.71		**
Hydrocynusspp 25% salt	1428.57	-3.550	
Commercial fessiekh	13285.71		**
Hydrocynusspp 30% salt	785.71	-3.741	
Commercial fessiekh	13285.71		**

** High significant differences

According to (Sugumar *et al.,* 2004)unhygienic handling is one of the main factors contributing to poor quality of fish in the retails; this could be due to the quality of fish used for Fessiekh, and the amount of salts added and the techniques used in commercial production. No yeast or mould was detected in our fresh and salted samples.

CONCLUSION

From the results, it may be concluded that the total viable bacterial counts of *Hydrocynus froskalii* after salting were decreased due to the course of salting. It was seen that the main factors affecting fessiekh quality are related to the amount of salt used for the process. The final

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quality can be largely attributed to the effect of various conditions upon the fermenting agents and activities. Commercial fessiekh samples contained higher microbial load than experimentally prepared products. Therefore, strict control measures are recommended to be applied for producers, and a good guidance should be provided for household producers.

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تأثير المواسم وتركيزات الملح على الحمولة الميكروبية للفسيخ

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المستخلص : تهدف الدراسة إلى التعرف على الميكروبات الموجودة في السمك المملح والمخمر والذي يعرف محليا باسم الفسيخ، فقد تم تحديد العدد الكلي للبكتريا الموجودة في الأسماك المملحة بتراكيز مختلفة (20٪، 25٪، 30٪ من وزن الأسماك) وذلك خلال المواسم المختلفة (الصيف، الخريف والشتاء)، وتم إعداد الفسيخ لنوع من الأسماك والمعروف محليا في السودان باسم الكاس (*Hydrocynus froskalii*) تم عزل وتعريف البكتيريا وكذلك فحص خميرة العفن في الفسيخ لمعرفة نوعية المحتوى الميكروبي في الفسيخ . أظهرت النتائج أن إجمالي عدد البكتيريا الحيوي في الأسماك الطازجة (*Hydrocynus froskalii*) المستخدمة كمادة خام في إعداد الفسيخ. تراوحت من $2x10^3$–$5.5x10^3$ ثم تغير المحتوى الميكروبي لسمكة *Hydrocynus froskalii* بعد التمليح بتراكيزه المختلفة وذلك خلال فترة التمليح والمواسم. وكانت هناك زيادة ملحوظة في إجمالي العدد الحيوي البكتيري خلال الأيام الخمسة الأولى في الصيف والخريف والعشرة أيام الأولى في الشتاء. بدأ العد البكتيري في الانخفاض كلما تقدمت عملية التمليح. و عُزلت خمسة أنواع من جنس *Staphylococcus* spp (*Staph. aureus*, *Staph. rostri*, *Staph.lentus*, *Staph.epidermidis* & *Staph.pyogen*) والتي عزلت من جميع عينات الأسماك المملحة حيث بلغت حوالي (46.77٪)، وعُزلت أيضاً ثلاثة أنواع من جنس *Micrococcus* spp (*Micro.lactis* و *Micro.roseus*, *Micro .leuteus*) وصلت إلى (38.17٪) في حين وصلت بكتيريا *Aerococcus* spp إلى (12.9٪). العدد الحيوي البكتيري للفسيخ التجاري كان عالياً ($p<0.05$) بالمقارنة مع فسيخ التجربة. لم يُعزل أي نوع من الفطريات سواء من فسيخ التجربة أو التجاري.

الكلمات المفتاحية: تركيز الملح، الموسم ، التحليل الميكروبيولوجي، الفسيخ.

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Seroprevalence of Brucellosis in Small Ruminants Assayed by The Rose Bengal Test, Al-Jabal Al-Akhdar Libya

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Abstract: This study aimed at determining the prevalence of anti- *Brucella* antibodies in small ruminants in Al- Jabal Al- Akhdar area, Libya. Nine regions were selected for the investigation (Al- Goba, Al- Wasata, Side Kahled, Lamloda, Al-Hesha, Marawa, Al-Gagab, Gandola and Ain Mara). Seroprevalence was assayed using the Rose – Bengal Plate Test (RBPT). Four hundred blood samples were collected randomly from 247 sheep and 153 goats with a history of abortion and reproductive disorders, during the period from January 2015 to June 2016. Approximately 10 ml blood sample was taken from each animal, in vacutainers. Serum samples were separated and subjected to examination by the RBPT. Samples showing visible agglutination within 4 mins. were regarded as positive for anti- Brucella antibodies. Data were analyzed statistically by the Chi- square test using the SpSS software, at $p \leq 0.05$ level of significance. Out of the 400 ovine and caprine sera tested, 125 (38%) were positive for anti – *Brucella* antibodies by the RBPT (Table 3). The rate of seropositivity was higher in goats (69.3%) than in sheep (18.6%) (Table 2). There were variations in seroreactivity from different regions. For instance, sera from Al- Hesha and Gandola exhibited 100% positivity, whereas those from both species in Al- Gagab were remarkably sero-negative (0%) (Table 3). Striking differences were shown by the sera from Gandola and Ain- Mara. Where all the caprine sera from Gandola were positive for anti – *Brucella* antibodies, all the 18 sera from Ain- Mara were serologically negative. Serum reactivity from both goats and sheep in other regions ranged between 60 and 83.3% in goats and 11.5 and 23.3% in sheep (Table 3). It can be concluded that the prevalence of anti- *Brucella* antibodies is high in small ruminants of Al- Jabal Al- Akhdar, Libya and may indicate a possible existence of Brucellosis in goats and sheep.

Keywords: Rose Bengal test, Small ruminants, brucellosis.

INTRODUCTION

Brucella is a Gram - negative facultative intracellular organism responsible for a variety of disease conditions and has a zoonotic significance. Brucellosis is caused by bacteria of the genus *Brucella* and is reported worldwide causing abortion, infertility, retained placenta, endometritis in females and to a smaller extent, orchitis, and infection of the accessory sex glands in males (Mustafa *et al.,* 2011). Ten species are recognized within the genus *Brucella*. There are six 'classical' species: *B. abortus, B. melitensis, B.*

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suis, B. ovis, B. canis and *B. neotomae* and other four species have been recognized more recently (Atluri V.L. *et al.,* 2011). Brucellosis is a worldwide re-emerging zoonosis that causes severe disease in humans, with non-specific clinical signs affecting numerous organs (Seleem *et al.,* 2010).

Contact with infected animals, ingestion of contaminated animal products and handling of Brucella isolates in laboratories are risk factors. Brucellosis in livestock and humans is still common in the Middle East, Asia, Africa, South and Central America, the Mediterranean Basin and the Caribbean. *B. melitensis* is particularly common in the Mediterranean basin, and it has also been reported from Africa, India, and Mexico (Kassahun *et al.,* 2010). Ovine brucellosis can be divided into classical brucellosis and ram epididymitis. Ram epididymitis is caused by non-zoonotic agent *B. ovis*, while classical brucellosis is caused by *B. melitensis* and constitutes a major public health threat equal to caprine brucellosis (Acha and Szyfres 2003). About 500,000 new human cases of brucellosis are reported annually worldwide making it the most common zoonosis (Seleem *et al.,* 2010). Status of the disease in small ruminants in a country can be known only through effective sero-monitoring using serological tests and random sampling methods for the disease. The economic importance of brucellosis in sheep and goats requires the use of sensitive and rapid diagnostic methods.

Diagnosis of *B. ovis* and *B. melintensis* infection is based on clinical examination, serological tests, biotechnological techniques, and cultural isolation (Webb *et al.,* 1980). The laboratory isolation and identification of *Brucella* organisms are the most reliable methods of diagnosis but are not always successful. And they are not practicable in terms of time and labor for field and laboratory personnel when large numbers of animals are involved and also cumbersome and pose a great risk to the laboratory personnel. The biotechnological procedures require trained persons and the establishment of advanced laboratories. Consumption of unpasteurized milk and milk products from cows, small ruminants or camels is considered to be the main route of infection as well as an occupational hazard (Almuneef *et al.,* 2004). In the North African region, as in sub-Saharan countries, social and economic factors play a major role in the spread of brucellosis (Makita K *et al.,* 2008). Brucellosis is considered to be endemic in Libya (Pappas *et al.,* 2006), although little information is available; previous studies are limited to food-producing animals such as cattle and goats (Gameel *et al.,* 1993) and reports of human brucellosis in Libya are limited to a few cases (Tiller *et al.,* 2009).

MATERIALS AND METHODS

Study area

A total of 400 blood samples were randomly collected from nine different regions of Libya (Al-Goba, Al- Wasata, Side Kahled, Lamloda, Al-Hesha, Marawa, Al-Gagab, Gandola and Ain Mara); 247 samples from sheep and 153 samples from goats (Table 1). The samples were collected during the period from January 2015 to June 2016.

Serum sample collection and submission

Approximately 10 ml of blood was collected from each animal using a Vacutainer and needle. The sample containers were tilted horizontally, overnight at room temperature to allow clotting. Serum from each animal was decanted into a single sterile cryogenic vial, labeled and transported to the laboratory of clinical pathology, Omer Almukter University, for investigation. The sera were stored at −20°C until tested.

Samples

A total of 400 serum samples of small ruminants comprising 247 from sheep and 153 from goats (Table 1), having the history of abortion and reproductive disorders like endometritis, retention of placenta, infertility and repeat breeding, were randomly collected from nine different locations. All the serum samples were tested for the presence of Anti-*Brucella* antibodies by using the serological test Rose Bengal Plate Test (RBPT).

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Table (1): Samples distribution from different regions in Al- Jabal Al- Akhdar , Libya.

Animal species	No. samples	Regions								
		Al- Goba	Al- Wasata	Sidi Khaled	Lamluda	Al-Heisha	Mrawh	Al-Gagab	Qandula	Ain Mara
Sheep	247	139	23	0	30	6	26	5	0	18
Goats	153	45	30	10	9	6	20	0	8	25
Total	400	184	53	10	39	12	46	5	8	43

RBPTprotoc

The RBPT (Cromatest, Spain) was performed according to the procedure described by Alton *et al.,* .(1988). To perform the test, antigen and serum were thawed and then brought to room temperature. The bottle containing antigen was shaken well to ensure homogenous suspension. Then, one drop (0.03 ml) of serum sample and one drop of antigen were put on the same slide using different micropipettes and mixed thoroughly using a spreader. The slide was rotated for 4 min. and observed immediately. Then after further 4 min. for results, a result was considered positive when there was noticeable agglutination after 4 min.

Data analysis

All data were analysed by Chi–square test, using the SPSS statistical software. All statistical tests were conducted at $p < 0.05$ level of significance.

RESULTS

The sero-prevalence of brucellosis in small ruminants is summarized in (Table 2). A total of 400 serum samples (from 247 sheep and 153 goats) were collected and tested. Of the 400 ovine and caprine sera tested, 152 (38%) were positive for Anti-*Brucella* antibodies by RBPT. Rates of seropositivity were higher in goats (69.3 %) than in sheep (18.6%) (Figure 1). Consequently, the incidence rate of brucellosis based on RBPT showed a high percentage of positive reactors in the overall prevalence of *Brucella* seropositivity among goats.

Table (2): Prevalence of Anti-*Brucella* antibodies in small ruminants species assayed by the Rose Bengal test, Libya.

Animal species	Animals tested	Seropositive animals	Proportion of posit animals
Goats	153	106	69.3 %
Sheep	247	46	18.6 %
Total	400	152	38%

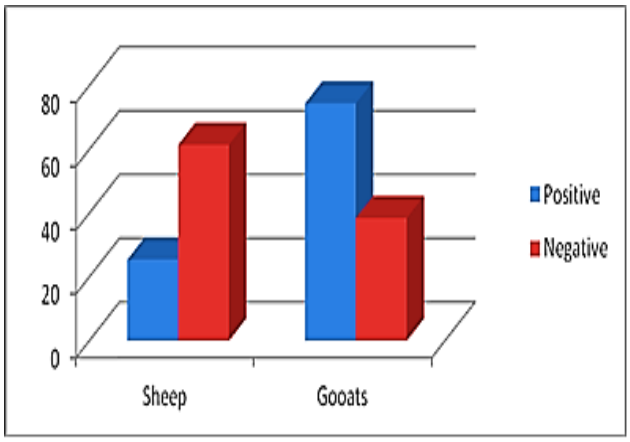

Figure (1): Prevalence of Anti-Brucella antibodies in small ruminants species assayed by the Rose Bengal test, Libya.

Test sera from Al-Hesha and Gandola municipalities showed the highest seropositivity (100%), whereas test sera from both species in Al-Gagab exhibited remarkable seronegativities by the RBPT (0%). It is worth mentioning that only 5 ovine samples from Al- Gagab were collected and investigated. Striking differences were exhibited by the caprine sera from Gandola and Ain- Mara. Where the caprine samples from Gandola showed 100% seropositivity, all the 18 ovine sera from Ain-Mara were serologically negative compared to 52% positivity by the 25 caprine samples from

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the same region. It is also noticeable that the 6 caprine serum samples from Al-Hesha were 100% positivity whereas the 6 ovine samples from the same area gave 50% seropositivity. Serum reactivity from both goats and sheep from other regions ranged between 60 - 83.3% in goats and 11.5 - 23.3% in sheep (Table 3) (Figure 2).

Table (3): The incidence rate of Brucellosis among small ruminants at different regions in Al- Jabal Al- Akhdar , Libya.

Region	Number of samples		Positivity of samp		Animals Species					
					Goats (153)			Sheep (247)		
	No	%	No	%	No	Pos	%	No	Pos	%
Al- Goba	184	46%	56	30.4%	45	28	62.2%	139	28	20.1%
Al- Wasata	53	13.3%	30	56.6%	30	25	83.3%	23	5	21.7%
Side Kahled	10	2.5%	7	70%	10	7	70%	0	0	0%
Lamloda	39	9.6%	14	35.9%	9	7	77.8%	30	7	23.3%
Al-Hesha	12	3%	9	75%	6	6	100%	6	3	50%
Marawa	46	11.5%	15	32.6%	20	12	60%	26	3	11.5%
Al-Gagab	5	1.3%	0	0%	0	0	0%	5	0	0%
Gandola	8	2%	8	100%	8	8	100%	0	0	0%
Ain Mara	43	10.8%	13	23.3%	25	13	52%	18	0	0%
Total	400	100%	152	38%	153	106	69.3%	247	46	18.6%

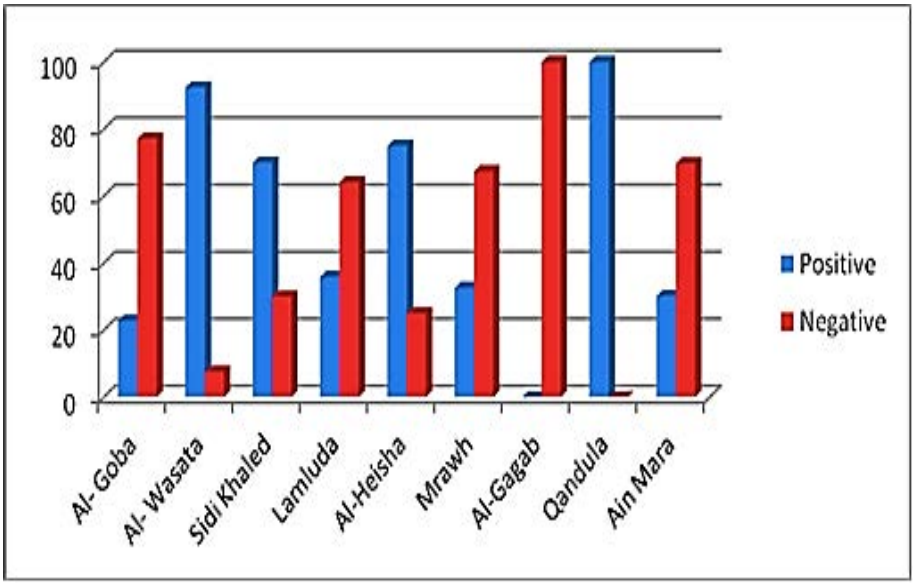

Figure (2): The incidence rate of Brucellosis among small ruminants at different regions in Al- Jabal Al- Akhdar , Libya.

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DISCUSSION

The prevalence of brucellosis observed in small ruminants in Al-Jabal Al-Akhdar in Libya was lower than most values reported in other African countries. This may be attributed to the low level of intensification, breed differences, flock size and composition, or the tests used to make the diagnosis. Brucellosis is a worldwide zoonotic disease that is recognized as a major cause of heavy economic losses to the livestock industry and poses a serious human health hazard (Ocholi *et al.,* 2005). In the present study, Table (2) shows the incidence of brucellosis among small ruminants in Al-Jabal Al-Akhdar in Libya by using RBPT. The incidences of brucellosis were 69.3 % and 18.6 % in goats and sheep respectively.

A local serological survey at the Al Jabal al Gharbi University in the western mountains region in 1997 found that 8.5% of sheep and 28.4% of goats were positive for brucellosis (Elarbi 1997). The obtained result was nearly similar to that recorded by Samaha *et al.,* .(2009) but lower than that reported by Ali and Mahdey.(2010), Holt *et al.,* .(2011) and DaSilva *et al.,* .(2014). A higher seroprevalence in goats than in sheep has also been described by other authors (Gargouri et al., 2009), Prevalence values between two- and fourfold higher in goats have been described in Eritrea (Omer *et al.,* 2000), East Morocco, Tunisia and Egypt (Benkirane 2006) and Nigeria (S.I.B. *et al.,* 2006), and between one and two-fold higher in Sudan, the United Arab Emirates (Benkirane 2006) and in Kenya (Ndarathi and Waghela 1991). In other countries, a higher prevalence has been detected in sheep. For example, Somalia (Andreani *et al.,* 1983), Jordan (Benkirane 2006) and Oman (Ismaily *et al.,* 1988). Programs and control measures have been undertaken in many countries in North Africa and the Near East (e.g. Egypt and Kuwait) (Samaha *et al.,* 2009). However, underreporting and under diagnosis of other food-borne pathogens are problems around the Mediterranean (Gargouri *et al.,* 2009), particularly in North African countries where communication with local authorities is problematic and most of the available information is unpublished or limited to seminars and workshops (Refai 2002).

Generally, goats are more susceptible to *Brucella* infection than sheep, and this could be partly due to the fact that sheep excrete the organism for shorter periods compared with goats. This may reduce the potential for spread of the disease within and between sheep flocks (Radostits *et al.,* 2000). The prevalence and severity of disease may vary with the breed, geographic location, type of diagnostic test, husbandry and environmental factors (Amin *et al.,* 2005). Another interesting result of our study is that individual seroprevalence was significantly higher in goats than in sheep. Our results are consistent with others reported by Coelhoa and Coelho.(2013) who found that goats are more susceptible to the infection than sheep. However, these results are in contrast with (Reviriego *et al.,* 2000). In addition, the results from this study indicate that Brucellosis is more prevalent in Gandola (100%) followed by Al-Hesha (75 %) than in other investigation districts (Table. 3).

The difference in infection rates between different districts in Al-Jabal Al-Akhdar governorate may be due to the difference in applied management in each area, failure, or absence of vaccination program in some herds. Differences between the prevalence of Brucellosis obtained in this study and those obtained by other authors may be attributed to various factors such as the season during which this study was performed, the area from which animals were examined, as well as the evolutionary changes in the animal husbandry which affect the rate of exposure and the different serological tests used confirmed by bacterial isolation.

CONCLUSION

Brucellosis is still a major disease of worldwide distribution. There are many factors involved in both human and animal brucellosis that make the control and eradication of this disease an

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important challenge. We conclude that in Al-Jabal Al-Akhdar in Libya, Brucellosis seroprevalence is high in small ruminants. Our data highlight the need for further researches, including the isolation and characterization of the causative agents, reliable epidemiological studies and the need to implement a transparency policy and effective control measures in Libya. Today, we have very powerful tools to fulfill the requirements: excellent serological methods, very effective immunogens and an overall knowledge of the pathogenesis of this disease.

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الإنتشار المصلي لمرض البروسيلا في المجترات الصغيرة بواسطة اختبار روز بينقال في منطقة الجبل الأخضر- ليبيا

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المستخلص : هدفت هذه الدراسة إلى استقصاء احتمال وجود الأجسام المضادة للبكتريا المسببة لمرض البروسيلا في المجترات الصغيرة في منطقة الجبل الأخضر – ليبيا. اختيرت تسعة مواقع لإجراء الدراسة، هي: (القبة، الوسيطة ، سيدي خالد ، لملودة ، الهيشة ، مراوة ، القيقب ، قندولة وعين مارة). استخدم إختبار روز بنقال بالطبق لإجراء الدراسة. جمعت أربعمائة عينة دم عشوائية من 247 رأس من الأغنام و 153 رأس من الماعز، لها تاريخ مرضي مثل الإجهاض وإضطرابات الجهاز التناسلي وذلك خلال الفترة من يناير 2015 حتي يونيو 2016. أخذت حوالي 10 مل من الدم من كل الحيوانات، وخضعت للإختبار بواسطة اختبار روز بنقال. اعتبرت عينة المصل التي أعطت تراصا (تلازنا) خلال 4 دقائق موجبة لوجود الأجسام المضادة لبكتريا البروسيلا. استخدم اختبار "Chi – Square" لتحليل البيانات. من مجموع 400 عينة تم اختبارها أظهرت 152 عينة بنسبة 38% نتائج موجبة مع ملاحظة أن معدل الإيجابية كان أعلى في أمصال الماعز (69.3%) عنها في أمصال الضأن (18.6%). اتضح أن هناك تبايناً في نسبة الإيجابية في الأمصال من مناطق الدراسة المختلفة. مثلا: أظهرت الأمصال من الهيشة وقندولة نسبة 100% إيجابية، وكانت التي جُمعت من القيقب سلبية بنسبة (0%). ظهرت تباينات لافتة للنظر في نتائج الأمصال من قندولة وعين مارة ففي حين أعطت كل الأمصال المعزية من قندولة تفاعلا موجبا يومئ بوجود الأجسام المضادة لبكتريا البروسيلا أعطت كل الأمصال الـ8 من عين مارة تفاعلاً سلبياً. تراوحت نسبة التفاعلية المصلية في كل من الماعز والضأن في المناطق الإخري من 60-83.3 % في أمصال الماعز إلى 11.5-23.3 % في أمصال الضأن. ويستنتج من هذه الدراسة أن هناك احتمالاً لوجود الأجسام المضادة للبكتريا البروسيلا بمعدلات عالية في المجترات الصغيرة في منطقة الجبل الأخضر _ ليبيا ، مما يشير إلى احتمال وجود مرض البروسيلا في الضأن والماعز.

الكلمات المفتاحية: اختبار الروزبنقال، المجترات الصغيرة، مرض البروسيلا (الإجهاض السري).

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Effects of *Glomus intraradices* on the drought resistance and growth of corn plant (*Zea mays* L.)

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Abstract: A pot experiment was carried out at the greenhouse of Faculty of Agriculture (Saba bacha), Alexandria University. The experiment was conducted to investigate the role of *Glomus intraradices* fungi in the effects on the growth and water requirement of pot-grown corn (*Zea mays* L.). Four water regimes (20%, 30%, 40% and 50% of the available soil water content) were conducted. The Arbuscular Mycorrhizal Fungi AMF inoculation could significantly increase plant growth (including plant height, leaf area, and fresh and dry mass), enhance relative leaf water content, transpiration rates and stomatal conductance, and improve plant drought tolerance. The water consumption of the mycorrhizal plants producing 1 g of dry matter was 20%–35% of water content conditions. These findings indicate that the mycorrhizae enhanced the water utilization efficiency**.**

Keywords: *G. intraradices*, corn plant (*Zea mays* L.), drought resistance.

INTRODUCTION

Glomus can be associated with the roots of most plants and can strongly impact water retention properties and the subsequent drought responses of its hosts. Previous work indicated that mycorrhizal associated citrus rootstocks exhibited higher root hydraulic conductivity than non-mycorrhizal plants, resulting in enhanced drought resistance ((Graham and Syvertsen 1984). The effects of The Arbuscular Mycorrhizal Fungi (AMF) on water absorption and utilization in apple, cherry and birch-leaf pear trees were studied under normal water status and drought-stressed conditions(Liu and Luo 1988, Liu 1989a, b, Wu *et al.,* 2006). Under normal water conditions, AMF enhanced the relative leaf water content and transpiration rates and decreased stomatal resistance in apple and cherry trees. Under drought conditions, AMF also enhanced stomatal conductance, transpiration rate and relative leaf water content, and decreased leaf water potential and the permanent wilting point. When mycorrhizal and non-mycorrhizal plants were watered under continuous drought conditions, the pressure inflation of mycorrhizal plants recovered fast and the plants exhibited an enhanced growth compared with the nonmycorrhizal plants(Liu and Luo 1988, Liu 1989a, b, Kaya *et al.,* 2003, Pinior *et al.,* 2005, Wu *et al.,* 2006). Plant growth Promoting rhizobacteria (PGPR) may also contribute to drought amelioration(Rubin *et al.,* 2017). Corn (*Z. mays* L) is an important crop and one of two sources for cereal flour used in the world for making bread. For example in Egypt, the average area of corn in 1999 was 1.648 million feddan which produced 5.438 million tons. Although Corn is relatively drought-resistant (ACSRT,1999), regular access to water sources ensures adequate corn production. Previous research indicated that AMF could improve drought resistance in different plants. In the current study, Corn seedlings were used to study the mechanism underlying drought tolerance and water retention in seedlings inoculated with AMF under various soil water content

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conditions. The findings here provided a rationale cause for applying mycorrhizae to cereal flour.

MATERIALS AND METHODS

The mycorrhizal inoculum, (*Glomus intraradiaces*) used as stock culture prepared from Faculty of Agriculture., Ain Shams University of Cairo. Seeds of corn (*Zea mays* L) were surface-sterilized for 3 min in 0.1% $HgCl_2$ solution, then washed three times in water and soaked for 24 h in water at 40°C. Seeds were subsequently germinated at 28°C in an incubator. Water tests were conducted in plastic test pots, each containing 3 kg of sandy loam and has the following general properties: pH, 8.00; organic matter, 2.34 g kg^{-1} ,the available nitrogen content was 45.22 mg kg^{-1}available P, 3.5 mg kg^{-1} soil. The procedures used for soil analysis were those described by(Page and Page 1982). In this soil, the maximum water holding capacity was 16%. All soil samples were steam-sterilized at 121°C for 2 h. Water control began after the seeds were sown. The eight treatment groups included inoculated or non-inoculated seeds maintained in soil with water content controlled at 20%, 30%, 40% or 50% of the available water. All experiments were conducted in a greenhouse in the specimen garden at Agriculture faculty (Saba bacha), Alexandria University.

The mycorrhizal treatments were carried out by adding 50g of inoculum per pot of these treatments which was placed below the seeds. Non Mycorrhiza treatments received the same quantity of autoclaved inoculums.(Khan *et al.*, 2003) Inoculum (50g) was consisted of external mycelium, spores and colonized roots mixed with soil. Five of corn seeds were sown in each pot. After the seeds sprouted, two plants were selected from each pot based on their growth level. During the experiment, the evaporated water per pot was measured daily by weighting pots, and the young seedlings were provided with a specific amount of water so that the water content in the soil was maintained at 20%, 30% 40% or 50% of the available water, respectively. After 60 days, the plants were harvested. Root samples for determination of root colonization with AM fungi were cleared with 10% KOH and stained with 0.05% trypan blue in lactophenol as described by (Phillips and Hayman 1970), and microscopically examined for AMF colonization by determining the percentage of root segments containing arbuscules and vesicles using a gridline intercept method(Giovannetti and Mosse 1980).

The plant height, the number of leaves, the fresh and dry weight of leaves, shoots, and roots per pot were measured. Leaf water saturation deficits were calculated by the saturation water content method. (Baker, 2010), transpiration rates and stomatal conductance were measured by a photosynthesis surveying instrument (Dutra *et al.,* 1996). The content of proline in leaves was measured with a Daojin UV-120 spectrometer (Pinior *et al.,* 2005). Water requirement (ml) was calculated through measuring the water consumption yielding 1 g of dry matter plants per pot.

Statistical analysis

Data were subjected to analysis of variance using the ANOVA procedures according to (Snedecor and Cochran 1972) Statistical significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

The AM fungi root colonization was noted in roots of corn, No colonization occurred in non-mycorrhizal corn seedlings. In the AM-inoculated plants, 96% of roots were colonized when the water content of the soil was at 50%of the available water. When the water content decreased, the percentage of AMF colonization likewise decreased. Specifically, the AM colonization of plants at 20% of the available water was less than that of plants at 30%, 40% and 50% of the available water (Table 1). The percentage of colonized seedlings remained above 76.8%. These results indicate that when corn seedlings are inoculated with *G. intraradiaces*, they are highly susceptible to colonization. These findings are consistent with the previous conclusion from field studies reporting a high

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natural occurrence of AMF mycorrhizal fungi in *Malus hupehensis* plant(Liu 1989a).

This colonization by AM fungi may explain, in part, the ability of corn plant to resist the damage of stressed conditions. The plant height, the number of leaves, and the fresh weight and dry weight of mycorrhizal-inoculated plants were greater than those of the non-mycorrhizal-inoculated plants grown under the same soil water content conditions (Tables 2 and 3). Thus, the mycorrhiza significantly promoted corn seedling growth. The growth of mycorrhizal plants grown at 20% of the available water did not differ significantly from the growth of non-mycorrhizal plants grown at 30%of the available water. When compared with the non-mycorrhizal plants, the dry weight of mycorrhiza plants increased by 78%, 100% 121% and 150% at soil water contents of 20%, 30%, 40% and 50%of the available water, respectively.

Table (1). Mean percentage of mycorrhizal colonization in corn seedlings under various treatment conditions

Inoculation	The available Water content of soil/%	Mycorrhizal colonization/%
Glomus intraradiaces	20%	76.8c
	30%	82.6b
	40%	95.2a
	50%	96.1a
non-inoculated	20%	0
	30%	0
	40%	0
	50%	0

Table (2). Effect of fungi on the growth of corn seedlings under various soil water contents

Inoculation	The available water content of soil/%	Plant height/cm	Numbers leaves
Glomus intraradiaces	20%	8.69d	10.20c
	30%	14.32c	13.65b
	40%	16.40b	14.12b
	50%	19.20a	16.84a
non-inoculated	20%	6.74e	6.43d
	30%	7.96d	7.87d
	40%	10.12f	9.89c
	50%	11.85f	10.34c

Mycorrhizal plants can significantly increase corn seedlings growth. When the soil water content was at 20% of available water, the mycorrhizal plant dry weight was 78% of the weight of non-mycorrhizal plants. Moreover, when the soil water content was 50% of available water, the mycorrhizal plant dry weight was 150% of the weight of non-mycorrhizal plants. This effect of mycorrhiza on the growth of corn seedlings is more effective than its impact on the growth of other plants such as peach and *Avena sativa*(Khan *et al.,* 2003). The fresh weight and the dry weight of mycorrhizal plants grown under the condition of 20% of available water were not significantly different from the weights of non-mycorrhizal plants grown in that of 30% and 40% water content.

Mycorrhizal corn seedlings required less water than the non-mycorrhizal plants to produce 1 g of dry matter. Specifically, when compared with the non-mycorrhizal plants, mycorrhizal plants required less water at 20%, 24%,28% and 35%of available water when grown in soil with a water content of 20%, 30% , 40% and 50%of available water , respectively (Fig. 1). These findings indicate that the mycorrhizae enhanced the water utilization efficiency. Leaf water saturation deficits in the mycorrhizal plants were lower than in the non-mycorrhizal plants grown under identical water content conditions.

Proline is an osmoregulatory key element in plants experiencing conditions of water stress. Under some

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conditions, various plants produce a large amount of proline to enhance osmosis and prevent dehydration. Thus, the quantity of proline produced can reflect the degree of water stress(Pinior *et al.,* 2005). Our results in table (4) shows that concentration of proline in leaves increased as the soil water content decreased. Furthermore, proline concentrations in the mycorrhizal plant leaves were significantly lower than in the non-mycorrohizal plant leaves. These results suggested that the quantity of proline in corn leaves increased as the water content of the soil decreased. When grown under the same water conditions, mycorrhizal plant leaves contained less proline than the non-mycorrhizal plant leaves. Thus, under the same degree of water deprivation, the mycorrhizal plants are less physiologically stressed than the non-mycorrhizal plants. Notably, when the soil water content was 40% or 50%of available water, the stomatal conductance in the mycorrhizal plants was significantly higher than that in the non-mycorrhizal plants. When the soil water content was at 20%of available water, the stomatal conductance in mycorrhizal plants was still higher than that in the non-mycorrhizal plants, but the difference did not reach significance. Furthermore, when compared with the non-mycorrhizal plants grown under the same water content conditions, the mycorrhizal plants exhibited higher transpiration rates (Table 4). Stress from dehydration can cause stomata close, and a decrease in stomatal conductance and transpiration rates in leaves, which may prevent the roots from absorbing and transporting water(Kaya *et al.,* 2003). This may eventually result in a decrease in the dry weight of plants. Our study indicated that mycorrhizae may improve stomatal conductance, transpiration rates in leaves and biomass, and significantly enhance drought tolerance in corn seedlings. Findings in the current study suggest three mechanisms by which mycorrhizae enhance drought tolerance in plants. First, hyphae can absorb soil water directly. Under drought-stressed conditions, hyphae can utilize the soil water that is not accessible by the roots. Thus, the water supply to the plant is improved and effectively enhancing drought tolerance. Second, hyphae can absorb nutrients including phosphorus, zinc and many other elements. In this way, plant nutrition can be improved, and plant growth can be increased likewise. Ultimately, the mycorrhizal plants have more roots capable of absorbing water. Finally, mycorrhizae can regulate the balance of internal hormones in plants to indirectly influence water metabolism in the affected plant (Murakami-Mizukami *et al.,* 1991, Dutra *et al.,* 1996, Lu *et al.,* 2007).

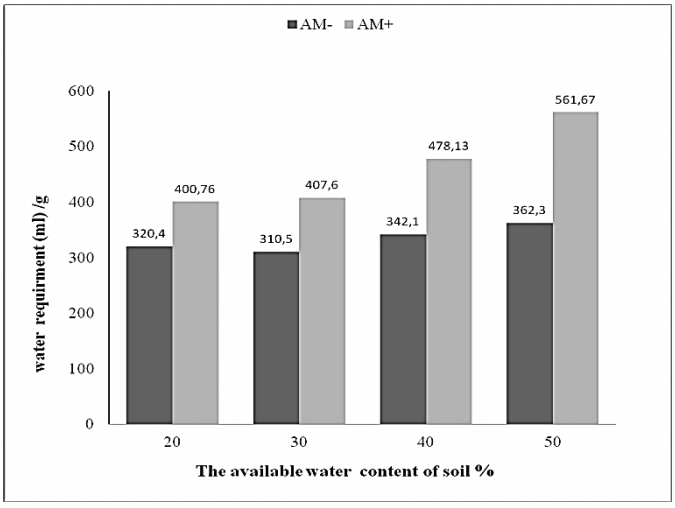

Fig. (1) The amount of water required to produce 1 g of dry matter in AM and non-AM corn seedlings grown in the soil water content of 20%, 30%,40% and 50%; respectively

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Table (3).Effect of AM fungi on the biomass of corn seedlings under various soil water contents ($gpot^{-1}$)

Inoculation	The available water content of soil/%	Leaf		Shoot		Root		Plant DW
		FW	DW	FW	DW	FW	DW	
Glomus intraradiaces	20%	4.19e	2.01d	2.31d	1.76d	6.22d	2.71e	6.48e
	30%	8.26c	3.46c	4.35c	3.05c	15.52c	7.00c	13.51c
	40%	12.01b	5.24b	5.92b	4.18b	26.40b	10.63b	20.05b
	50%	13.65a	6.54a	7.11a	5.42a	31.22a	14.20a	26.16a
non-inoculated	20%	2..31g	1.34e	1.65e	1.21d	2.56de	1.09f	3.64f
	30%	3.66f	1.76e	2.95d	1.87d	4.63d	3.12e	6.75e
	40%	4.22e	2.60d	3.01d	1.96d	6.15d	4.50d	9.06d
	50%	6.33d	3.31c	5.22c	3.06c	7.33d	4.11d	10.48d

Table (4). Effect of AM fungi on water physiology of corn seedlings

Inoculation	The available water content of soil/%	Leaf waterSaturation deficit/%	Concentration of proline in leaves/%	Stomatal conductance /(mmol.m^{-2} s^{-1})	Transpiration rates/(mmol · m^{-2} s^{-1})
Glomus. Intraradiaces	20%	13.06b	0.056b	25.23b	2.11b
	30%	10.13b	0.032c	46.86a	3.40a
	40%	7.39c	0.027c	50.72a	4.11a
	50%	5.67c	0.016cd	53.62a	4.62a
non-inoculated	20%	32.54a	0.320a	14.70b	1.68c
	30%	15.56b	0.082b	21.68b	2.32b
	40%	9.63b	0.067b	23.63b	2.67b
	50%	6.34c	0.046bc	26.12b	2.89b

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تأثيرات فطر *G. intraradices* على مقاومة الجفاف ونمو نبات الذرة (*Zea mays* L.)

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المستخلص : أجريت هذه التجربة فى صوبة زجاجية بكلية الزراعة (سابا باشا) جامعة الإسكندرية لاختبار فرضية دور فطر الميكوريزا *Glomus intraradices* في مقاومة الجفاف ونمو نبات الذرة، ولتحقيق ذلك تم تنمية نباتات الذرة في تربة ملقحة بفطر الميكوريزا وإضافة أربعة أنظمة من المحتوى المائي 20% و30% و40% و50% من الماء االمتاح، أوضحت النتائج أن عزلة فطريات الميكوريزا لها تأثيرات معنوية مختلفة على نمو نبات الذرة من حيث (ارتفاع النبات، مساحة الورقة، وزن النبات الطازج والجاف) بالإضافة إلى تحسين المحتوى المائي في الورقة، ومعدلات النتح وبالتالى قدرة النبات على مقاومة الجفاف، ولوحظ أن الاستهلاك المائي النباتات الملقحة بفطر *G.intraradices* قليل لإنتاج واحد جرام من المادة الجافة بنسبة 20%- 35% بالمقارنة مع النباتات غير الملقحة تحت نفس الظروف، ونستنتج من هذه الدراسة إن لفطر الميكوريزا آليات حماية النباتات من الجفاف.

الكلمات المفتاحية : *G. Intraradices* ، نبات الذرة (*Zea mays* L)، مقاومة الجفاف.

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دراسات فسيولوجية ونسيجية على تأثير الهيدروكورتيزون على الكلى في الأرانب البيضاء النيوزيلاندية

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المستخلص: استهدفت هذه الدراسة اختبار تأثير عقار الهيدروكورتيزون سكسينات الصوديوم على كل من بعض وظائف الكلى وكذلك على التركيب النسيجي للكلى في الأرانب النيوزيلاندية البيضاء Newzealand White Rabbits . شملت التجربة على 30 أرنب من الذكور بوزن يتراوح مابين 1500– 2500 جرام، وتم تقسيمها إلى 4 مجاميع ضابطة وأخرى معاملة بالدواء لفترات زمنية مختلفة؛ وذلك على حسب مدة الحقن. بعد معاملة الأرانب بعقار الهيدروكورتيزون بجرعة 10 مجم/كجم بشكل يومي تبين أن العقار لم يؤدى إلى تغير أوزان الأرانب المعاملة لمدة أسبوع. وحدثت زيادة غير معنوية في أوزان الأرانب المعاملة لمدة أسبوعين والمعاملة بالتدريج، ولكن المعاملة الفجائية سجلت انخفاضاً غير معنوي في أوزان الأرانب مقارنة بوزنها قبل الحقن. سجل ارتفاع معنوي في تركيز اليوريا، الكرياتينين، البروتين الكلي، الألبومين وأيونات الصوديوم والبوتاسيوم بعد المعاملة لمدة أسبوع، ولكن لوحظ انخفاض معنوي بسيط في تركيز اليوريا وعدم وجود فرق معنوي في تركيز الكرياتينين وأيونات الصوديوم والبوتاسيوم بعد المعاملة لمدة أسبوعين. في حين كانت هناك زيادة معنوية في البروتين الكلي والألبومين قد سجلت بعد أسبوعين من المعاملة. مع ملاحظة أن المعايير المدروسة في المعاملة الفجائية لم تتغير مقارنة بالمجموعة المعاملة لمدة أسبوعين ولكنها عادت إلى المعدلات الطبيعية بعد المعاملة التدريجية أظهر الفحص النسيجي للقشرة الكلوية ظهور مادة حامضية الاصطباغ داخل تجاويف بعض الأنيبيبات القاصية الملفوفة في المجموعة المعاملة لمدة أسبوعين. كما أظهر النخاع الكلوي في المجموعتين المعاملتين وجود نفس المادة داخل تجاويف بعض الأنيبيبات الجامعة. وبزيادة مدة المعاملة ظهرت فجوات في سيتوبلازم العديد من الخلايا المبطنة للأنيبيبات الجامعة. وتم مشاهدة نفس التغيرات النسيجية المرضية في كلى الأرانب التي تم إيقاف المعاملة فيها فجأةً، وكذلك المجموعة التي تم إيقاف معاملتها تدريجياً ولكن ظهرت هذه التغيرات بصورة أخف في المجموعة الأخيرة.

الكلمات المفتاحية: الهيدروكورتيزون ، الكلى ، فيسيولوجي ، نسيجي ، الأرانب.

المقدمة

إن كثرة استخدام العقاقير الاستيرويدية في علاج العديد من الأمراض الحادة والمزمنة، أصبح من الأمور الشائعة، وحيث إن معظم هذه الأمراض دائمة التكرار وتستوجب العلاج بشكل يومي ولفترات طويلة ومتكررة؛ لذا كان من الضروري التعرف على الآثار الجانبية لهذه العقاقير، ومن هنا تم اقتراح مشروع هذا البحث لدراسة تأثير هذه العقاقير على التركيب النسيجي للكلى ووظائفها الحيوية. وقد تم اختيار عقار هيدروكورتيزون سكسينات الصوديوم كمثال لهذه العقاقير. الجلوكوكورتيكويدات Glucocorticoids هي مجموعة هرمونات إستيرويدية أهمها هرمون الكورتيزول Cortisol وتؤثر أساساً على تمثيل الكربوهيدرات، ومن هنا جاءت التسمية (خليل، 2005). ووظائف الهيدروكورتيزون الرئيسة هي رفع مستوى الجلوكوز في الدم من خلال تخليق الجلوكوز Gluconeogenesis؛ وهو اصطناع الكبد

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للجلوكوز من مصادر غير سكرية مثل الأحماض الأمينية والأحماض الدهنية، والمساعدة في أيض البروتينات والكربوهيدرات (Lipworth 1999) ، كما تساعد هذه الهرمونات في تمكين الفرد من مقاومة الأنواع المختلفة من الضغوط Stresses والصدمات التي يتعرض لها (خليل، 2012). ويتم هدم الهرمونات الإسترويدية أساسا في الكبد ويحدث الهدم في الكلية وتفرز معظم النواتج في البول (خليل، 1997). تمكن الباحثون من تصنيع الاستيرويدات التي تتميز بفعاليتها العالية بالمقابلة مع الاستيرويدات الطبيعية. حيث إن لها ألفة كبيرة للمستقبلات الخاصة بالقشرانيات السكرية الموجودة في خلايا الجسم، وتبقى فترة أطول في الدم. ومن الهرمونات الاستيرويدية المصنعة الدكساميثازون والبريدونيسولون (محيي الدين وآخرون، 1990). والهيدروكورتيزون هو جلوكوكورتيكويد صناعي يستخدم على نطاق واسع لعلاج العديد من الأمراض (Elshennawy and Elwafa 2011). وقد أجريت بعض الدراسات للتحقق من شدة التأثيرات السلبية للهيدروكورتيزون؛ كمثال لعقار الجلوكوكورتيكويد الصناعي، على بعض أعضاء الجسم مثل البنكرياس (Gloor وآخرون، 2001)، الجوانب التناسلية لإناث الفئران (Piffer and Pereira 2004)،الغدة التيموسية(Rodrigues-Mascarenhas وآخرون، 2006)، الكبد (Gevorgyan وآخرون، 2008)، جهاز تحت المهاد – النخامي – قشرة الغدة الكظرية (Yarushkina 2008)، والحصين (Tata and Anderson 2010). يستخدم الجلوكوكورتيكويد على نطاق واسع في علاج أمراض الكلى، ومع ذلك فإن الأطباء أقل دراية بالتأثيرات الفسيولوجية لهرمون الكورتيزول على الكلى (Mangos وآخرون، 2003)، ونظراً لان المراجع المتاحة والدراسات السابقة على تأثير الهيدروكورتيزون على التركيب النسيجي للكلى قليلة جداً، لذلك فإن الدراسة الحالية تهدف إلى إلقاء الضوء على مثل هذه التأثيرات على أنسجة هذا العضو في ذكور الأرانب البالغة. تهدف هذه الدراسة إلى اختبار تأثير عقار هيدروكورتيزون سكسينات الصوديم على وظائف الكلى، ودراسة التأثيرات النسيجية المرضية التي قد تنتج من استخدام هذا العقار على الكلى.، بالإضافة إلى معرفة مدى ارتباط الأثر الضار لهذا العقار بطول الفترة الزمنية للمعاملة.

المواد وطرق البحث

حيوانات التجارب: استخدمت في هذه الدراسة الأرانب البيضاء Newzealand White Rabbits، التى تم تربيتها لفترة قبل بدء الدراسة لغرض التأقلم على الظروف البيئية الجديدة وضمان خلوها من أي أمراض.

العقار المستخدم: هيدروكورتيزون سكسينات الصوديوم Hydrocortisone sodium succinate هى مادة صلبة متجانسة بيضاء اللون عديمة الرائحة وشديدة الذوبان في الماء والكحول، ويوجد في أمبولات 100vials ملليجرام، 250 ملليجرام، 500 ملليجرام، 1000 ملليجرام (Ali وآخرون، 2000) وذلك عن طريق شركة سيجما للكيماويات Sigma Chemical Company.

تحديد الجرعة المستخدمة: تم حقن الأرانب بعد تحديد أوزانها بجرعة 10مج/كجم (Walker and Schnitzer 1980)، عن طريق الحقن العضلي ولفترات زمنية مختلفة؛ وهي جرعة تعادل الجرعة التي يتعاطاها انسان وزنه 70 كجم، وتم ذبح الحيوانات بعد كل فترة زمنية.

تصميم التجربة:أدخلت الأرانب التجربة بوزن يتراوح مابين 1500- 2500 جرام، وشملت هذه التجربة عدد 30 أرنباً، تم تقسيمها إلى مجاميع ضابطة وأخرى معاملة بالدواء لفترات زمنية مختلفة؛ وذلك على حسب مدة الحقن:

- المجموعة الأولى: تم استخدام 10 أرانب وقسمت إلى مجموعة ضابطة Control 5 أرانب وحقنت مرة واحدة يومياً بماء مخصص للحقن (ماء مقطر) Water for injection، ومجموعة معاملة 5 أرانب تم حقنها يومياً بالهيدروكورتيزون سكسينات الصوديوم حسب الوزن 10مجم / كجم وذبحت بعد7 أيام من بداية المعاملة.
- المجموعة الثانية: تم استخدام 10 أرانب وقسمت إلى مجموعة ضابطة Control 5 أرانب وحقنت يومياً بماء مخصص للحقن ، ومجموعة معاملة 5 أرانب حقنت يومياً

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بالهيدروكورتيزون سكسينات الصوديوم 10مجم/كجم وذبحت بعد 14 يوماً من بداية المعاملة.

– المجموعة الثالثة: تم استخدام 5 أرانب وتم حقنها يومياً بالهيدروكورتيزون سكسينات الصوديوم 10مجم/كجم لمدة 14 يوماً من بداية المعاملة ثم تركت 6 أيام بدون حقن ثم ذبحت.

– المجموعة الرابعة: تم استخدام 5 أرانب وتم حقنها يومياً بالهيدروكورتيزون سكسينات الصوديوم 10مجم/كجم لمدة 14 يوماً من بداية المعاملة ثم حقنت تدريجيا بجرعات تنازلية وهي 7½ مجم لمدة يومين ، ثم 5 مجم لمدة يومين ثم 2½ مجم لمدة يومين ثم الذبح.

الفحص الظاهري: بعد تحديد وزن الأرانب قبل وبعد عملية الحقن وملاحظة أي أعراض سريرية قد تظهر عليها طوال فترة التجربة تم ذبحها وتجميع الدم في أنابيب خاصة كما تم استخراج الكلى.

الفحص الفسيولوجي:

– **فحص اليوريا في الدم:** تم تقدير اليوريا باستخدام جهاز Beckman–BUN–Analyzer 2 وباستخدام مجموعة كواشف من شركة بيكمان الآيرلندية Beckman,Ireland ، حيث تمر اليوريا الموجودة في الم Urease التالي :–

$$Urea + H_2O \longrightarrow (NH_4)_2CO_3 \rightarrow NH_4 + HCO.$$

ونتيجة هذا التفاعل هو تحول اليوريا من صورة غير أيونية إلى صورة أيونية (كربونات الأمونيوم) ، وقد أخذت القراءة بالميللجرام لكل 100 مل من الدم .

– **الكرياتينين:** يتفاعل الكرياتينين في الظروف القاعدية مع Picric acid مكوناً معقداً أحمر، باستخدام جهاز Linear المصنع من قبل شركة Biosystems الإسبانية ومجموعة من الكواشف المجهزة من نفس الشركة، ويمكن قياس معدل تكوين هذا المعقد بزيادة شدة الامتصاص الضوئي عند طول موجي 405 نانوميتر ويتناسب طردياً مع تركيز الكرياتينين الموجود في العينة باستخدام ضابط Standard يحتوي على كرياتينين معلوم التركيز. يمكن قياس تركيز الكرياتينين في العينة بوحدة قياس ملليجرام لكل 100 مل من الدم.

$$\text{Creatinine + Picric acid} \xrightarrow[37^{o}C]{pH>12} \text{Red addition complex}$$

– **مستوى أيونات الصوديوم والبوتاسيوم:** باستخدام جهاز Beckman– Na+ & K+ – Analyzer 2 ومجموعة كواشف مجهزة من قبل شركة بيكمان الآيرلندية Beckman,Ireland، وقد تمت عملية القياس عند طول موجي قدره 545 نانوميتر وأخذت القراءة مباشرة من لوح العداد الرقمي بالمللي مول لكل لتر.

الفحص النسيجي: وفيه تم أخذ قطع من الكلى ووضعت فوراً في المثبتات النسيجية التالية: مثبت فورمالين 10% Formalin محلول بوان – Bouin's fluid محلول زنكر – Zenker's fluid محلول سوزا Susa fluid. تم تمرير العينات في المحاليل الكحولية التصاعدية ثم الترويق و التشفيف بالزايلين ثم طمرت العينات في شمع البرافين المنصهر وصبت في قوالب الشمع وتركت حتى تصلبت ثم قطعت بجهاز التقطيع الشمعي Microtome إلى شرائح رقيقة بسمك 4–6 ميكرون وثبتت على شرائح زجاجية ، وصبغت الشرائح بصبغة الهيماتو كسلين والايوسين Harries haematoxyline and eosin (H&E)، وقد تم حفظ وتمرير العينات وصبغها استناداً إلى(Bancroft and Gamble 2008). بعد الصبغ تمت تغطية الشرائح بغطاء زجاجي Cover slide بعد وضع قطرات من مادة كندا بلسم Canada balsam ثم تم فحص وتصوير الشرائح النسيجية المصبوغة بواسطة مجهر ضوئي (Olympus) مزودة بآلة تصوير نوع Olympus Camedia C-(7070).

التحليل الإحصائي: حللت النتائج إحصائياً باستخدام برنامج Minitab 13 وذلك عن طريق تحليل التباين الأحادي One way Analysis of Variance (ANOVA) عند مستوى احتمالية (0.05) وفقا لما ذكره (Ott,1984) .

النتائج

أولاً: الفحص الظاهري: بعد معاملة الأرانب بعقار الهيدروكورتيزون بجرعة 10 مجم/كجم بشكل يومي لمدة

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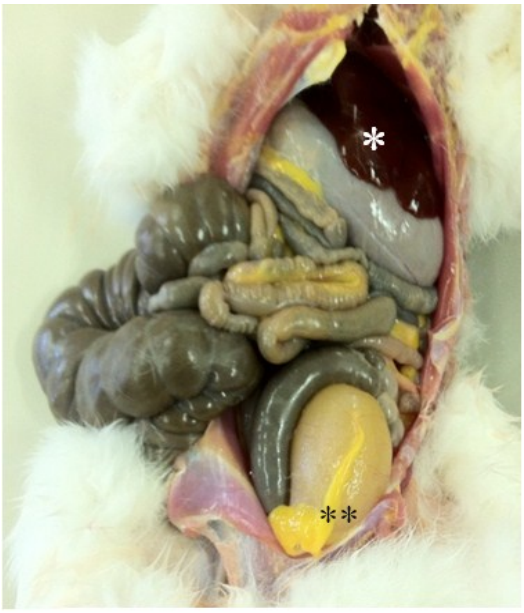
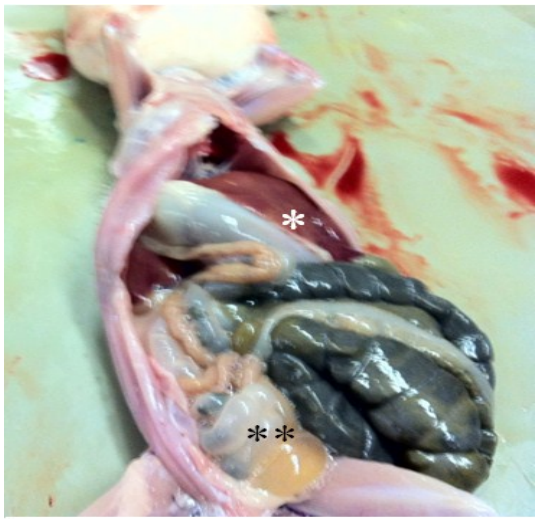
أسبوع وأسبوعين، لوحظ على الأرانب كثرة التبول، كما لوحظ بالعين المجردة بعد ذبح الحيوان انتفاخ في المثانة البولية واحتقان بالكبد (شكل 1) مقارنة بالمجموعة الضابطة (شكل 2).



شكل (1) صورة ضوئية لأرنب معامل لمدة أسبوعين يوضح احتقان الكبد (*) وانتفاخ المثانة البولية (**)



شكل (2) صورة ضوئية لأرنب بالمجموعة الضابطة يوضح اللون الطبيعي للكبد (*)وعدم انتفاخ المثانة (**)

عدم التغير في أوزان الأرانب المعاملة لمدة أسبوع (0.17±1.72) مقارنة بوزنها قبل الحقن (0.19±1.74)، كما حدث زيادة في أوزان الأرانب ولكن لم تصل الي مستوى المعنوية في الأرانب المعاملة لمدة أسبوعين (0.63±2.13) مقارنة بوزنها قبل الحقن (0.71±1.64) وذلك عند مستوى احتمالية (0.05)، كما سجلت نتائج المعاملة الفجائية (جدول-2) انخفاضاً في الوزن إلا أنها كانت غير معنوية (0.37±1.63) مقارنة بوزنها قبل الحقن (0.83±1.99)، و كذلك لوحظ زيادة في الأوزان ليست بالزيادة المعنوية في الأرانب المعاملة بالتدريج (0.289±2.11) مقارنة بوزنها قبل الحقن (0.53±1.77) عند مستوى احتمالية (0.05).

جدول (1) : تأثير عقار الهيدروكورتيزون على وزن الجسم في الأرانب لمدة أسبوع وأسبوعين مقيساً بالجرام.

العينة	عدد المعاملات (N)	المتوسط ± الانحراف القياسي X ± S.D	الفروق المعنوية عند 0.05
المجموعة الضابطة	5	0.19 ±1.74	a
معاملة لمدة أسبوع	5	0.179 ±1.72	a
المجموعة الضابطة	5	0.719 ±1.64	a
معاملة لمدة أسبوعين	5	0.63 ± 2.14	a

- الحروف المختلفة تعني وجود فروق معنوية عند مستوى احتمالية 0.05

جدول (2) : تأثير عقار الهيدروكورتيزون على وزن الجسم بعد الإيقاف التدريجي والفجائي في الأرانب مقيساً بالجرام.

العينة	عدد المعاملات (N)	المتوسط ± الانحراف القياسي X ± S.D	الفروق المعنوية عند 0.05
المجموعة قبل الحقن	5	0.531 ±1.77	a
المعاملة التدريجية	5	0.289 ±2.11	a
المجموعة قبل الحقن	5	0.838 ± 1.99	a
المعاملة الفجائية	5	0.372 ± 1.63	a

- الحروف المختلفة تعني وجود فروق معنوية عند مستوى احتمالية 0.05

ثانياً: الفحص الفسيولوجي

مستوى اليوريا في المصل: من خلال الجدول (3) أظهرت نتائج التحليل الإحصائي وجود فرق معنوي عند مستوى احتمالية (0.05) في مستوى اليوريا بالدم لدى الأرانب المعاملة بالهيدروكورتيزون المقاسة بالملليجرام لكل 100مل من الدم، كما سجل ارتفاع معنوي في تركيز اليوريا بعد

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المعاملة لمدة أسبوع (54.9±22.8) مقارنة بالمجموعة الضابطة (39.8±14.8)، كما لوحظ انخفاض معنوي بسيط بعد المعاملة لمدة أسبوعين (34.9±7.1) مقارنة بالمجموعة الضابطة (40±11.4). إلا أن المعاملة التدريجية والفجائية رجعت إلى معدلها الطبيعي.

جدول (3) : تأثير عقار الهيدروكورتيزون على مستوى اليوريا في مصل الأرانب لمدة أسبوع وأسبوعين مقيساً بالملليجرام / 100 مل من الدم.

العينة	عدد المعاملات (N)	المتوسط ± الانحراف القياسي X ± S.D	الفروق المعنوية عند 0.05
المجموعة الضابطة	5	14.809 ± 39.80	a
معاملة لمدة أسبوع	5	22.760 ± 54.9	b
المجموعة الضابطة	5	11.379 ± 40.00	a
معاملة لمدة أسبوعين	5	7.109 ± 34.86	b

- الحروف المختلفة تعني وجود فروق معنوية عند مستوى احتمالية 0.05

مستوى الكرياتينين في المصل: يوضح الجدول (4) نتائج التحليل الإحصائي لمستوى الكرياتينين في مصل الأرانب المعاملة بالهيدروكورتيزون مقيساً بالملليجرام لكل 100مل من الدم ، ولوحظ وجود فروق معنوية بين الأرانب المعاملة لمدة أسبوع والأرانب الضابطة ،وسجل ارتفاع معنوي في تركيز الكرياتينين بعد المعاملة لمدة أسبوع (0.3±1.7) مقارنة بالمجموعة الضابطة (0.2±0.56)، كما لوحظ عدم وجود فرق معنوي في تركيز الكرياتينين بعد المعاملة لمدة أسبوعين (0.3±0.8) مقارنة بالمجموعة الضابطة (0.3±0.6)، في حين إنّ المعاملة التدريجية والفجائية عادت إلى معدلها الطبيعي وذلك عند مستوى احتمالية (0.05).

جدول (4) : تأثير عقار الهيدروكورتيزون على مستوى الكرياتينين في مصل الأرانب لمدة أسبوع وأسبوعين مقيساً بالملليجرام/100 مل من الدم.

العينة	عدد المعاملات (N)	المتوسط ± الانحراف القياسي X ± S.D	الفروق المعنوية عند 0.05
المجموعة الضابطة	5	0.2302 ± 0.56	a
معاملة لمدة أسبوع	5	0.2612 ±1.67	b
المجموعة الضابطة	5	0.3000 ± 0.60	a
معاملة لمدة أسبوعين	5	0.3416 ± 0.76	a

- الحروف المختلفة تعني وجود فروق معنوية عند مستوى احتمالية 0.05

مستوى تركيز أيونات الصوديوم: يوضح التحليل الإحصائي في الجدول (5) مدى تأثير الهيدروكورتيزون على مستوى تركيز أيونات الصوديوم (Na^+) في الأرانب مقيساً بالملللي مول، حيث أوضحت النتائج وجود ارتفاع معنوي لأيونات الصوديوم بعد المعاملة لمدة أسبوع (138.6±0.89) مقارنة بالمجموعة الضابطة (135.5±2.3)، إلا أن المعاملة لمدة أسبوعين أدت إلى ارتفاع في مستوى تركيز أيونات الصوديوم ولكن هذا الارتفاع لم يصل إلى مستوى المعنوية (136.6±2.5) مقارنة بالمجموعة الضابطة (134.2±1.5). إلا أن المعاملة التدريجية والفجائية رجعت إلى معدلها الطبيعي وذلك عند مستوى احتمالية (0.05).

جدول (5) : تأثير عقار الهيدروكورتيزون على مستوى تركيز أيونات الصوديوم في مصل الأرانب لمدة أسبوع وأسبوعين مقيساً بالملي مول / 100مل من الدم.

العينة	عدد المعاملات (N)	المتوسط ± الانحراف القياسي X ± S.D	الفروق المعنوية عند 0.05
المجموعة الضابطة	5	135.5 ± 2.26	a
معاملة لمدة أسبوع	5	138.6 ±0.89	b
المجموعة الضابطة	5	134.2 ±1.52	a
معاملة لمدة أسبوعين	5	136.6 ± 2.53	a

- الحروف المختلفة تعني وجود فروق معنوية عند مستوى احتمالية 0.05

مستوى تركيز أيونات البوتاسيوم:جدول (6) يبين تأثير الهيدروكورتيزون على مستوى تركيز أيونات البوتاسيوم (K^+) مقيساً بالملللي مول ، حيث سجل ارتفاع معنوي في تركيز أيونات البوتاسيوم في الأرانب المعاملة لمدة أسبوع (6.9±0.5) مقارنة بالمجموعة الضابطة (5.5±0.9)، إلا أن المعاملة لمدة أسبوعين أدت إلي عدم وجود فرق معنوي (6.3±0.9) مقارنة بالمجموعة الضابطة (5.2±0.6). في حين إنّ المعاملة التدريجية والفجائية عادت الي معدلها الطبيعي وذلك عند مستوى احتمالية (0.05).

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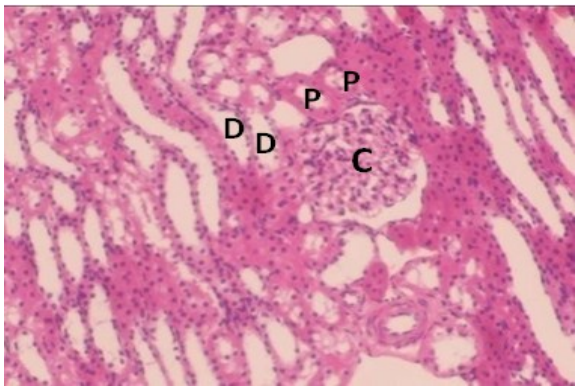
جدول (6) : تأثير عقار الهيدروكورتيزون على مستوى تركيز أيونات البوتاسيوم في مصل الأرانب لمدة أسبوع وأسبوعين مقيساً بالمللي مول/ 100مل من الدم.

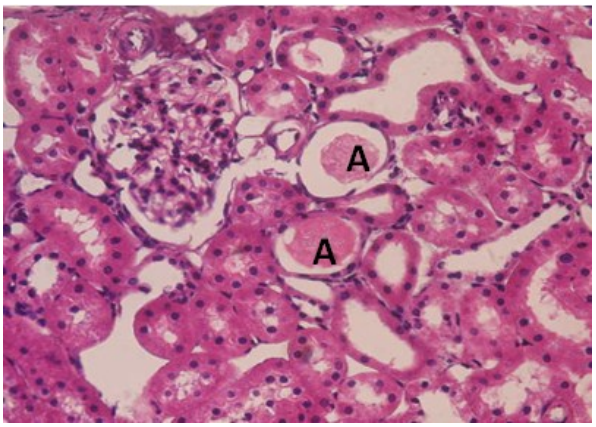
العينة	عدد المعاملات (N)	المتوسط ± الانحراف القياسي X ± S.D	الفروق المعنوية عند 0.05
المجموعة الضابطة	5	0.957 ±5.48	a
معاملة لمدة أسبوع	5	0.517 ± 6.91	b
المجموعة الضابطة	5	0.665±5.20	a
معاملة لمدة أسبوعين	5	0.945±6.27	a

- الحروف المختلفة تعني وجود فروق معنوية عند مستوى احتمالية 0.05

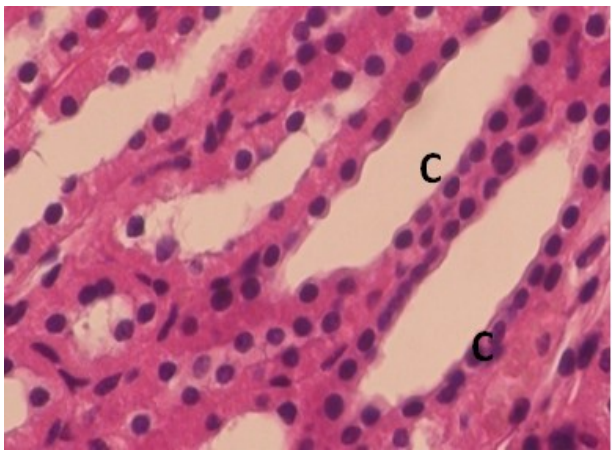
ثالثاً: الفحص النسيجي أظهر الفحص النسيجي للقشرة الكلوية Renal cortex لكلى أرانب المجموعة الضابطة التركيب الطبيعي للكريات الكلوية Renal cortex ،الأنيبيبات الدانية الملفوفة Proximal convoluted tubules والأنيبيبات القاصية الملفوفة Distal convoluted tubules (شكل 3). وفي المجموعة المعاملة لمدة أسبوع لم تظهر تغيرات واضحة في قشرة الكلية بينما ظهرت مادة حامضية (ايوسينية) الاصطباغ داخل تجاويف بعض الأنيبيبات القاصية الملفوفة في المجموعة المعاملة لمدة أسبوعين (شكل 4). يوضح (شكل 5) التركيب الطبيعي لنخاع الكلية Renal medulla المأخوذ من أرنب المجموعة الضابطة، حيث ظهرت الأنيبيبات الجامعة Collecting tubules مبطنة بخلايا مكعبة ذات أنوية كروية، فى حين أظهرت كلى الأرانب في المجموعتين المعاملتين بالهيدروكورتيزون ظهور مادة حامضية الاصطباغ داخل تجاويف بعض الأنيبيبات الجامعة، وبزيادة مدة المعاملة ازداد انتشار هذه المادة في كلى المجموعة المعاملة بالهيدروكورتيزون لمدة أسبوعين علاوة على ظهور فجوات في سيتوبلازم العديد من الخلايا المبطنة للأنيبيبات الجامعة (شكل 6). وتم مشاهدة نفس التغيرات النسيجية المرضية في كلى الأرانب التي تم إيقاف المعاملة فيها بالهيدروكورتيزون فجأةً، وكذلك المجموعة التي تم إيقاف معاملتها تدريجياً ولكن ظهرت هذه التغيرات بصورة أخف في المجموعة الأخيرة، كما قلت الفجوات داخل الخلايا المبطنة للأنيبيبات الجامعة كما قل انتشار المادة الحامضية داخل تجاويف هذه الأنيبيبات مقارنة بالمجموعة المحقونة لمدة أسبوعين.



شكل (3) قطاع في القشرة الكلوية لأرنب بالمجموعة الضابطة يوضح الكرية الكلوية (C) ، الأنيبيبات الدانية الملفوفة (P) والأنيبيبات القاصية الملفوفة (D) . صبغة (H&E)×400.



شكل (4) قطاع في القشرة الكلوية لأرنب معامل لمدة أسبوعين يظهر مادة حامضية الاصطباغ (A) داخل تجاويف بعض الأنيبيبات القاصية الملفوفة . صبغة (H&E)×400.



شكل (5) قطاع في النخاع الكلوي لأرنب بالمجموعة الضابطة يبين الأنيبيبات الجامعة (C) . صبغة (H&E)×400.

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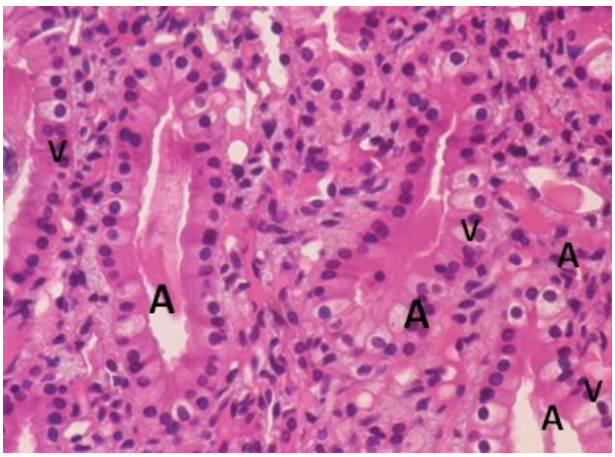

شكل (6) قطاع في النخاع الكلوي لأرنب معامل لمدة أسبوعين يوضح ظهور مادة حامضية الاصطباغ (A) داخل تجاويف بعض الأنيبيبات الجامعة وظهور فجوات (V) في سيتوبلازم الخلايا المبطنة لهذه الأنيبيبات . صبغة (H&E)×400 .

المناقشة

لقد أدى حقن الأرانب بعقار الهيدروكورتيزون إلى كثرة التبول كما لوحظ بالعين المجردة بعد ذبح الحيوان انتفاخ في المثانة البولية ولكن في هذا البحث لم نلاحظ العطش الشديد. تتفق هذه المشاهدات مع(Baas وآخرون، 1984)، الذين لاحظوا أن الآثار المترتبة على زيادة الكورتيزول في وظائف الكلى في الكلاب هي زيادة كمية البول بنسبة 23% وزيادة معدل الترشيح الكبيبي. وأفاد زايد وتوني (1998) أن لهذه الهرمونات تأثيراً مدراً للبول في حالة احتفاظ الجسم بالماء لأن لها فعلاً مضاداً للهرمون المضاد لإدرار البول على الكلية، وتعمل على سحب السوائل من الخلايا إلى خارج الخلايا والدم. ومن المثير للاهتمام أن بعد إعطاء الجرعة العلاجية من ديكساميثازون لمدة 3 أسابيع لاحظ الباحثون كثرة التبول Polyuria وعطشاً شديداً Polydipsia خلال فترة العلاج بأكملها (Abraham وآخرون، 2005)، ومع ذلك فإنه بعد إيقاف العلاج بالديكساميثازون فقد توقفت حالة كثرة التبول والعطش بعد 7 أيام واعتبر الباحثون أن هذه الملاحظات علامة من علامات الشفاء بعد التوقف عن العلاج(Abraham وآخرون، 2005). في حين لاحظ(Parker وآخرون، 2003) أن إعطاء الكورتيزول يؤدي إلى زيادة التبول، ولكن ليس له تأثير على شرب الماء، وعلى النقيض من ذلك فقد قرر زايد وتوني (1998) أن هرمونات القشرانيات السكرية تسبب حجز الماء، وأضاف (Boykin وآخرون، 1978) أن الكورتيزول يعمل كهرمون مضاد لإدرار البول Antidiuretic hormone.

أشارت نتائج هذه الدراسة إلى زيادة في وزن الأرانب ولكنه غير معنوي مقارنة بالمجموعة الضابطة. من المعروف أن الهرمونات الاستيرويدية تسبب احتباس السوائل في الجسم.

إن الإفراز المفرط للقشرانيات السكرية يسبب مرض كوشنج (Cushing's disease) ويسبب هذا المرض التعب وفقدان كتلة العضلة نتيجة للتحويل المفرط للأحماض الأمينية إلى الجلوكوز وإعادة توزيع دهون الجسم مسبباً ما يسمى بالوجه القمري أو الوجه الذي يشبه القمر Moon Face . بالرغم من أن السبب غير معروف إلا أنه تم اقتراح أن هذه السمنة ناتجة من التحفيز المفرط لاستهلاك الغذاء بحيث يصبح إنتاج الدهون في بعض الأنسجة أسرع من تحللها في تلك الأنسجة (Negi, 2009). كما أن للاستيرويدات تأثيراً على تراكم الدهون خصوصاً في الخلايا العظمية(Kawai وآخرون، 1985)، وقد لوحظ بالعين المجردة بعد ذبح الحيوان ازدياد كمية النسيج الدهني حول الكبد والكلى في المجموعة المعاملة بالتدريج، وأفاد (Kawai وآخرون، 1985) أن الهرمونات الاستيرويدية تؤدي إلى الكبد الدهني Fatty liver في أربعة أسابيع.

أوضحت نتائج هذه الدراسة ارتفاعاً في مستوى اليوريا في مصل دم الأرانب المعاملة لمدة أسبوع، وأدت في الأرانب المعاملة لمدة أسبوعين إلى انخفاض معنوي. وعلى العكس من ذلك فإن مستوى اليوريا، كان ضمن النطاق الفسيولوجي الطبيعي، ولم يتأثر بالمعالجة بالجلوكورتيكويد في أي من الحيوانات المعالجة (Abraham وآخرون، 2005)، وربما تعود الزيادة في مستوى اليوريا في دم الأرانب المعاملة بالهيدروكورتيزون إلى التأثيرات الجانبية للدواء على نسيج الكلى. وتعضد دراستنا الحالية هذا التعليل وأظهر الفحص النسيجي لكلية الأرانب المعاملة حدوث انسداد لتجاويف بعض الأنيبيبات الكلوية في أماكن متفرقة من القشرة والنخاع بواسطة مادة حامضية الاصطباغ، علاوة على ظهور فجوات في سيتوبلازم العديد من الخلايا المبطنة للأنيبيبات الجامعة،

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وهذا مع العلم بأن القصور في وظائف الكلى يسبب احتجاز مكونات البلازما التي تخرج بواسطة الكلية مثل اليوريا (Carlton وآخرون، 1995). وللكورتيزول تأثير مباشر على إخراج أيونات الهيدروجين عن طريق الكلى؛ وهذا لتحفيز إخراج أيونات الأمونيا من خلال تثبيط إنزيم جلوتامينيز الكلوي Tai Renal glutaminase enzyme)وآخرون، 1981(. ولهذه الهرمونات تأثير مدر للبول لأن لها فعلاً مضاداً للهرمون المضاد لإدرار البول Antiduretic hormone (ADH) على القنوات الجامعة Collecting ducts، مما أدى إلى تناقص إعادة امتصاص الماء واليوريا، وربما أدى هذا التناقص في إعادة امتصاص اليوريا إلى قلة دوران اليوريا في النخاع الكلوي، وبناء عليه انخفاض تركيز اليوريا في الدم بعد أسبوعين (Baas وآخرون، 1984)، إن الزيادة في تركيز الأحماض الأمينية في البلازما وزيادة نقل الأحماض الأمينية إلى خلايا الكبد بواسطة الكورتيزول قد تجعل خلايا الكبد تزيد من عملية إزالة المجموعة الأمينية Deamination من الأحماض الأمينية وبالتالي زيادة الأمونيا واليوريا (Negi, 2009).تبين الدراسة الحالية وجود ارتفاع معنوي في الكرياتينين في مصل الأرانب المعاملة لمدة أسبوع ثم رجوعها إلى معدلها الطبيعي في الأسبوع الثاني.

يتم إنتاج الكرياتينين في الكبد من Methionine و Glycine و Arginine. في العضلات الهيكلية تتم فسفرتة إلى Creatinephosphate، الذي هو عبارة عن مخزون مهم للطاقة لإنتاج ATP. وبما أن للقشرانيات السكرية تأثيراً على هدم البروتينات في خلايا الجسم وخصوصاً خلايا العضلات وطرحها في البلازما فمن المتوقع ملاحظة زيادة في معدل الكرياتينين في الدم. وهذا يتفق مع (Mandal 2007)الذي لاحظ أن للقشرانيات السكرية تأثيراً على تقليل محتوى البروتين والكرياتينين في عضلات الجرذان وبالتالي انطلاقها إلى الدم. سجل في هذه الدراسة ارتفاع معنوي في تركيز أيونات الصوديوم بعد أسبوع من المعاملة، إلا أن المعاملة لمدة أسبوعين أدت إلى ارتفاع تركيز هذه الأيونات لكن هذا الارتفاع لم يصل إلى المعنوية عند المقارنة بالمجموعة الضابطة. كما أشارت النتائج إلى وجود ارتفاع في تركيز أيونات البوتاسيوم بعد المعاملة لمدة أسبوع، وعادت هذه الايونات إلى معدلها الطبيعي بعد أسبوعين من المعاملة. تعمل الجرع المتزايدة من الكورتيزول على حجز وإبقاء الصوديوم داخل الجسم وطرح البوتاسيوم. يؤدي استمرار إعطاء هذه الجرع العالية إلى الخزب Edema وزيادة الصوديوم ونقص البوتاسيوم في الدم والقلاء الأيضي Metabolic alkalosis (محيي الدين وآخرون، 1990). ويتجلى الضعف في وظائف الأنيبيبات الكلوية في اضطراب التفاعل مع الإلكتروليتات والذي يمكن أن يؤدي إلى زيادة امتصاص الصوديوم (Smets وآخرون، 2010)، وعلى النقيض من ذلك فقد أشار (Baas وآخرون، 1984)إلى أن تركيزات الصوديوم والبوتاسيوم في البلازما قد انخفضت نتيجة لزيادة الكورتيزول في الكلاب.

وجد (Parker وآخرون، 2003) أن إعطاء الكورتيزول يقلل من إخراج الصوديوم؛ ولكنه ليس له تأثير على مستوى الإلكتروليتات في البلازما. وأفاد نفس الباحثين أن فقد الماء يؤدي إلى زيادة تركيز الصوديوم في البلازما. وأكد (Müller and O'Connor 1958) أن الكورتيزول يساعد على إخراج البوتاسيوم بواسطة الكلية. ومن جهة أخرى فقد وجد (Abraham وآخرون، 2005)أن نسبة الشوادر (الأكتروليتات) كانت ضمن النطاق الفسيولوجي الطبيعي، ولم تتأثر بالمعالجة بالجلوكورتيكويد في أي من الحيوانات المعالجة. وعلى مستوى الفحص النسيجي للكلى فقد أدت المعاملة بالعقار إلى ظهور مادة حامضية (إيوسينية) الاصطباغ Acidophillic (eosinophilic) material داخل تجاويف بعض الأنيبيبات الكلوية في كل من القشرة والنخاع الكلوي. وتم وصف هذه المادة بواسطة شيفيل (1982) على أنها قوالب زجاجية Hyaline casts .

كما تم تفسير وجود هذه المادة على أنها بروتين مخاطي Mucoprotein نتيجة لنخر الأنيبيبات الكلوية وزيادة نفاذية الشعيرات الدموية المكونة للكبيبة (Jubb 1985).في حين تم تفسيره بواسطة (Levenson وآخرون، 1982) على أنها مادة بروتينية Proteinaceous material نتيجة لزيادة تدفق البروتين إلى الراشح الكبيبي ثم تجميعه خارج الكبيبة،

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وهذا ربما يسبب ضيق أو انسداد الحيز المحفظي. وقد أكد Carlton)وآخرون، 1995(أن كمية كبيرة من بروتين البلازما ترشح خلال حاجز الترشيح الكبيبي المتضرر ويفوق ذلك كمية البروتين الممتصة. وفي الجهة الأخرى اعتبر (DiScala وآخرون، 1967) أن هذه المادة عبارة عن وذمة، وفي الجرذان والكلاب والأغنام يزداد كلٌ من معدل الترشيح الكبيبى وتدفق الدم الكلوي Renal blood flow بواسطة الجلوكوكورتيكويدات (McDougall وآخرون,2000)، وأكد(Smets وآخرون، 2010) أن الجلوكوكورتيكويدات تؤثر على وظيفة الكلى بشكل غير مباشر من خلال التأثير على الجهاز القلبي الوعائي، ومباشرةً عن طريق تأثيرها على وظائف الكبيبات والأنيبيبات الكلوية؛ وإنها تسبب زيادة معدل الترشيح الكبيبي في الإنسان والأغنام والجرذان والكلاب. وعلى النقيض من كل ذلك فقد أقر (Cassano وآخرون، 1964، Katz وآخرون، 1975) أن هذه المادة تتواجد نتيجة ترسيب عديدة السكريات المخاطية Mucopolysaccharides كما أنها تعتبر مسؤولة عن نقص الراشح الكبيبي والتغيرات الأخرى في وظائف الكلى.

الخلاصة

من خلال هذه الدراسة التي تضمنت اختبار تأثير عقار الهيدروكورتيزون سكسينات الصوديوم على كلٌ من وظائف الكلى وكذلك على التركيب النسيجي للكلى في الأرانب البيضاء نجد أنه من الضروري الإشارة إلى أهم الاستنتاجات والتوصيات وهي: أن المعاملة بالهيدروكورتيزون سكسينات الصوديوم في الأرانب قد أدت إلى ارتفاع معنوي في تركيز كلٌ من اليوريا، الكرياتينين، البروتين الكلي، الألبومين، أيونات الصوديوم و البوتاسيوم، وهذا قد يشير إلى احتمال عواقب سلبية للجلوكوكورتيكويدات. ؛ لذا ينبغي أن يستخدم الهيدروكورتيزون في المجالات الطبية بحذر وتحت احتياطات وقيود. ويجب موازنة الفوائد والضرر الذي يمكن أن ينتج عن ذلك في عملية الاتزان والثبات الداخلي Homeostasis في الجسم، في الوقت ذاته فإن الهيدروكورتيزون قد أدى إلى تأثير واضح على التركيب النسيجي للكلى، اشتملت هذه التغيرات على تغيرات تنكسية Degenrative changes ونخرية Necrotic changes في العديد من الخلايا المكونة لهذه الأعضاء. وقد تنعكس هذه التغيرات على وظائف هذه الخلايا؛ مما قد يؤدي إلى نقص وعوز في أدائها وكفاءتها. وبناء عليه لابد أن يوضع في الاعتبار المضاعفات التي ربما قد تنتج في وظائف بعض الأعضاء أثناء العلاج بواسطة الجلوكوكورتيكويد والتي يجب أن ينظر إليها خلال عمليات المعالجة لحماية صحة الإنسان من آثارها، بالرغم من أن إفراز الكورتيزول كاستجابة للضغوط هو وظيفة طبيعية، إلا أن إفراز الكورتيزول لفترات طويلة بسبب التوتر المزمن ربما يؤدي إلى تغيرات فسيولوجية معنوية. وهذا ما قد تسببه الجلوكوكورتيكويدات والتي تعتبر إحدى هذه الضغوط ولها آثار جانبية واسعة.

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Physiological and Histological Studies on the Effect of Hydrocortisone on Kidneys of Rabbits

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Abstract: This study aimed to test the effect of hydrocortisone sodium succinate drug on blood serum components and kidneys tissues in white rabbits.The experiment included 30 male rabbits weighing between 1500-2500g. They were divided into 4 control and treated groups, for different periods of time, depending on the duration of injection. Treatment of the rabbits with hydrocortisone dose of 10 mg/kg daily did not lead to change in the weights of rabbits treated for a week. Non significant increase occurred in the weights of rabbits treated for two weeks and treated gradually, but in sudden treated group a non significant decrease in rabbits weights was recorded in comparison with their weights before injection .A significant increase in the concentration of urea, creatinine, total serum protein, albumin, sodium and potassium ions after treatment for a week, but a significant reduction was noted in the concentration of urea and no significant difference was noted in the concentration of creatinine, sodium and potassium ions after treatment for two weeks. While there were a significant increase in the total serum protein and albumin after two weeks of treatment. Noting that, in the suddeny treated group, the biochemical parameters did not change compared to that present in the two weeks treated rabbits. While, in the gradually treated group, most of the biochemical parameters returned to their normal values. Histological examination of the renal cortex showed acidophilic material accumulated in the lumina of some distal convoluted tubules in the group treated for two weeks. The renal medulla also showed the presence of the same material inside the collecting tubules in the both treated groups. With the increase of the duration of treatment; the intracytoplasmic vacuoles appeared in many of the cells lining the collecting tubules. The same histopathological changes were observed in the kidneys of rabbits that their treatment was suddenly stopped, as well as the group that their treatment was gradually stopped, but these changes became low in the last group.

Keywords: hydrocortisone, kidney, physiological, histological, rabbits.

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تأثير الفطر الأحيائي *Pythium oligandrum* وراشحه على نمو بعض ممرضات النبات الفطرية

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المستخلص : في هذه الدراسة اختبر قدرة الفطر *Pythium oligandrum* التضادية على بعض الفطريات الممرضة للنبات وهي *Botrytis cinerea*، *Fusarium oxysporum*، *Alternaria ocharcenus* و *Sclerotinia sclerotioum*، *Fusarium solani* و *Rhizctonia solani* باستخدام تقنيات الزرع المزدوج والطبق المسموم براشح الفطر، بينت النتائج حدوث تثبيط في النمو الطولي لجميع الفطريات المختبرة، كما أشارت النتائج إلى فعالية راشح الفطر المضاد في خفض نمو الفطريات بزيادة التركيز المستخدم.

الكلمات المفتاحية : *Pythium oligandrum* ، الراشح الفطري، التضاد، فطريات ممرضة للنبات.

المقدمة

يمتلك الفطر *P. oligandrum* خصائص مميزة تجعله قادراً على كبح نمو ونشاط بعض الممرضات النباتية (Benhamou وآخرون، 1997، Le Floch وآخرون، 2007، Vesely 1979)، لقدرته التنافسية العالية وقابليته التضادية على تثبيط نمو الفطريات الممرضة، إلا أن التأثير المضاد لفطر *P.oligandrum* يعتمد على نوع الفطر الممرض و كثافة المضاد والنبات العائل. من الصفات المميزة لهذا الفطر أنه غير قادر على إنتاج المضادات الحيوية (Benhamou وآخرون، 1997)، ولا يمتلك أي صفات تجعل منه فطراً ضاراً للنبات العائل(McQuilken, Mark P وآخرون، 1998)، غير ممرض، ولا ينجم عنه أي تلوث للبيئة، هذه الأسباب جعلته مؤهلاً لاستخدامه في مجال المكافحة لأنه يؤثر مباشرة على الممرضات بواسطة التطفل، التضاد أو التنافس على المكان والغذاء(Foley and Jones and Deacon, 1995؛Deacon, 1986؛ Benhamou وآخرون1997 , Rey وآخرون 1998 ؛ Wulffوآخرون2005) فطر *P.oligandrum* يتطفل على *Phytophthora parasitica* ، وينتج انزيمات تؤدى دوراً في التضاد (Picard وآخرون، 2000)، ووصفت العديد من الدراسات هذا الفطر بأنه متطفل بواسطة اختراقه لهيفات الفطريات الممرضة (Laing and Deacon, 1991 ؛ Orlikowski and ؛Ribeiro and Butler, 1995 .(Jaworska-Marosz, 2003

لفطر *P. oligandrum* تأثير على الرتب الفطرية المختلفة الفطريات الاسكية (Bradshaw-Smith وآخرون، 1991)، الفطريات البيضية (Benhamou وآخرون، 2012)، والفطريات البازيدية (Ikeda وآخرون، 2012)، وعلى التراكيب الساكنة مثل السكليروشيا(Rey وآخرون، 2005)، وقد سجل وجود تفاعلات بين الفطر البيضيّ *P.oligandrum* والعديد من الفطريات المحمولة بالتربة والممرضة للنبات (*P.ultimum*، *P.aphanidermatum*، *Fusarium oxysporum*، *Verticillium albo-atrum* و *R.solani*، *P.megasperma* (Benhamou, وآخرون1999 ,)، أيضا تفاعلات ضد *Septoria*، *Fusarium*، *Rhynchosporium* و *Helminthosporium* (HÝSEK 2004)، وضد *A. alternata*، *F. culmorum*، *Fusarium* spp.، *F. oxysporum*، *F. solani*، *Gliocladium*، *P. irregulare*، *Trichoderma* و *R. solani* (Patkowska 2008) ، كذلك ضد *B.*

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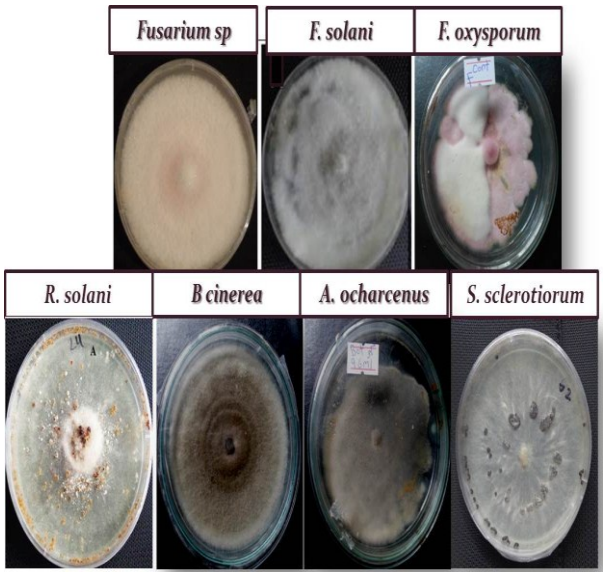
، *Ascochyta* spp ،*Alternaria* spp ،*cinerea* ،*Peronosplasmopara* spp ،*Fusarium* spp ، *P.viticola* ،*P. infestans* ،*Phoma* spp *S.* ،*R.solani* ،*Pythium* spp. ،*Puccina* spp. *Verticillium* و *U. necator* ،*sclerotiorum* spp(Kabaluk وآخرون، 2010).

المواد وطرق البحث

مصدر الفطر *P. oligandrum* المستخدم في هذه الدراسة عزل محليا من تربة حقول منطقة راس الترب وفقا لما ذكره (Mohamed and Akila 2014)، تم إعداد مترشحه الفطريّ بتنمية الفطر على بيئة ثمانية خضروات لمدة 10 أيام من التحضين جفف النمو الميسليومي جيدا، حيث نقل إلى البيئة السائلة جلوكوز اسبارجين التي أعدت وفقا لما ذكره (McQuilken, MP وآخرون، 1992)، وموزعة في دوراق Roux بمعدل 100مل، بعد 21 يوماً من التحضين تمت عملية الترشيح باستخدام شاش جاف ومعقم، يليه أوراق ترشيح No 1 . whatman، المترشح الفطريّ خزن إلى حين الحاجة إليه عند 4 °م.

الفطريات الممرضة للنبات : في هذه الدراسة اختبر الفطريات التالية الموضحة في الشكل (1) وهي فطر *A.ocharcenus* (Ao) المستورد من جمهورية مصر العربية بواسطة (أجى، 2010)، أما باقي الفطريات فتحصل عليها من مصادر محلية وتشمل: الفطر *A.solani* المسبب لمرض اللفحة المبكرة على الطماطم(محمد، 1997)، فطر (Bot) *B.cinerea* المسبب لمرض العفن الرمادي على البصل (الأربد، 2014)، فطر (F1) *F.oxysporum*المسبب لذبول الفيوزارمى على شتلات طماطم (محمد وإدريس، 2009)، (F2) *F.solani* المسبب لعفن جذور الفول (بيانات غير منشورة)، (F3) *Fusarium sp* و فطر(Sc) *S.sclerotiorum* المعزولة من بذور البازلاء (عبدالعالي، 2015)، sp *Pythium* المسبب لموت بادرات الخيار (Mohamed and Ibrahim 2009)، *R.solani*، (Rhiz) المعزول من بذور الفاصوليا (El–Gali، 2003).



شكل (1):الفطريات الممرضة المستخدمة في الدراسة والمتحصل عليها من مصادر محلية

اختبار تضاد المستعمرات الفطرية :تم التضاد معمليا بين الفطر *P.oligandrum* و الفطريات الممرضة معاً وفقا لطريقة (Bala وآخرون، 2009، Sinclair and Dhingra 1995) على الوسط نفسه ، بحقن قرص قطره 5 مم من ميسيليوم الفطر *P. oligandrum* عمره 3 أيام، يبعد مسافة 2.7 سم من حافة الطبق و 3سم من الفطر الممرض، بمعدل 4 مكررات لكل معاملة، بالإضافة إلى أطباق الشاهد لكل الفطريات المدروسة، حضنت الأطباق على درجة حرارة 25°م، مسافة النمو الطوليّ حسبت في الأطباق المعاملة والمقارنة للفطريات الممرضة ومنها حسبت نسبة التثبيط بتطبيق معادلة(Hajieghrari وآخرون، 2008):

نسبة تثبيط النمو الميسيليومى للفطر الممرض = [(النموالطولي الفطر الممرض (الشاهد) – النموالطولي الفطر الممرض (المعاملة)/ النموالطولي الفطر الممرض (الشاهد)] *100

تأثير راشح فطر *P. oligandrum* مترشح الفطر عُقم على البارد بواسطة مرشح زايتس، حيث طبقت تقنية الأطباق المسمومة (Sinclair and Dhingra 1995) والتي تحوي الوسط الغذائي بطاطس دكستروز اجار والمضاف إليه تركيزات مختلفة من الراشح الفطري (2.4، 4.8 و9.6 %)، ثم صبت في الأطباق 15مل/ طبق وتركت لتتصلب، ووضعت أقراص

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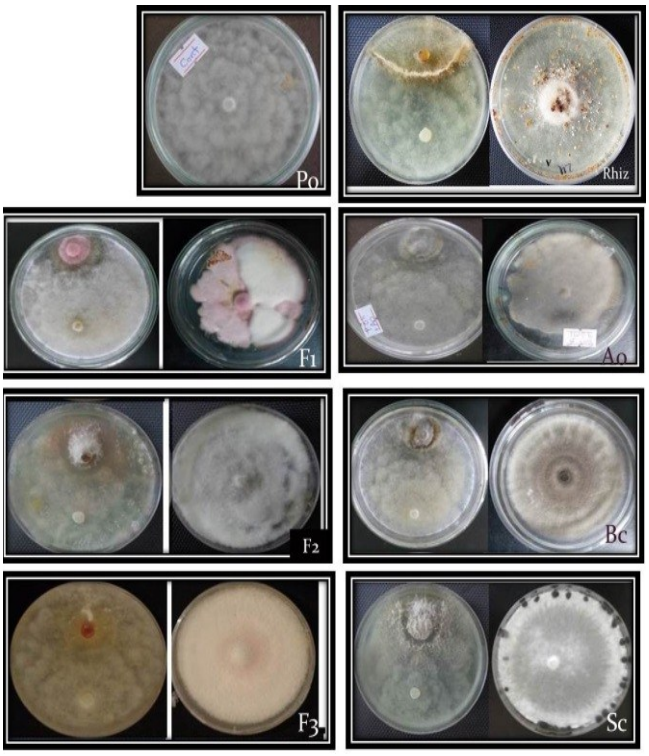
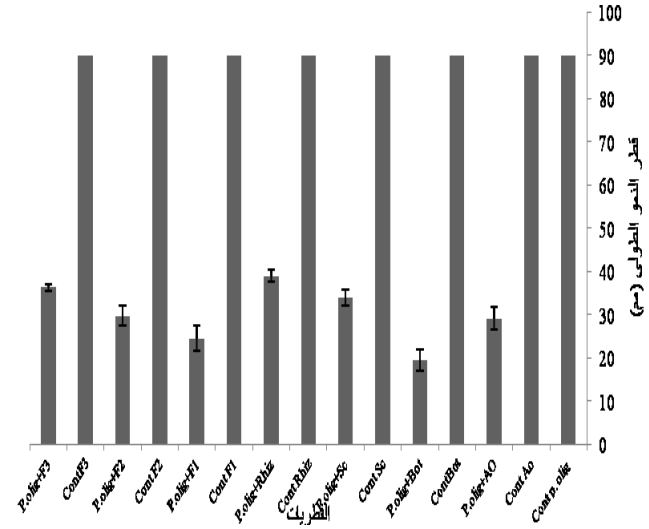
الفطريات الممرضة بقطر 5 مم في مركز كل الطبق، وغلفت الأطباق بشمع البرافليم وحضنت عند25°م، أما أطباق الشاهد فأضيف إليها التركيز نفسه من بيئة جلوكوز اسبارجين، قيس النمو الطولي لكل فطر باستخدام المسطرة وقياس قطرين متعامدين للمستعمرة ، وانتهت بحساب نسبة تثبيط الناتج عن المترشح الفطريّ.

النتائج

اختبار تأثير الفطر *P. oligandrum* :تشير النتائج المبينة بالشكل (2) إلى التأثير الملحوظ للفطر *P. oligandrum* على الفطريات المختبرة في أطباق الاجار، حيث سجل توقف نمو *A. ocharcenus*، *B. cinerea*، *S. sclerotiorum*، *R. solani* (F1)، *F. oxysprum* (F2)، *F.solani* و *Fusarium* sp (F3) عند تلامسها مع فطر *P.oligandrum*، يتضح من النتائج أن الفطر *P.oligandrum* غطى الطبق حتى تلامس مع الفطريات الممرضة، وعدم وجود هالة أو مسافة تثبيط بينها، كما سجل في هذه الدراسة عدم تكون أجسام حجرية للفطرين *S.sclerotiorum* و *R. solani* مقارنة بأطباق الشاهد. وعند حساب النمو الطولي للفطريات النامية منفردة أو في وجود الفطر المكافح بعد 7 أيام، أظهرت نتائج التحليل الإحصائي أن وجود الفطر المضاد أدى إلى توقف نموها الميسيليومى معنويا لجميع الفطريات كما هو موضح بالشكل (3) وسجل انخفاض نمو كل من (*F.oxysporum* و *B.cinerea*) إلى 19.5 و 24مم على التوالي، ولم يتجاوز 29مم لكل من الفطرين *A. ocharcenus* و *F.solani*، في حين وصل نمو *R. solani*، *Fusarium* sp و *S.sclerotiorum* إلى 39 ، 36 و34 مم على التوالي (شكل 4).



الشكل (2) تضاد بين فطر *P. oligandrum* (Po) والفطريات المختبرة على أطباق تحوي الوسط الغذائي *A.ocharcenus* (Ao) *R. solani* (Rhiz) *S.sclerotiorum* (Sc)، *B.cinerea* (Bot)، *F. solani* (F2)، *F.oxysporum* (F1)، و *Fusarium* sp (F3) مقارنة بأطباق الشاهد



شكل (3) قطر النمو الطولى للفطريات الممرضة في وجود فطر *P. oligandrum* النامية على الوسط الغذائي (PDA) عند (LSD 5% الفطريات*الزمن* التركيز= 3.656)، التكرار 4 أطباق. Ao: *A. ocharcenus*, Bot: *B. cinerea* ,Sc: *Sclerotinia S.sclerotioum* , Rhiz: *R. solani*, F1: *F.oxysporum*, F2: *F. solani*, F3: *Fusarium* sp,

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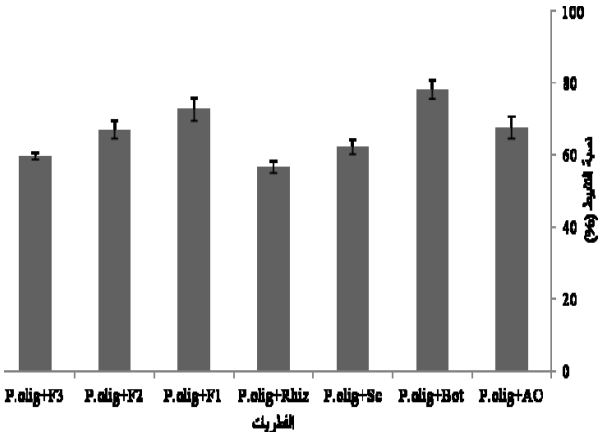

شكل (4) نسبة تثبيط لفطر *P. oligandrum* على النمو الطولي للفطريات الممرضة النامية على الوسط الغذائي. عند (LSD 5% : الفطريات*الزمن*التركيز =5.106)، التكرار 4 أطباق. Ao: *A. ocharcenus*, Bot: *B. cinerea* ,Sc: *Sclerotinia S.sclerotioum* , Rhiz: *R. solani*, F1: *F. oxysporum*, F2: *F. solani*, F3: *Fusarium* sp,

دور راشح الفطر في تثبيط نمو الكائنات الممرضة : بينت النتائج المدونة في جدول (1) أن للراشح الفطري *P. oligandrum* تأثيراً على النمو الميسيليومي للفطريات الممرضة للنباتات النامية على الأطباق المسمومة بالمترشح خلال أزمنة تحضين متباينة، حيث أنخفض نموها الميسيليومي بعد 3 أيام، وظهر تأثيره بوضوح على كل من: *F. oxysporum*، *S.sclerotioum* و *R.solani* ، وبعد 5 أيام على *A.ocharcenus*، *F. solani* و *Fusarium* sp، بينما بعد 7 أيام كان التأثير على فطر *B. cinerea*، من جهة أخرى فالنتائج المبينة بالجدول تشير إلى أن للتراكيز العالية تأثيراً ملحوظاً على النمو الفطري، وأن زيادة تركيز المترشح في البيئة يؤثر على النمو الطولي مقارنة بأطباق الشاهد، وكان التأثير أكثر وضوحاً بعد 5 و7 أيام من التحضين، والجدير بالملاحظة أن التركيز 2.4% كان أقل تثبيطاً بعد 3 أيام على الفطر *Fusarium* sp بنسبة 2.4%، وبعد 5 أيام على فطر *B.cinerea* بنسبة 3.9% و بعد 7 أيام على فطر *F. solani* بنسبة 15.1%. وكان أعلى تثبيطاً على الفطر *F.oxysporum* بعد 3 أيام حيث بلغ 55%، وفطر *F.solani* بعد 5أيام بنسبة 45% ، في حين أصبح أعلى تثبيطاً على هذا الفطر بعد 7 أيام بنسبة وصلت إلى 61.7%.

المناقشة

أشارت نتائج الدراسة إلى فاعلية الفطر *P. oligandrum* على الممرضات النباتية المختبرة تحت الظروف المعملية، مؤديا إلى توقف نمو هذه الفطريات عند تلامسها معه، وعدم وجود هالة أو مسافة تثبيط بين جميع الفطريات الممرضة والفطر المختبر و عدم تكون أجسام حجرية للفطرين *S.sclerotiorum* و *R. solani* مقارنة بأطباق الشاهد، كما أظهرت نتائج التحليل الإحصائي أن وجود الفطر *P.oligandrum* أدى إلى توقف نموها الميسيليومي معنويا لجميع الفطريات، تختلف مقدرة الفطر على إظهار قدرته التضادية حسب الأنواع من الجنس نفسه أو أجناس فطرية أخرى أو حسب طبيعة تأثيره عليها تطفلية أو تضادية حسب ما ذكر (Takenaka, S and Kawasaki 1994) ،لذا تتفق هذه النتائج مع (Jones and Deacon 1995) الذي أكد أن الفطر يثبط نمو كل من *Phialophora*، *B.cinerea*، *F. oxsporium*، *F.culorum*، *Botryotrich piluliferum*، *T. aureoviride*، *P. cinnamomi*، *R.solani*، *Pythium* spp. ، *V.dahlia* و *P.parasitica* (Al-Rawahi and Hancock, 1998؛ Picard وآخرون 2000 .,) الذين توصلوا إلى قدرة هذا الفطرعلى التأثير في نمو *T.aureoviride*، *P.graminicola*، *P.vexans*، *R. solani*، *Botrytis* spp.، *Fusarium* spp. (Laing and Deacon 1991)، وضد فطر *B. cinerea* (Rey وآخرون، 1998)، وفسر التفاعل بين الفطريات الممرضة والفطر المضاد *P. oligandrum* النامية معاً على أطباق بترى بتأثيره المباشر في إنتاج مواد طيارة مؤديا إلى خفض معدل النمو (Bradshaw-Smith وآخرون، 1991)، وعدد كبير من الدراسات السابقة ذكرت أن المقدرة التضادية لهذا الفطر عالية وأنه فطر فعال ضد *P.ultimum*، *R.solani* ، *Mycocentrospora acerina* (Lutchmeah and

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Cooke 1984)، وضد *F.oxysporum, P.ultimum, R.solani ،P.aphanidermatum, V.albo – atrum* ، *P.megasperma*(Benhamou وآخرون، 1999)، ويحد من نشاط كل من: *P.ultimum*، *T.roseum*، *B.piluliferum*، *F.culmorum* ، *R.solani*، *Phialophora*(Foley and Deacon 1986) مؤدى إلى تناقص نمو *F. oxysporum* عند التلامس المباشر بينهما (Laing avd Deacon, 1991؛ Le Floch وآخرون 2005) وعزا (El-Katatny وآخرون، 2006) توقف النمو عند منطقة التقاء الفطرين إلى أن فطر *P.oligandrum* ينتج إنزيمات محللةً للجدر الخلوية و مواد طيارة، أما(Mulligan وآخرون، 1995) فقد أشاروا إلى تنافس *P.oligandrum* على الاجار مع *B.cinerea*، *Phialophora* sp و*F. culmorum*، وفسر أسباب سيطرته على أطباق النامى معه الفطريات الممرضة لقدرته التنافسية على المكان والغذاء(Al-Rawahi andHancock, 1997؛ LeFloch وآخرون2007؛ Vallance وآخرون 2009؛ Gerbore وآخرون2014).

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جدول (1) النمو الطولي للفطريات الممرضة المعاملة براشح فطر *P. oligandrum* عند تراكيز وأزمنة تحضين مختلفة

الفطريات	التركيز	النمو الطولى (مم) ونسبة التثبيط (%) خلال ازمنة التحضين مختلفة*					
		3 أيام		5أيام		7أيام	
		النمو الطولي	التثبيط (%)	النمو الطولي	التثبيط (%)	النمو الطولي	التثبيط (%)
A. ocharcenus	0.0	1.8± 31.5*		.0 ±70.0		3.8 ±80.8	
	2.4	1.0 ±30.2	3.3± 4.2	2.6 ±42.0	3.8±40.0	2.5 ±60.0	3.1±25.7
	4.8	1.3±27.0	4.2±14.3	1.4 ±36.7	2.1±47.6	6.0 ±54.7	7.5±32.3
	9.6	1.5 ±22.2	4.8± 29.6	2.0 ±34.5	2.9± 50.7	3.0 ±45.3	3.7± 43.9
B. cinerea	0.0	0.6±32.7		9.8 ±56.2		1.4 ±84.2	
	2.4	0.8 ±30.2	2.3± 7.7	1.0 ±54.0	1.8±3.9	6.3 ±68.3	7.5± 18.8
	4.8	0.9 ±29.5	2.6± 9.8	0.6 ±53.2	1.0± 5.4	1.3 ±56.2	1.5± 33.3
	9.6	3.5 ±24.5	10.7± 25.1	2.8 ±46.7	5.1± 17.0	2.5 ±47.5	3.0±43.6
S. sclerotioum	0.0	0.6± 55.3		0.0± 85.0		0.0 ± 85.0	
	2.4	2.6 ± 43.0	4.6±24.6	0.6± 65.3	0.7± 23.1	0.6 ± 65.3	0.7± 23.1
	4.8	1.5 ± 41.7	2.7± 26.9	1.5± 61.7	1.8± 27.5	1.5 ± 61.7	1.8± 27.5
	9.6	1.0 ± 38.0	1.8± 33.3	1.0± 60.0	1.2± 29.4	1.0 ± 60.0	1.2± 29.4
R. solani	0.0	3.1 ± 55.7		0.0± 85.0		0.0 ± 85.0	
	2.4	2.3 ± 42.7	4.1± 23.4	1.5± 60.3	1.8± 29.0	1.5 ± 60.3	1.8± 29.0
	4.8	0.6 ± 41.7	1.0± 25.2	1.0± 63.0	1.2± 25.9	1.0 ± 63.0	1.2± 25.9
	9.6	0.6 ± 38.3	1.0± 31.2	1.0± 58.0	1.2± 31.8	1.0 ± 58.0	0.7± 31.4
F. oxysporum	0.0	1.8 ± 31.5		2.0± 67.3		4.3 ± 77.5	
	2.4	1.4 ± 36.7	3.7± 6.7	2.9± 45.8	4.3± 31.9	2.1 ± 65.8	2.7± 15.1
	4.8	1.0 ± 31.3	2.6± 20.3	3.2± 40.2	4.8±40.3	2.5 ± 65.0	3.2 ± 16.1
	9.6	1.3 ± 17.7	3.2± 55.0	0.9± 32.0	1.3± 52.5	1.8 ± 70.5	2.3 ± 9.0
F. solani	0.0	1.0 ± 33.0		5.0± 60.0		0.0 ± 85.0	
	2.4	2.1 ± 31.3	4.6± 8.1	1.0± 33.0	1.7±45.0	4.0 ± 59.3	4.8± 30.2
	4.8	0.6 ± 27.7	1.7± 16.2	1.0± 36.0	1.7±40.0	1.0 ± 51.0	1.2± 40.0
	9.6	0.6 ± 20.3	1.7±38.4	1.0± 23.0	1.2± 45.9	1.0 ± 46.0	1.7± 61.7
Fusarium sp	0.0	2.3 ± 42.7		2.6± 56.0		0.0 ± 85.0	
	2.4	0.6 ± 41.7	1.4± 2.4	1.7± 42.0	3.1±25.0	1.5 ± 60.3	1.8 ± 29.0
	4.8	0.6 ± 38.3	1.4± 10.2	0.6± 41.7	1.0± 25.6	1.0 ± 63.0	1.2 ± 25.9
	9.6	1.0 ± 38.0	2.3± 11.0	1.0± 38.0	1.8±32.1	1.0 ± 58.0	1.2 ± 31.8

	LSD نمو الطولي	نسبة التثبيط
الفطريات	1.055492	1.702014
الزمن	0.690982	1.11423
التركيز	0.797877	1.11423
الفطريات*الزمن	1.828166	2.947974
الفطريات*التركيز	2.110985	2.947974
التركيز *الزمن	1.381964	1.929902
الفطريات*الزمن* التركيز	3.656333	5.106041

*(القطر ± SD)

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واعتبر (Benhamou وآخرون، 2012) أن هذه الصفة من نقاط القوة التي يمتلكها فطر *P.oligandrum* .

بينت نتائج قياس النمو الطولي للفطريات النامية على اطباق مسمومة بتراكيز من الراشح الفطري إلي وجود تأثير على النمو الميسيليومي، وأن التركيز الأعلى للمترشح 9.6% ثبط نمو جميع الانواع الفطرية المختبرة، فأعطت انخفاضاً معنوياً مقارنة بأطباق الشاهد، فسر هذا الأنخفاض إلي المواد الكيميائية المنتجة بواسطة الفطر *P.oligandrum*، كأحتواء هذا الراشح على مواد سامة (Benhamou وآخرون، 2012)، والإنزيمات المحللة للجدر الخلوية وإنتاج بعض المثبطات الميتابولزمية (Takenaka, Shigehito وآخرون، 2003) من بين تلك الإنزيمات إنزيم proteases، إنزيم cellulases و إنزيم glucanase (El-Katatny وآخرون، 2006، Horner وآخرون، 2012) و إنزيم Cellulose (Takemotoوآخرون، 2007) وقد عزا (Benhamou 2009) أن الفطر *P.oligandrum* يفترس عوائله الفطرية في وجود الإنزيمات محللة، أما الإنزيمات: chitinase - endo، ß-glucanase، protease، ß-glucosidase و cellobiohydrolase، فيفرزها الفطر لتعمل على تحطيم الجدر الخلوية للأجسام الحجرية sclerotia المنتجة بواسطة الفطريات الممرضة (Boominathan and Sivakumaar 2012).

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Effect *P. oligandrum* and its filtrated on growth of some phytopathogenic fungi

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Abstract: In this study, the antagonism effect of *P. oligandrum* against some plant pathogenic fungi such as *A. ocharcenus*, *B. cinerea*, *F. oxysporum*, *F. solani*, *R. solani* and *S. sclerotiorum* was studied. By using dual culture and poisoned plates techniques. Direct confrontation between the colonies of *P. oligandrum* and all pathogenic fungi resulted in an inhibition their growth. The results of the activity fungus filtrate at different concentrations indicated to the radial growth inhibition of all pathogenic fungi at all concentrations of the antagonist filtrate, and increased the inhibition with concentration increase.

Key words: *Pythium oligandrum,* fungus filtrate, antagonism, plant pathogenic fungi.

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