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THE ROLE OF K\(^+\) CHANNEL SUBTYPES IN CATECHIN INDUCED VASORELAXATION IN RAT’S AORTIC RINGS

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Abstract;

The current study aimed to investigate the role of K\(^+\) channels subtypes in relaxant effects of catechin on rat’s aortic rings. The isometric tension of the thoracic aorta was measured using ADI PowerLab Data Acquisition System. Catechin at concentrations (10\(^{-5}\) to 5x10\(^{-1}\)M) produced more potent relaxant effects in phenylephrine (10\(^{-6}\) M) precontracted aortic smooth muscle with Log IC\(_{50}\)'s of -1.159 mg/ml as compared to potassium chloride (60 mM) with Log IC\(_{50}\)'s of -6.373 mg/ml. The catechin induced relaxation in aortic rings precontracted with phenylephrine was 17.464 ± 0.068 %, whereas for aortic rings precontracted with potassium was only 5.574 ± 0.131%. In aortic rings preincubated with glibenclamide (10\(^{-5}\)M) and tetraethylammonium (1mM), catechin relaxant effect was enhanced with Log IC\(_{50}\)s of -3.119 and -3.001 mg/ml, respectively. On the other hand, in aortic rings precontracted with PE and preincubated with BaCl\(_2\) and 4-AP, catechin induced statistically non-significant relaxant effects as compared with aortic rings of the control aortic ring which was precontracted with PE. From the results of the current study, it can be concluded that catechin produced more potent vasorelaxant effect on aortic rings precontracted with phenylephrine that KCl. This vasorelaxant effect of catechin is produced via the activation of both K\(_{ATP}\) and K\(_{ca}\), but not Kir and Kv channels subtypes.

Keywords: K\(^+\) channel blockers, Catechin, vasorelaxation, aorta.

Introduction

Catechins, which are also known as flavan-3-ols, are simple forms of flavonoid; based on their structure, they are classified as flavanols which include catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate.

Epicatechin concentrations are high in apples, blackberries, broad beans, cherries, black grapes, pears, raspberries, and chocolate. The remaining catechins such as epigallocatechin, epicatechin gallate, and epigallocatechin gallate are found in high concentrations in both black and green tea (Williamson and Manach, 2005 and Ghayur et al., 2007).

Catechins has an important protective role against many diseases such as heart disease, stroke, atherosclerosis, hypertension, kidney disorders, obesity, diabetes (Crespy and Williamson, 2004) and act as antithrombic agent (Yang, et al, 1999). Furthermore, the antioxidant effects of Catechins has been recognized as they prevent the oxidation of antioxidants such as vitamin E and even some times elevate its level (Alessio, et al, 2003 and Tijburg, et al, 1997). In vitro studies on Catechins have shown that it inhibit catechol O-methyltransferase (COMT), the enzyme that degrades norepinephrine; and thus, reflecting the important role of the sympathetic nervous system and its neurotransmitter norepinephrine in the control of thermogenesis and fat oxidation (Dulloo et al., 1999).

Catechin can induce vasorelaxant responses via the stimulation of endothelial Nitrous oxide (NO) and nitrous oxide synthase (NOS) (Huang et al., 1999 and Lorenz et al., 2004) or increased the production of PGI\(_2\) (Mizugaki et al., 2000). Catechin-evoked vasorelaxant effects appear to be both endothelium-dependent and independent (Huang et al., 1998). It has been indicated that the effect of Epigallocatechin-3-gallate on rat’s aorta may be used as an interesting model for the subsequent development of new PDE- inhibitory drugs for improving the pharmacological treatment of diseases such as cardiovascular pathology (Alvarez, et al, 2006).

Taking into the consideration the above informations on Catechin effects, and since no attempt have been made to study the role of K\(^+\) channels subtypes in vasorelaxation effect of Catechins, the current work was undertake to shed light on the role of above channel subtypes in vasorelaxation induced by catechin in rat’s aortic rings.
Materials and Methods

Materials

Albino Rats

Adult male albino rats, Rattus rattus norvegicus (250-350g) used in the current study were bred in the Department of Biology, Faculty of Science, University of Zakho. The rats were reared in standard PVC rat cages, maintained in the laboratory under controlled temperature (22 ± 2 °C), and photoperiod of 12-hours light/dark cycle using automated light-switching device. All rats were provided with standard food pellets prepared as described by Shekha, (2010) with a free access to water ad libitum. The animals were acclimatized to the laboratory conditions for 1-2 weeks before using them in the experiments.

Methods

Isolated Aorta Preparation

The animals were injected intraperitoneally with heparin (1500 units/ kg body weight) and left for 30 min, to avoid blood clotting and possible damage of endothelial layer of the aorta (Fulton et al., 1996). Animals were then anesthetized with Ketamine (40 mg /kg) and Xylazine (10 mg/Kg) intraperitoneally.

The chest cavity was opened and after the removal of excess tissue and fat, the aorta was isolated and transferred to a beaker containing Krebs solution aerated with carbogen [95 % oxygen (O_2) and 5 % Carbon dioxide (CO_2)]. The beaker was placed in the water bath at 37 °C and the aorta cut into small rings of about 2-4 mm long.

Measurement of Isometric Contraction in Isolated Rat Aorta

The procedure of Shekha and Al-Habib, (2012) was followed with some modifications to study the vascular reactivity in the isolated aorta. Two stainless steel wires were carefully passed through the lumen of the aortic rings. One of them was anchored to the base of glass organ chamber and the other was connected to a force transducer (Model MLT0201/RAD) coupled to the transbridge amplifier connected to a PowerLab Data Acquisition System and computer running chart software (Version 7) used for isometric tension measurement. Special attention has been taken during the preparation to avoid damaging the endothelium. The extents of contraction and relaxation were expressed by the tension recorded by the system. The relaxation rate was defined as \( T_{\text{relaxation}} / T_{\text{contraction}} \) expressed as a percentage.

Prior to the experiment, 10 ml of Kreb’s solution was placed inside the glass tissue chamber and the organ tissue bath system was maintained at 37 °C by circulating water through the water jacket from a circulating water bath set at 37 °C. The aorta was continuously aerated by carbogen (95 % O_2 and 5 % CO_2). The initial tension was set at 2 g weight and left for 60 minutes. The aortic segments were initially exposed to 60 mM K+ to test their functional integrity. After that, the chamber medium was changed several times until a stable resting tone was recorded, then the experiments were started.

Statistical Analysis

The statistical analysis was performed using two-way analysis of variance (ANOVA) supported by Bonferroni test when carrying out a pairwise comparison between the same dose of different groups using Graphpad prism program, version 6.01. Analysis of variance for repeated measurements was applied to data consisting of repeated observations at successive time points. P-values less than 0.05 were considered as statistically significant. In all figures, the symbols (*, ** and *** ) representing mean differences are significant at the 0.05, 0.01 and 0.001 levels, respectively. The maximum contractile responses to catechin were calculated as a percentage of the contraction produced by PE or KCl and expressed as the means ± standard error of the mean (SEM). The tension produced by vasoconstrictors (PE and KCl) was defined as 0% tension, and the baseline tension before adding vasoconstrictors was 100% tension.

Results

Relaxant Effect of Catechin on Aortic Rings Contracted by PE and KCl

Typical traces from representative experiments on the relaxing effect of different concentrations of catechin on PE and KCl precontracted aortic rings are shown in figure (1). Dose-response curves for the effect of catechin on PE- and KCl-induced contractions are shown in figure (2). Catechin at concentrations from 10^{-2} to 5X10^{-1} M resulted in a highly significant relaxant effect (P<0.001) in PE (10^{-6} M) as compared with KCl (60 mM) precontracted thoracic aortic rings.

Catechin produced a more potent inhibitory effect on PE than KCl induced contractions,
with Log IC$_{50}$'s of -1.159mg/mL (Log IC$_{50}$ of CI 95% between -6.708 to 4.389) and -6.373 mg/mL (LogIC$_{50}$ of CI 95% between -22.430 to 9.686), respectively. Catechin produced highly significant relaxant effects on PE-induced contractions which was 17.464 ± 0.068%, whereas catechin produced a mild relaxant effect on the aortic ring precontracted with KCl (5.574 ± 0.131%).
Role of Potassium Channels Subtypes in Vasorelaxation Induced by Catechin

The role of K⁺ channels subtypes in the relaxant effect of catechin was investigated by preincubation of aortic rings in GLIB (10⁻⁵), TEA (1mM), BaCl₂ (1mM) and 4-AP (1mM) and precontracted with PE. The Dose-response curves for Catechin effect on PE precontracted aortic rings preincubated with K⁺ channel blockers are shown in figures (3, 4, 5 and 6).

In aortic rings precontracted with PE and preincubated with GLIB in the presence of catechin showed a highly significant (P<0.001) relaxant effect at doses (3X10⁻³-5X10⁻¹) and with TEA also showed a significant (P<0.01) relaxant effect at concentrations (10⁻²-5X10⁻¹) on aortic rings. The percentages of relaxation were 31.089±0.136% and 58.392±0.110%, and with Log IC₅₀’s of -3.119 mg/ml (with a Log IC₅₀ of CI 95% between -3.994 to -2.245) and -3.001 mg/ml (with a Log IC₅₀ of CI 95% between -3.875 to -2.128), respectively. On the other hand, aortic rings precontracted with PE and preincubated with BaCl₂ and 4-AP showed limited relaxant effects which were statistically non-significant to catechin as compared with aortic rings precontracted with PE as control with Log IC₅₀’s of -1.159 mg/ml (with a Log IC₅₀ of CI 95% between -6.708 to 4.389) and the percentages of relaxation were 4.247 ± 0.280 and 17.464±0.068%.

![Fig. 3: Cumulative dose-response curve for the relaxant effects of catechin in aortic rings preincubated with GLIB, precontracted with PE.](image-url)
Fig. 4: Cumulative dose-response curve for the relaxant effects of catechin in aortic rings preincubated with TEA, precontracted with PE.

Fig. 5: Cumulative dose-response curve for the relaxant effects of catechin in aortic rings preincubated with BaCl₂, precontracted with PE.
Discussion

The results of the study showed that catechin in a dose-dependent mode inhibited the contractions induced by PE and high K⁺ concentration in isolated rat thoracic aorta. The flavonoid tested in this study was found to be more sensitive in relaxing the contraction induced by PE. The results of one of the previous study suggested that the reduction of transmembrane Ca²⁺ influx and/or agonist induced release of intracellular Ca²⁺ in these cells may contribute to the vasorelaxant action of catechin, although these effects do not seem to be important for lower concentrations of the catechin (Alvarez, et al., 2006).

From the results of the current study, it has been observed that vasorelaxation-induced by catechin was enhanced significantly by TEA and GLIB, but not by 4-AP and BaCl₂. These results suggest that vasorelaxant responses to catechin are mediated through increasing K⁺ efflux at least, via K_{ATP}, K_Ca channels. There is growing evidence that the K_{ATP} channels activity may be modulated by NO. Kubo et al., (1993) demonstrated that nitric oxide donors cause activation of K_{ATP} channels in rat aorta by means of a cyclic GMP-dependent mechanism, possibly cyclic GMP-dependent PK.

Álvarez et al., (2006) demonstrated that catechin-induced relaxation in rat aortic rings was endothelium-independent and mediated by the inhibition of phosphodiesterase (PDE) 1-5 isoform activity. Moreover, it was reported that catechin has the ability to induce contractile responses in isolated rat aorta (Sanae et al., 2002; Shen et al., 2003 ; Álvarez-Castro et al., 2004).

From the results of the present study it can be concluded that the relaxant effect of catechin on aortic smooth muscle is mediated via the activation of K_{ATP} and K_Ca, but not Kir and Kv channels.

References


پویش:

لیکوئیدهایی که در ابتدا در سال ۱۹۹۴ توسط Catechin، با نام خریداری می‌گردیده‌اند، تاکنون بیش از ۵۰۰ مولار، با توجه به فیتولین در محصولات مختلف، با هدف بهبود همان‌گونه که در بالاترین مقدار تولید می‌شود. در این مطالعه، لیکوئیدهایی که نام خریداری می‌گردیده‌اند، با توجه به فیتولین در محصولات مختلف، با هدف بهبود همان‌گونه که در بالاترین مقدار تولید می‌شود.

PowerLab Data Acquisition System

گرزبرونه‌کنی در هر نسخه‌ای از ماسولککنی به دانشگاهی که دریافت می‌شود، لیکوئیدهایی که نام خریداری می‌گردیده‌اند، با توجه به فیتولین در محصولات مختلف، با هدف بهبود همان‌گونه که در بالاترین مقدار تولید می‌شود.

الماتراک با هدف بهبود همان‌گونه که در بالاترین مقدار تولید می‌شود، لیکوئیدهایی که نام خریداری می‌گردیده‌اند، با توجه به فیتولین در محصولات مختلف، با هدف بهبود همان‌گونه که در بالاترین مقدار تولید می‌شود.

MOL (۵۰۰ مولار)، با توجه به فیتولین در محصولات مختلف، با هدف بهبود همان‌گونه که در بالاترین مقدار تولید می‌شود، لیکوئیدهایی که نام خریداری می‌گردیده‌اند، با توجه به فیتولین در محصولات مختلف، با هدف بهبود همان‌گونه که در بالاترین مقدار تولید می‌شود.
EFFECT OF RENAL STONE FORMATION ON SOME RENAL RELATED PARAMETERS IN KOYA CITY

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(Accepted for publication: May 27, 2015)

Abstract:
The thirty stone former patients (12 men with 18 women) involved in the current study, were attended the Shaheed Khalid Hospital in Koya city between April to November 2013. The cases diagnosed in the hospital by clinical examination followed by kidney urinary bladder KUB x-ray and ultrasonography. Further more thirty healthy person (15 men with 15 women) were also involved in this study as control group.
The data of a current study revealed the dominant prevalence of the renal stone type both in men and women was for uric acid stone type, and calcium carbonate, calcium phosphate and calcium oxalate represent moderate types, and cysteine stone type represent the lowest type among involved patients. The stone formation was more prevalence in age group 51-60 that represent 27% of the total included formers.
The results showed significant (p< 0.05) elevation in serum creatinine, urea and uric acid levels in both sexes stone formers, whereas serum total protein and albumin levels were significantly lower in both men and women stone formers as compared with the levels in normal healthy group. The hematological results showed significantly lower numbers of RBC number and Hb content in both men and women stone formers, whereas, WBC count and platelets count showed higher numbers in both men and women stone formers as compared normal healthy group.

Key words: (Renal stone formers, Hepatorenal parameters, Heamatological parameters)

Introduction
Urolithiasis is the most common urological disease affecting human worldwide and stone formation contributed to genetic and environmental factors (Harpreet Kaur et al., 2012). Stone disease is an increasing and major public health problem with more predominance in men (Memon et al., 2009).
Pathogenesis of urinary stones formation is thought to be multifactorial, the infection of urinary tract is a major factor. Kidney stones may contain various combinations of chemical forms, that occur when salts in the urine precipitate and form solid materials that resulted from a wide variety of metabolic and environmental disturbances (Sharda and Zia, 2006). Urinary stones are typically classified by their chemical composition into calcium-containing, struvite, uric acid, or other compounds (Lieske and Segura, 2004). Stones were predominantly of mixed type, with calcium oxalate as commonest constituent (Quaader et al., 2006).
Creatinine, urea and uric acid are frequently used to assess kidney function, and their elevated serum levels indicate kidney dysfunction (Lawrence et al., 2003). Creatinine is released into the blood at a constant rate and freely excreted by the kidneys. For this reason, creatinine test is frequently used to assess kidney function its elevation indicate kidney dysfunction (Hamid, 2008).
Urea is one of the major nitrogenous wastes in blood, which are toxic end products of catabolism and they are normally removed from the blood and excreted by kidneys at a rate that balances their rate of production.
Renal disease is one of the major disease states that are associated with elevated plasma uric acid concentration because its filtration and secretion are impaired (Lawrence et al., 2003).
The kidneys maintain the blood volume and regulate the mineral content in the bloodstream. The liver converts nutrients into energy, forms proteins and stores carbohydrates. While these organs can be remarkably resilient in the elimination of toxins, their other functions can be damaged in the process.
Kidneys as the common endocrine organs are responsible for controlling red cell production through erythropoiesis by release of erythropoietin (EPO) hormone, which its deficiency leads to the decrease of RBCs and Hb (Suressh et al., 2012). Renal diseases are
associated with a variety of haemopoietic changes. Anemia parallels the degree of renal impairment and its most important cause is failure of renal erythropoietin secretion. Other factors include chronic blood loss, hemolysis and bone marrow suppression by retained uremic factors (Suresh et al., 2012). The current investigation aimed measurement of the hepatorenal functions tests and hematological parameters in stone formers.

Materials and Methods

This study included (30) patients of renal stone former (18 men with 12 women), who attended the Shaheed Khalid Hospital in Koya city from April to November 2013. The diagnosis of the cases was done in the hospital based on the clinical examination followed by kidney-urinary bladder KUB x-ray, ultrasonography, intravenous urography IVU and urinalysis. Furthermore this study also included 30 healthy (15 men with 15 women) subjects at same ages free of kidney disease signs, they were randomly selected as a healthy group for comparison.

Five ml of venous blood samples were obtained from both patients and healthy group by sterile disposable syringe. After coagulation of the blood, each blood sample was centrifuged for 3 minutes at 4000 rpm to get a clear and cell free serum. The serum was used for biochemical parameters assay. The colorimetric Elitech Diagnostic kits were used for measurement of renal function test parameters (serum total protein, creatinine, urea, uric acid, albumin and bilirubin. In this method, 1 ml of serum was added to flexor tube and the concentrations of the parameters were analyzed using automatic chemical analyzer (Benchtop automatic biochemistry analyzer (ELITech) (FLEXOR EL200, ELITech clinical systems), France.

An aliquot of blood was immediately removed and mixed with ethylene diamine tetra acetic acid (EDTA) as an anticoagulant. The blood samples were analyzed for blood parameters using a full automated hematological analyzer according to the manufacturer's protocol. The hematological parameters (WBC, RBC, Hb, Pt, and Hct) were analyzed on the same day a blood samples collection using Coulter Counter.

Statistical analysis

The SPSS (statistical package for social science) (V 20) T- test was used to analyze the data. The P value (P<0.05) was considered to be statistically significant.

Results

The results showed that the prevalence of the uric acid renal stone type more dominant both in men and women and represent 38.09% of stones. The percentage of calcium carbonate, calcium phosphate and calcium oxalate types were 19.04% for each type and the remaining 4.76% was composed of cysteine stone (Table 1).

Table 1: The prevalence of the renal stone types

<table>
<thead>
<tr>
<th>Type of stones</th>
<th>Cysteine stone</th>
<th>Calcium carbonate</th>
<th>Calcium phosphate</th>
<th>Calcium oxalate</th>
<th>Uric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of Patients</td>
<td>4.76</td>
<td>19.04</td>
<td>19.04</td>
<td>19.04</td>
<td>38.09</td>
</tr>
</tbody>
</table>

Results showed that renal stone formations are more prevalence in age groups 51-60 which represent 27% of the total stone formers (Table 2).

Table 2: The prevalence of the renal stone in relation to age groups

<table>
<thead>
<tr>
<th>Age groups</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51-60</th>
<th>61-70</th>
<th>71-80</th>
<th>81-90</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of patients</td>
<td>3.57</td>
<td>10.71</td>
<td>21.42</td>
<td>27</td>
<td>23</td>
<td>14.28</td>
<td>3.57</td>
</tr>
</tbody>
</table>
The results showed significant elevation in serum creatinine level 1.137±0.143, 1.185±0.184 in both men and women stone formers respectively as compared to normal men and women subjects 0.751±0.047, 0.630±0.057 respectively, also serum urea level showed significant elevation in both men and women 35.08 ± 1.415, 34.41 ± 1.881 respectively, as compared to its level in normal men and women subjects 21.21 ± 1.419, 20.5 ± 2.121 respectively. The level of the uric acid was showed significant elevation in both men and women stone formers 5.528 ± 0.165, 5.191 ± 0.211 respectively, as compared to its level in normal men and women subjects 3.33 ± 0.21, 3.1 ± 0.271 respectively. Whereas both men and women stone former showed significant lower serum total protein 6.725±0.172, 6.626±0.166 respectively, as compared to their levels in normal men and women subjects 7.422±0.141, 7.283±0.148 respectively. The level of the albumin was showed significant lower in men and women stone formers 4.250±0.259, 4.262±0.208 respectively, as compared to their levels in normal men and women subjects 5.021±0.103, 4.868±0.142 respectively. The level of bilirubin in men and women stone formers showed non-significant lowering 0.725±0.138, 0.403±0.045 respectively, as compared to their levels in normal men and women subjects 0.803±0.104, 0.495±0.046 respectively (Tables 3 & 4).

Table 3: Renal related parameters in renal stone former men compared to healthy men

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls M±S.E.</th>
<th>Patients M±S.E.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (gm/dl)</td>
<td>7.422±0.141</td>
<td>6.725±0.172</td>
<td>0.005</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>5.021±0.103</td>
<td>4.250±0.259</td>
<td>0.012</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.803±0.104</td>
<td>0.725±0.138</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.751±0.047</td>
<td>1.137±0.143</td>
<td>0.029</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>21.21 ± 1.419</td>
<td>35.08 ± 1.415*</td>
<td>0.004</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>3.33 ±0.21</td>
<td>5.528 ± 0.165*</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

NS= non-significant

Table 4: Renal related parameters in renal stone former women compared to healthy women

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls M±S.E.</th>
<th>Patients M±S.E.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (gm/dl)</td>
<td>7.283±0.148</td>
<td>6.626±0.166</td>
<td>0.008</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>4.868±0.142</td>
<td>4.262±0.208</td>
<td>0.030</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.495±0.046</td>
<td>0.403±0.045</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.630±0.057</td>
<td>1.185±0.184</td>
<td>0.014</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>20.5 ± 2.121</td>
<td>34.41 ±1.881*</td>
<td>0.005</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>3.1 ±0.271</td>
<td>5.191 ± 0.211*</td>
<td>0.002</td>
</tr>
</tbody>
</table>

NS= non-significant

The results showed significantly lower numbers of RBC and Hb content in both men and women stone formers, as compared with normal subjects, whereas WBC and platelets showed higher numbers in both men and women stone formers, as compared with normal subjects (Tables 5 and 6).

Table 5: Hematological parameters in renal stone former men

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls M±S.E.</th>
<th>Patients M±S.E.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC x 10^6 cell/mm³</td>
<td>5.008±0.096</td>
<td>4.410±0.219</td>
<td>0.020</td>
</tr>
<tr>
<td>WBC cell/mm³</td>
<td>6.876±0.250</td>
<td>8.525±0.615</td>
<td>0.021</td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td>14.79±0.231</td>
<td>13.76±0.439</td>
<td>0.039</td>
</tr>
<tr>
<td>Platelet x 10^9 cell/mm³</td>
<td>227.3±11.10</td>
<td>273.8±14.73</td>
<td>0.019</td>
</tr>
</tbody>
</table>
Table 6: Hematological parameters in renal stone former women

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls M±S.E.</th>
<th>Patients M±S.E.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCx10^6 cell/mm³</td>
<td>4.778±0.154</td>
<td>3.508±0.190</td>
<td>0.0001</td>
</tr>
<tr>
<td>WBC cell/mm³</td>
<td>6.891±0.499</td>
<td>8.446±0.433</td>
<td>0.032</td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td>12.66±0.328</td>
<td>11.08±0.310</td>
<td>0.003</td>
</tr>
<tr>
<td>Platelet x 10^3 cell/mm³</td>
<td>207.5±10.61</td>
<td>243.4±11.75</td>
<td>0.035</td>
</tr>
</tbody>
</table>

Discussion

The data of the current study showed that the prevalence of the renal stone types both in men and women are higher for uric acid stone type than calcium carbonate calcium phosphate and calcium oxalate types. This results are in agree with previous results reported that approximately 80% of stones are composed of calcium, 10% of struvite, 9% of uric acid, and the remaining 1% are composed of cystine (Fredric et al., 2005). In another study reported that calcium oxalate monohydrate is the most frequent stone component (Alaya et al., 2011). In anther study results indicated that calcium stone predominated with male predominance of the most prevailing in Egypt (Al-Ali et al., 2002).

The results showed significant elevation of serum creatinine, urea and uric acid levels in both men and women stone formers. Elevation of serum creatinine in the current study confirms the results of (Kasem (2004), who explained that this increase in creatinine level may be due to reduced renal function and increased acidic medium in renal tubules and impaired metabolism as well as kidney complications lead to higher creatinine. Recently, another study revealed a significant increase in serum levels of urea, creatinine, and uric acid and a significant decrease in total protein level in kidney stone former patients (Kaniaw, 2013). Kidney stone cause dysfunction of the kidney as a result of cell injuries and increasing the accumulation of nitrogenous waste products in the blood (Worcester et al., 2006). Elevation of urea as a result of the damage of some renal nephrons units leads to a defect in urea filtration and excretion that reported in previous study (Hayder Bawa, 2008). High serum uric acid level might lead to stone formation and a decreases in the solubility of calcium oxalate salts in urine (Mehdi, 2008).

In the current study, serum total protein and albumin levels showed significant lowering levels in both men and women stone formers. These results are in agreement with another study in Sulaimani governorate (Kaniaw, 2013), she reported lower level of serum total protein and albumin levels in stone former patients cause of kidney dysfunction as a result of stone formation and kidney tissue damage.

The results showed significantly lowering in the numbers of RBC and Hb content in both men and women stone formers, whereas, WBC and platelets showed higher numbers in both men and women stone formers. This may be caused by impaired erythropoietin production and other factors which suppress marrow erythropoiesis and shortened red cell survival (Suresh et al., 2012). In the absence of EPO, DNA cleavage is rapid and leads to cell death (Suresh et al., 2012). RBC survival is decreased in uremic patient’s in proportion to the blood urea nitrogen concentration and, it improves significantly after intensive hemodialysis. Uremic plasma increases the expression of phosphatidylserine on the outer cell surface in red blood cells. This enhances the recognition of damaged red blood cells by macrophage, leading to their subsequent destruction and decreased survival. (Michael et al., 2004). Red blood cells survival is presumed to be a toxic substance normally excreted or metabolized by the kidneys, one such substance is guanidine and its derivatives which appear to be a subset of the many retained metabolites, adversely affect erythrocyte survival (Robert and means, 2004).

In conclusion renal stone patients have lower hematological indices, due to impaired production of erythropoietin, and other factors like increase haemolysis, suppression of bone marrow erythropoiesis, hematuria and gastrointestinal blood loss. The concentration of serum creatinine shows negative correlation with all the
haematological parameters. And the degree of changes depends on the severity of renal failure.

Reference


لا يمكنني قراءة أو فهم النص العربي في الصورة. أنا بحاجة إلى نص باللغة الإنجليزية للمساعدة.
TOXIC EFFECTS OF ESSENTIAL OILS OF ELATTARIA CARDAMOMUM L. AND LAMBDA- CYHALOTHRIN ON TRIBOLIUM CONFUSUM (DUVAL)

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Abstract:
The toxic effect of Lambda-cyhalothrin and essential oils of cardamom seeds of Elettaria cardamomum (L.), individually or in combination, on the larva, pupa and adult stages of confused flower beetle, Tribolium confusum, through the topical application were studied. The percentages of mortality of larval stage exposed to Lambda-cyhalothrin, at concentrations of 0.25, 0.35, 0.50 and 0.75 µl/ larva were 13.33, 20, 36.67 and 46.67 % respectively. While, the percentages of mortality of pupa stage at concentrations of 0.25, 0.35, 0.50 and 0.75 µl/ pupa were 13.33, 16.67, 26.67 and 43.33 respectively. For adults, exposed to Lambda concentrations of 0.25, 0.35, 0.50 and 0.75 µl/ adult were 10.0, 13.33, 23.33 and 40.0 respectively. The percentages of mortality of larvae exposed to cardamom oil at concentrations of 0.1, 0.3, 0.5 and 1 µl/ larva were 60, 76.67, 90 and 96.67% respectively, while the mortality of pupa exposed to concentrations of 0.1, 0.3, 0.5 and 1 µl/ pupa were 46.67, 63.33, 83.33 and 90 respectively. The percentages of mortality of the adults exposed to concentrations 0.1, 0.3, 0.5 and 1 µl/ adult were 43.33, 60.0, 76.67 and 83.33 respectively.

The synergistic ratios for larvae, pupae and adults exposed to mixture 1 (0.03 µl / insect cardamom oil and different concentrations of pesticide), were 2.01, 1.70 and 1.60, respectively, where as the synergistic ratios of larvae, pupae and adults exposed to mixture 2 (0.07 µl / insect cardamom oil and different concentrations of pesticide) were 2.61, 2.34 and 3.10, respectively.

This indicates that a combination of cardamom seeds oil with different concentrations of the insecticide Lambda produced synergistic effects.

Keywords: Cardamom, Tribolium confusum, Lambda- cyhalothrin, Synergistic.

Introduction

The broad incorrect and uses of insecticide had led to the development of resistance in several species of insect to insecticide. Since the number of insect species resistance to insecticides was about 460 species until 2003. The side effects of insecticides use prompting many conservationists call to stop the use of insecticides and prevent their production. This call was unrealistic as evidenced by increased production and use of insecticides throughout the world, which of course refers to the insecticides still is the effective means adopted by human to control the pests, (Meister, 2010).

The most convenient alternative to reduce the use of insecticides is the rational and correct methods of use in order to reduce the damage produced from their side effects. In addition anabolic substances of insecticides increase the effectiveness when used at low concentrations. (Metcalf, 1972).

The cardamom, Elettaria cardamomum L. (Zingiberales: Zingiberaceae), called the "Queen of spices" in India, is a tall, perennial, reed-like herb that grows wild and cultivated in India and Sri Lanka. The essential oils have therapeutic benefits such as gastrointestinal, cardiovascular and neural disorders (Lewis, 1984; Aneja and Sharma, 2010; Abbasipour et al. 2011). Some studies showed that cardamom essential oils have toxic effect against some stored insects (Jacobson, 1989). A study by Sivakumar et al. (2010) revealed that cardamom essential oils showed toxicity on cowpea weevil, Callosobruchus maculates (F.).

The chemical composition of cardamom varies considerably with the variety, region, and age of the product. The content of volatile oil in the seeds is strongly dependent on storage conditions. Some studies showed that the main components found in the volatile oil from E. cardamomum were 1,8-cineole (29.7 to 36.3%) and α-terpinyl acetate (26.1 to 31.3%) (Olivero-Verbel et al. 2010 and Korikontimath et al. 1999). The later also reported that the volatile oil of E. cardamomum contained about 11.6% limonene, and 3% linalool,. In addition, the basic cardamom aroma is produced by a combination of the major components, 1,8-cineole and α-terpinyl acetate (Lawrence 1979).

Lambda-cyhalothrin is a synthetic pyrethroid insecticide, which is the analogues of pyrethrins, and naturally occurring insecticidal compounds produced in the flowers of chrysanthemums (Chrysanthemum cinerariaefolium). Insecticidal
products containing pyrethroids have been widely used in pest control (Amweg and Weston 2005; Oros and Werner 2005; He et al. 2008).

The aim of the present study was to investigate the effect of lambda and *Elattaria cardamomum* essential oils applied individually or in combination on larva, pupa and adult of *T. confusum*. In addition, this paper describes for the first time the toxicity of the essential oils of cardamom against important stored product pests: the confused flour beetle, *Tribolium confusum* Duval (Coleoptera: Tenebrionidae).

**Materials and Methods**

**Insects** The insect species, *T. confusum*, was used during the present study. Larvae, pupae and adults were obtained from laboratory cultures origin incubated in dark at 30±1°C and relative humidity 70±80% using incubators (LAB TECH. Korea). *T. confusum* was reared on wheat flour mixed with yeast (1:10, w:w). The 5th instar larvae of *T. confusum*, newly formed pupae (few hours – 24 hours) and adults (one week after emergence) were used in contact toxicity experiments.

**Extraction of Cardamom essential oil**

Seeds of Cardamom (50g) were grinded with electrical grinder (Tata 300 w). and the obtained powder was extracted with 300 ml petroleum ether (boiling point 60-80°C) in Soxhlet apparatus. The extraction was condensed using thin film Vacuum rotary evaporator to evaporate the solvent from cardamom oils. The extracted essential oils was transferred to glass bottles and kept in refrigerator until needed.

**Methods of Treatment (contact toxicity)**

Each developmental stage (larva, pupa or an adult confused flour beetles) was treated superficially (Topical application) with 5 µl of the desired concentration of the tested material, which was added on the upper surface of the insect thorax (Notum) using 50 µl capacity micro-syringe (Huang et al. 2000). The control group was treated with appropriate solvent (water in the case of the pesticide and petroleum ether in extract case). After 15 minutes, the treated groups were placed in petri dishes and saved in incubator to avoid contamination by any abnormal insect. The percentages of mortality for larvae and adult beetles were recorded after 24 hours, and pupa until adult emergence.

**Effect of a combination of cardamom extract and Lambda.**

The surface treatment for larvae, pupae and adults of confused flour beetles were tested using MSDOS system according to Probit program which gave the lethal doses (5% (LD5) = 0.03 µl and 15% (LD15) = 0.07 µl) of the extract and then treated with various concentrations of the pesticide. The mortality rate was recorded after 24 hours of larval and adult, and pupa until adult emergence.

**Estimating Synergistic Ratio (SR)**

To calculate synergy ratios the formula of Brattesten and Metcalf (1970) was used as follows

\[
\text{Synergistic Ratio} = \frac{\text{LD50 or LC50 to pesticide only}}{\text{LD50 or LC50 to pesticide + synergistic}}
\]

The synergistic ratio is equal to the number of times the increase in pesticide toxicity caused by the synergistic.

**Results and Discussion**

Effect results of effect of cardamom seeds extract on of various life cycle stages of confused flour beetle are shown in Table (1), which indicate that the essential oil of the cardamom seeds extracts has toxic effects on the larvae, pupae and adult of the confused flour beetle. The percentages mortality of the larvae exposed to at concentrations of 0.1, 0.3, 0.5 and 1 µl/ larva were 60, 76.67, 90 and 96.67% respectively, where as the percentages of mortality of pupae exposed to concentrations of 0.1, 0.3, 0.5 and 1 µl/ pupa were 46.67, 63.33, 83.33 and 90%, respectively.

The percentages of mortality of the adult insects exposed to concentrations 0.1, 0.3, 0.5 and 1 µl/ adult were 43.33, 60.0, 76.67 and 83.33% respectively. The mortality ratio values revealed that the essential oil of cardamom seeds extract is more toxic to the larvae as compared with pupae and adults (Fig. 1).
These results are in agreement with other studies which showed the toxicity test of cardamom oil and different vegetable oils against many types of insects. Ngamo et al., (2007) found that oils extracted from black sesame *Hyptis spicigera* were toxic to adults of various insects such as Rice weevil *Sitophilus oryzae*, Maize weevil *Sitophilus zeamais*, cowpea weevil *Callosobruchus maculatus* and the red flour beetle *Tribolium castaneum*. Furthermore, essential oils extracted from Neem (*Azadirachta indica*), Tulasi leaves (*Ocimum sanctum*), and seeds of pongamia (*Pongamia pinnata*) have toxic effects to the wax worm larvae (*Galleria mellonella*), and their percentages of mortality ranged between 93-52% (Surendra et al., 2010).

It was found that the essential oils extracted from leaf laurel (*Laurus nobilis*), myrtle (*Myrtus communis*) and thyme (*Thymus sipyleus*) were toxic to adult of beans weevil (*Acanthoscelides obtectus*) and red flour beetle (Karaborklu *et al.*, 2010). Also, Padin and his coworker investigate the toxicity effects of essential oils extracted from nine different types of plants. They found that all extracts were toxic to red flour beetle adults, and the mortality rate of Ethanolic extract from *Viola arvensis* was 68% (Padin *et al.*, 2013).

**Effect of insecticide Lambda-cyhalothrin on stages of confused flour beetle**

The percentages of mortality of various developmental stages of the flour beetle *Tribolium confusum* exposed to various concentrations of Lambda-cyhalothrin are shown in (Table 1). The percentages of mortality of larval stage exposed to concentrations of 0.25, 0.35, 0.50 and 0.75 µl/ larva were 13.33, 20, 36.67 and 46.67%, respectively; while, the percentages of mortality of pupae exposed to concentrations of 0.25, 0.35, 0.50 and 0.75 µl/ pupa were 13.33, 16.67, 26.67 and 43.33%, respectively. Adults beetles exposed to concentrations of 0.25, 0.35, 0.50 and 0.75 µl/ adult, the percentages of mortality were 10.0, 13.33, 23.33 and 40.0%, respectively. These results clearly indicate that the pesticide has relatively more toxic effect against larvae as compared with pupae and adults (Figure 2).
The results of the current study on the toxic effect of Lambda on flour beetles agreed with other studies on the toxicity of lambda insecticide and other insecticides against different insects species. Umeda et al., (2000) found that the insecticide lambda is extremely toxic against cabbage looper (Trichoplusia ni) and diamondback moth (Plutella xylostella). Furthermore, the rates of mortality in both insects were generally increased with the increasing of the duration of exposure. Additionally, the insecticide Aktara was very toxic to 1 day aged adults of Khapra beetle (Trogoderma granareum) and cowpea weevil Callosobruchus maculatus and 4 weeks aged adult of red flour beetle and confused flour beetle (Tribolium confusum).

![Figure (2)](image_url)

Figure (2) Effect toxic effect of Lambda-cyhalothrin on various developmental stages of the flour beetle Tribolium confusum.

The effectiveness of the toxicity of lambda insecticide was proven to be very toxic against red flour beetle (Uddin and Ara, 2006). Chavan et al. (2012) proved that the lethal dose of insecticide lambda against teak skeletonizer larvae (Eutectena machaeralis) after 12 hours of treatment was 81.14 %. Naz et al. (2013) revealed that the lambda insecticide is highly toxic to four types of berry bug, (Halys spp.) in comparison with Chloropyriphos insecticide and Neem extract.
Table (1) Effect of Cardamom extract, Lambda insecticide and mixture ratios in mixtures 1 and 2 on the percentages of mortality of Larva, pupa and adult of flour beetles

<table>
<thead>
<tr>
<th>phases</th>
<th>Insecticide</th>
<th>Extract</th>
<th>Mixture 1</th>
<th>Mixture 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. µl</td>
<td>Mortality %</td>
<td>Conc. µl</td>
<td>Mortality %</td>
</tr>
<tr>
<td>5th instar Larvae</td>
<td>0</td>
<td>0.00 z</td>
<td>0</td>
<td>16.67 v</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>13.33 w-y</td>
<td>0.1</td>
<td>60.00 i-m</td>
</tr>
<tr>
<td></td>
<td>0.35</td>
<td>20.00 u-x</td>
<td>0.3</td>
<td>76.67 e-h</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>36.67 p-t</td>
<td>0.5</td>
<td>90.00 cd</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>46.67 m-q</td>
<td>1</td>
<td>96.67 ab</td>
</tr>
<tr>
<td>Pupae</td>
<td>0</td>
<td>0.00 z</td>
<td>0</td>
<td>6.67 y</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>13.33 w-y</td>
<td>0.1</td>
<td>46.67 m-q</td>
</tr>
<tr>
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<td>0.35</td>
<td>16.67 v-x</td>
<td>0.3</td>
<td>63.33 h-l</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>26.67 s-v</td>
<td>0.5</td>
<td>83.33 d-f</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>43.33 n-r</td>
<td>1</td>
<td>90.00 cd</td>
</tr>
<tr>
<td>Adults</td>
<td>0</td>
<td>0.00 z</td>
<td>0</td>
<td>10.00 xy</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>10.00 xy</td>
<td>0.1</td>
<td>43.33 n-r</td>
</tr>
<tr>
<td></td>
<td>0.35</td>
<td>13.33 w-y</td>
<td>0.3</td>
<td>60.00 i-m</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>23.33 t-w</td>
<td>0.5</td>
<td>76.67 f-h</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>40.00 o-s</td>
<td>1</td>
<td>83.33 d-f</td>
</tr>
</tbody>
</table>
In the present study, the toxicity of the insecticide varied with the developmental stages (larvae, pupae and adult) of confused flour beetle. The insecticide was more toxic to larvae than pupae and adults. This difference in sensitivity to the insecticide could be mainly attributed to the variation of the levels of the insecticide metabolic enzymes.

This result is in line with Saidana et al. (2007) as it was found that the petroleum ether extracts for several types of plants were very effective against larvae and adults of the confused flour beetle, but the larvae were more sensitive than the adults. Also, the present results agreed with Soummane et al. (2011), they investigated the effect of the methanol extract of aerial parts of some medicinal plants on Mediterranean fruit fly, Ceratitis capitata, it was found that the extract was more toxic to larvae as compared to adults.

By comparing the effect toxic effect of insecticide with that of cardamom oil on the developmental stages of the flour beetle, the results clearly indicated that the oil extract was more effective than the insecticide lambda.

**Effect of combination cardamom oil extract and Lambda on larvae, pupae and adults.**

The results of the toxic effect of a combination cardamom seed oil and Lambda showed the presence of seed oil enhanced the toxicity of the insecticide lambda on larvae, pupae and adults of the confused flour beetle, which was increased with increasing the synergistic ratio.

The effect on 5th instar Larvae.

As shown in Figure 1, the highest percentage of mortality rate (73.33%) was observed in larvae exposed to mixture (1) (0.03µl extract and 0.75 µl insecticide). The percentage of mortality was with increasing the concentration of the oil extract. When the oil extract (0.07 µl) mixed with the four insecticide concentrations (ranging 0.25-0.75 µl), the highest percentage of mortality (83.33%) was observed at an insecticide concentration of (0.75 µl).

The effect on Pupae.

The highest mortality rate (56.67%) was obtained with the mixture (1), which consists of 0.03 µl seed oil extract and 0.75 µl Lambda. There is a proportional relationship between the percentage of mortality oil extract concentration. When oil extract (0.07 µl) mixed with the four insecticide concentrations (ranging 0.25-0.75 µl), the highest percentage of mortality (70.00%) was observed at an insecticide concentration of (0.75 µl).

The effect on Adults.

The highest percentage of mortality (53.33%) was observed in adult insects exposed to mixture (1), (0.03 µl extract and 0.75 µl Lambda). The percentage of mortality was increased with increase the concentration of the oil extract. When oil extract (0.07 µl) mixed with the four insecticide concentrations (ranging 0.25-0.75 µl), the highest mortality rate (73.33%) was observed at an insecticide concentration of (0.75 µl).

Results in Table (2) showed that the synergistic ratios were 2.01, 1.70, and 1.60 in the larvae, pupae and adults, respectively. Figure 3 shows the synergistic ratios of insects exposed to mixture (1), which consist of 0.03 µl /insect of cardamom seeds oil extract combined with various concentrations of Lambda.
Figure (3) Synergistic effect in mixtures 1 and 2 on various developmental stages of confused flour beetle stages

Whereas, in mixture (2) the synergistic ratios were 2.61, 2.34, 3.10 larvae, pupae and adults, respectively. Figures 4-6 shows the addition of 0.07 µl / insect of the cardamom seeds oil extract with various concentrations of Lambda, showed increased mortality ratios as compared to insecticide alone and mixture (1).

Figure (4): Effect of Lambda, Mixture 1 and Mixture 2 on the percentage of mortality of larvae of confused flour beetle.
Figure (5): Effect of Lambda, Mixture 1 and Mixture 2 on the percentage of mortality of Pupae of confused flour beetle pupae.

Figure (6): Effect of Lambda, Mixture 1 and Mixture 2 on the percentage of mortality of adults of confused flour beetles.
The synergistic effect of cardamom seeds oil extract on the toxic effect of lambda may be attributed to that these materials increase the permeability of the pesticide through the cuticle and facilitate its incoming to the target site, this conclusion agrees with that of Sun and Johnson (1972); Shufeng et al. (2005) whom suggested that synergistic effect may be due to facilitating the entry of the pesticide through cuticle.

On the other hand, Wilkinson (1976); Michalets (1998) and Mckinnon et al., (2008) have suggested another mechanism for the synergistic effect by inhibition of function oxidative enzymes which are responsible for the degradation (metabolized) to pesticide.

This confirms the previous conclusion that the variation of insects sensitive to pesticides is attributed to primarily to the contrast enzymes levels that metabolize the pesticide, This conclusion is in agreement with those of Cassida et al. (1966) and Franklin (1972) that the percentage of inhibition ratio depends on the synergistic concentration. Furthermore, Khalequzzaman and Rumu (2010) studied the effect of mixing cardamom essential oil with primiphos-methyl pesticide (1/20) and they showed a synergistic effect by increasing percentage of mortality against adult cowpea weevil.

Table (2) Synergistic ratios in both mixtures 1 and 2, and their mixing ratios

<table>
<thead>
<tr>
<th>Phases</th>
<th>Mixture 1</th>
<th></th>
<th>Mixture 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td>Insecticide</td>
<td></td>
<td>Insecticide</td>
</tr>
<tr>
<td></td>
<td>Extract</td>
<td>Conc. µl</td>
<td>Extract</td>
<td>Conc. µl</td>
</tr>
<tr>
<td></td>
<td>Insecticide Conc. µl</td>
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<td>Conc. µl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>5th Instar Larvae</td>
<td>0.03</td>
<td>0.35</td>
<td>2.01</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.50</td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75</td>
<td></td>
<td>0.75</td>
</tr>
<tr>
<td>Pupae</td>
<td>0.03</td>
<td>0.25</td>
<td>1.70</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35</td>
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<td>0.35</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.75</td>
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<td>.75</td>
</tr>
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</table>
Reference


مهدومة دياربو ريزا (الجاور) بو (يرقات و عزاري و بالغات) ل تيكول 1 دا (0.03 ميكرولي / ميلو زيت توقف (الجاور) لهذ ريزا جاواز درمان (اللامدا) كعمسه 18.01, 2.00, 1.70, 1.60, 1.50, 1.40, 1.30, 1.20, 1.10, 1.00, 0.90, 0.80, 0.70, 0.60, 0.50, 0.40, 0.30, 0.20, 0.10, 0.00. مايكرولي / برقة نسب القبل المذوبة لليرقات المعرضة للمبيد الكيميائيلامدا عند الواركي 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.25, 3.50, 3.75, 4.00, 4.25, 4.50, 4.75, 5.00 مايكرولي / عذراء. كانت نسب القبل المذوبة للترطر الفيري عند الواركي 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.25, 3.50, 3.75, 4.00, 4.25, 4.50, 4.75, 5.00، 0.75 مايكرولي / بالا. فكانت نسب القبل المذوبة 60, 63, 66, 69, 72, 75, 78, 81, 84, 87, 90, 93, 96, 99, 100 على الالوالي. نسب القبل المذوبة لليرقات المعرضة لمستخلص زيت بذور الجاور عند الواركي 0.1, 0.05, 0.1, مايكرولي / برقة. فقد كانت 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, 0.25. كانت نسب القبل المذوبة عند الواركي 0.01, 0.05, 0.1, مايكرولي / بالا. هي 0.33, 0.37, 0.41, 0.45, 0.49, 0.53, 0.57, 0.61, 0.65, 0.69, 0.73, 0.77, 0.81, 0.85, 0.89, 0.93, 0.97, 1.00. كما أظهرت نسب التأثرة لليرقات والمداي والبالغات المعرضة للغاية 0.1 مايكرولي / حشرة زيت بذور الرايز (ذات Tests جاواز، ميتا. 0.01 دا (0.00.12 مايكرولي / إنداني زيت بذور الرايز (ذات Tests لمبيد الكيميائيلامدا له تأثير تأري.
Trichodina sp. AS BIOINDICATOR FOR EVALUATION OF BIOCHEMICAL OXYGEN DEMAND (BOD5) IN AQUACULTURE FISH FARMS (PONDS)

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(Accepted for publication: May 18, 2015)

Abstract:
The present study include the using of the prevalence of the fish infestation by the protozoan Trichodina sp. as bioindicator for evolution of the biological oxygen demand BOD5 (lowering down of the dissolved oxygen DO) from Ainkawa fish hatchery. For this purpose, two handered and forty (240) finger ling fishes of Cyprinus carpio were collected from six ponds (40 samples from each pond) and fishes were examined from December, 2012 to the end of february, 2013. Biological oxygen demands were measured by azid modification of Winkler method (through measuring of dissolved oxygen DO) for each pond. Results reveal that there are a direct relationship between the prevalence of fish infestation by the Trichdina sp. and the values of BOD5. The prevalence of fish infestation in each pond increased (57.5, 40, 27.5, 45, 15, and 42.5% respectively) with the increase of the values of BOD5 (9.2, 8.0, 5.7, 8.1, 2.9 and 8.0 mg.l⁻¹, respectively).

Key words: BOD5, Trichodina, Water pollution, Erbil.

Introduction
The concept of aquaculture development as a potential source of food supply is receiving much attention in Iraq. This has resulted in rapid growth in fish farming practice. The intensification of such fish culturing creates disease problems that originate from overcrowdness or deteriorating water quality, such as unsuitable water temperature, pH, dissolved oxygen, BOD5 and organic mater (Dujin, 1973 and Kugel et al., 1990).

Trichodina (Ciliophora: Protozoa) are very common ectoparasites which in most cases are pathogenic to both freshwater and marine fish (Wellborn, 1967). Dogiel (1961) reported that trichodiniaasis caused by Trichodina was stimulated by water quality and the high density of fish in ponds. It causes irritation by feeding on the epithelial cells covering the surface of the gills and skin of the fish, which can result in hyperplasia (proliferation) of the epithelial cells, clubbing and even fusion of the gill filaments. This affects the abilities of both gills and the skin to maintain optimal respiratory and excretory activities, and the ability of the skin to maintain proper homeostatic osmoregulatory properties. Massive infestations of these parasites on fish can also directly result in superficial to deep ulcerative skin lesions which allow for secondary bacterial and fungal infections to develop at the affected site (Smith and Schwarz, 2009).

In Iraq, The first information concerning genus Trichodina was given by Shamsaddin et al. (1971), who recorded T. domerguei on eight species of fishes brought from different fish markets in Baghdad city. For the next years a total recorded number of Trichodina reached eighteen species (Mhaisen, 2014).

In the present study, only Trichodina sp. were selected because based on the previous study which were done on the different fish farms in Iraq and Kurdistan region appear that Trichodina sp. make a large problem in all aquaculture fish farm after Ichthyophthirius and Lernaea cyprinacea Mama and Abdullah (2010) and Al-Marjan and Abdullah (2009).

The aim of this study is to know the relationship between fish infestation by Trichodina sp. and the BOD5 as a step for evaluation of the water quality of the fish farms.

Description of the Study Area
Ainkawa fish hatchery is located northwest of Erbil city, Kurdistan region, Iraq. This project was built in 2000 on 27 hectare, and started working in 2005. In this region there are eighteen ponds of different sizes, among these six ponds were selected. Fishes of various sizes were stocked for greater growth and artificial reproduction (Fig. B).
Materials and Methods

This study includes two aspects, the first deals with the isolation of the *Trichodina* sp. which infect common carp *Cyprinus carpio* in Ainkawa fish hatchery farms, situated northwest of Erbil city, Kurdistan region-Iraq, for this purpose 240 fingerling fishes were collected from six ponds (40 samples from each pond) and transported alive in a cool box containing tank water (free from chloride) to the laboratory during the period from December, 2012 to the end of February, 2013. Each fish was examined by taking smears from skin, fins, and buccal cavity through slight scraping. The gills were removed, and placed in a Petri dish containing water then microscopically examined. Smears were stained by Klein’s dry silver method for observation of the adhesive disk as suggested by Asmat (2001). All slides were examined under a light compound microscope at 700X.

The second aspect included the measurement of the biological oxygen demand (BOD5) through measurement of dissolved oxygen based on azide modification of Winkler method (Maiti, 2004). Samples of water were collected by a special bottle (BOD bottle, 300ml capacity) from each fish farm separately.

Results and Discussion

The prevalence of fish infestation by *Trichodina* sp. in each pond were variable 57.5, 40, 27.5, 45, 15 and 42.5% respectively with the change of the BOD5 concentration in each ponds 9.2, 8.0, 5.7, 8.1, 2.9 and 8.0 mg.l⁻¹ respectively (Fig. 2).

The analysis of the results indicate that there are a relation ship between the BOD5 concentration and the prevalence of fish infestation by the *Trichodina* sp (Fig. 3). The highest prevalence of the fish infestation by the parasite (57.5%) and the highest concentration of the BOD5 (9.2 mg.l⁻¹) were recorded in the pond number one. Depending on these data, we can evaluate the quality of ponds water because high BOD5 values is an indicator for poor quality of water (Peter and Ludemann, 1972). On the other hand low concentration of the BOD5 value (2.9 mg.l⁻¹) and low prevalence of fish infestation by *Trichodina* sp. (15%) was recorded in pond number five.

Biological oxygen demand (BOD5) is defined as the amount of oxygen required by microorganisms in decomposing organic materials in a sample under "aerobic condition at 20°C over a period of 5 days (Maiti, 2004).
High BOD5 values means presence of microorganisms (like \textit{Trichodina}) in water and consumption of small amount of oxygen, which may supported by fecal coliform results in lowering the amount of dissolved oxygen and elevation of BOD5. Results of the present study agree with those of Othman, (2013).

Several parasitologists and ecologists mentioned the presence of a direct relationship between the concentration of organic materials and the prevalence of infection by the parasite especially Trichodinid ciliates, because they can be used as potential bioindicator fish farms (Yeomans, 1997). A strong relationship between ecological parameters and parasite infection were found from Lesser Zab and Greater Zab rivers in north of Iraq (Abdullh, 2002). Seasonal dynamics of \textit{Trichodina} sp. on whiting (\textit{Merlangius merlangus}) in relation to organic pollution on the eastern Black Sea coast of Turkey was reported by Palm and Harry (2005).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Shows percentage of infested fish by \textit{Trichodina} sp. and values of biochemical oxygen demand (mg.l$^{-1}$) in the studied ponds.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Shows relationship between percentages of infested fish by \textit{Trichodina} sp. and values of biochemical oxygen demand (mg.l$^{-1}$) in the studied ponds.}
\end{figure}
References


يله (BOD5) (Trichodina sp.) له Arduino ماسي

بقيمة تزريرية برتينج يليه يبتكرودينات بسياق لى جوزة 

وبه بعملية وفيرة دكم و لدى Trichodina sp. (BOD5) (نورمويل نوكسيجي تواوة) له كينيدي كاتي

بديكتداني ماسي له عيناقة. لبوي تتم مبربقية دوو مواد جل (240) بتجسماس لهجوزي كاري وناسب

كروكايده له شش كينيدي 40 راسش ماسي له هيريك له كينيدي للماء. 

كاناتي يلمكي عام 2013 بكوتيبي مانغي شوباتي سالي 2013. ووه هاملبات يلي نيوندن بى BOD5

زينكايا (BOD5) له 

(ريكيبيا دهنازدري كتاني نوكسيجي تواوة) له

هيريك له كينيدي كاتي. ووه مانجيكاكي ليكولينهوه ك ددركورتو ك يبرودندي راستورانتو هيمي له كنون ريزدي

ووه نغيز كل ريجوز توشيون زيان دهكت له هيريك

له كينيدي كاتي (BOD5) (40.5, 40.5, 40.5, 40.5, 40.5 و 15, 15 و 15 و 15 و 15 و 15 ) بيه زيادوري خسستي

لله كينيدي كاتي (BOD5) (0.6 مل/لتر, 0.6 مل/لتر, 0.6 مل/لتر, 0.6 مل/لتر, 0.6 مل/لتر, 0.6 مل/لتر, 0.6 مل/لتر)

بقدواي ينك (1).


كيليل حيوي لقييم المتطب الحيوي للأوكسجين (BOD5) في الأزواج الاستامك (Trichodina sp.)

الخلاصة: 

اجريت هذه الدراسة على استامك مقم بعينة لفحص استعمال نسب استعمال

بـ BOD5 (Trichodina sp.) (Triturina) (Cyprinus carpio)

الداب (DO) وهذا الفرع تم جمع ماتان واربون (240) خصوصا من جمعة الكارب (الرافعي)

من مزايا احساس (40 نوجد من كل حوز) خلال القراءة المخصرة بين كانون الأول 2012 مالي نهاية

الظاهرة BOD5 بطريقة تراكيز الأوكسجين المذاب (DO) جميع الاحساس.

خلال تحليل النتائج، ظهرت ترابط مباشرة بين نسب استعمال الاستامك بالواتروكودينا و قيمة ال

BOD5 حيث ازدادت نسبة استعمال الاستامك في كل حوز من الاحساس السنية (40.5, 40.5, 15, 15, 20, 20, 20)

و مع ازداد تراكيز المتطب الحيوي للأوكسجين في تلك الاحساس (7.2, 7.2, 8.0, 8.0, 8.0, 8.0

مل/لتر على التوالي).

كلمات المدخلة: BOD5, الوايكيودينيات، تتوع مياه، اربيل.
DIAGNOSIS OF TOXOPLASMOSIS IN SHEEP USING SEROLOGICAL (ELISA) AND MOLECULAR TECHNIQUE IN DUHOK GOVERNORATE - KURDISTAN REGION

Farhad Buzo Mikaeel, Jassim Abdo and Lokman Taib Omer
Duhok Research Center, Faculty of veterinary Medicine, University of Duhok, Kurdistan Region – Iraq.
(Accepted for publication: May 3, 2015)

Abstract:
Toxoplasmosis is an important zoonotic diseases in human and animals. The disease caused by the protozoan *Toxoplasma gondii*. It is an economically important disease of livestock, especially sheep and goats. The present work aimed to diagnose toxoplasmosis in sheep using two methods, serological (ELISA) and molecular tools (PCR) and comparing the serological data with the molecular results to determine the sensitivity and the specificity of the molecular tools.

The study was carried out at Duhok Research Center, University of Duhok. Ninety sex whole blood samples were collected from in aborted ewes in Duhok governorate during the period September 2013-September 2014. The samples were collected from different localities including Aqra, Dohuk district, Shikhan and Zakho. The serological tests showed that 22(22.91 %) of the samples were positive from examined sheep serum by ELISA, while 23(23.93%) by using PCR assay. PCR was performed on all DNA of sheep blood samples to amplify B1 gene as a target sequence.

Good correlation between the results of PCR and ELISA were detected, there’s no statistically significant difference, it can be concluded that ELISA combined with the PCR technique is a recommended tool for accurate diagnosis of Toxoplasmosis but PCR is more specific for detection of *T. gondii* with sensitivity of 95.45 % and specificity of 97.29%. When taking the ELISA as a reference test. The results of PCR assay showed it’s important in the diagnosis of the carrier infected cases more than ELISA techniques.

Keyword: Toxoplasmosis, Sheep, ELISA, PCR.

1. Introduction
Sheep are important to the economy of many countries because they are a source of food for humans. Sheep are commonly infected with the protozoan *Toxoplasma gondii* infection of sheep and goat poses a risk to public health, as well as economic losses due to reproductive failure (Dubey and Towel, 1986; Dubey and kirkbride, 1990; Edward and Dubey, 2013). The parasite was first described in 1954 (Buxton et al., 2007). Ovine toxoplasmosis has a worldwide distribution especially in temperate sheep rearing countries where the climatic conditions favor oocyst survival (Buxton and Rodger, 2008). Toxoplasmosis is a major cause of reproductive failure associated with abortion in sheep and goat. In sheep, abortion or prenatal mortality of lambs occur when ewes suffer a primary infection during pregnancy (Tenter et al., 2001).

Toxoplasmosis is diagnosed mainly by direct smear, Immunohistochemistry, serology testing and PCR (Mazumder et al., 1988; Ramadan et al., 2007; Dubey, 2009, 2010)

Various ELISA methods using crude, fractionated, or recombinant antigens have been used to detect *T. gondii* antibodies in ovine sera. Compared ELISA based on crude and recombinant antigens found to have varying degrees of specificity in naturally and experimentally-infected sheep (Caballero-Ortega et al., 2008).

The ELISA assay for *T. gondii* antibodies has been adapted for use in most domestic animals including sheep and goat (Dubey, 2008; Dubey, 2009). There is specific ELISA assays for both IgM and IgG subtypes. These ELISA assays are ideally suited to screen large numbers of samples and looking at the IgM/IgG ratio (Denmark and Chessum, 1978). Serological analysis using IFAT and ELISA has been widely employed in order to detect herds infected by toxoplasma, including swine and sheep flocks (Van der Puije et al., 2000).

Moreover, Molecular assays such as PCR make it possible to detect small quantities of target DNA and potentially provide an alternative sensitive diagnostic tool, specific molecular diagnostics of toxoplasmosis is generally based on the detection of a specific DNA sequence, using different assays and protocols, mostly from highly conserved regions such as the B1 gene repeated 35 times in the
genome, 529 bp repetitive element with about 200-300 copies in the genome, ITS-1 (internal transcribed spacer) that exists in 110 copies and 18S rDNA gene sequences (Jones et al., 2000; Habibi et al., 2012; Moazeni Jula et al., 2013; Tavassoli et al., 2013). In general, this technique has been proven as a useful method in diagnosis of clinical toxoplasmosis (Dubey, 2008), it is highly specific and sensitive and very useful together with serological tests to differentiate the chronic, acute or reactivated infections (Neil and Lappin, 1991; Switaj et al., 2005).

Early diagnosis of infection is of great consequence for reducing the severity of the disease and the risk of congenital toxoplasmosis (Behbehani and Al-Karmi, 1980; Edward and Dubey, 2013).

Few serological studies have been conducted on the prevalence of T. gondii antibody in farm animals in Kurdistan Region but no molecular study for detection of parasite DNA have been used. Therefore, the aims of the present study were to estimate the prevalence of the T. gondii antibodies in aborted ewe’s serum by using serological tests (ELISA) and to compared with the detection of parasite DNA using molecular tools (PCR) in order to determine the sensitivity and specificity of diagnostic techniques for the diagnosis of Toxoplasmosis in sheep Duhok Governorate,

2. Material and method:

The study was carried out in the Duhok Research Center /Faculty of veterinary medicine, university of Duhok. The sheep of this study were of local breeding (Karadi), only female aborted ewes were involved in this work.

2.1 Sampling:

Ninety sex whole blood samples were collected from aborted ewes in Duhok governorate during the period September 2013 to September 2014. The samples were collected from different localities including Aqra, Duhok district, Shikhan and Zakho.

The blood samples were collected from the jugular veins of the sheep, each sample was divided in to the two parts, one left few minutes to obtain serum for serological tests, the other part of the blood samples was collected in sterile tubes with EDTA for PCR investigation. The collected sera and blood samples were coded and preserved at -20 °C until used.

2.2 Serological assay:

Sheep IgG antibodies against T. gondii were tested using an enzyme-linked immunosorbent assay (ELISA). The indirect Elisa (ID. VET. Innovative diagnostics, France) was performed using commercial kit. Optical densities (OD) were read at 450 nm. The results were expressed as the percentage of the mean absorbance values of sample (S) to the mean absorbance value of the positive (P) control sample provided with the diagnostic kit. The resultant SP ratio was expressed as a percentage (S/P %). According to the manufacturer’s recommendation, sera with S/P% ≤ 40% should be regarded as negative, between 40 and 50% as doubtful, between 50% ≤ and < 200% as positive, and ≥ 200% as strong positive.

2.3 Polymerasechain reaction (PCR):

2.3.1 DNA extraction

Genomic DNA was extracted from blood samples using a commercially available kit, QIAamp DNA, blood and tissue kit (Qiagen, Hilden, Germany). DNA extraction and purification protocol was recommended by the manufacturer.

Amplification was conducted in a total volume of 25 μl. The reaction mixture contained 12.5 ul, 2X ready PCR mix (KAPA2G, PCR kit, KAPA Biosystems; USA ) which consisted of 1.25 ul Taq-Pol, 75 mM Tris-HCL (pH 8.8), 1.5 mM MgCl2, and 0.2 mM of each dNTP. The reaction mixture contained 12.5 ul master mix, 10 pmol of each forward and reverse primers (Table 1), 1 ug DNA template, and 8.5 ul RNase free water to a total volume of 25 ul.

2.3.2 Primer selection

The primer pairs used in the PCR experiment were TOX4 (CGCTGCAGGGAGGAAGACGAAAGTTG) and TOX5 (CGCTGCAGACACAGTGACATCTGGATT), as described by (Homan et al., 2000)

2.3.3 PCR technique

The amplification was carried out in a thermal cycler (Ependrof, Germany) according to the following program: an initial denaturation step at 95°C for 7 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing, primer at 55°C for 1 min, extension step at 72°C for 45 sec. and a final extension step at 72°C for 5 min. Amplified PCR products were the PCR products examined by electrophoresis in 1.5% agarose gel. The amplified PCR product was then extracted and purified using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). The PCR product was cloned into the pGEM-T Easy Vector System II (Promega, Madison, WI, USA). The positive colonies were selected and the inserts were sequenced by MWG-Biotech (Germany).
agarose gel, stained with ethidium bromide solution, visualized under UV transilluminator and photographed

4. Results

In the results of the diagnosis of *T. gondii* infection in sheep relied on the immunological (ELISA) and molecular tests, as presented in Table 1, ELISA detected anti-*T. gondii* antibodies in 22 out of 96 sheep (22.91%), whereas 23/96 (23.9%) of the samples were positive by PCR. Two of the samples were positive with PCR but they were negative with ELISA assay and one sample was seropositive but negative with PCR (Figure 1).

(Fig. 1). Gel agarose electrophoresis (1.5%) for *T. gondii* PCR products analysis M: 100 bp DNA size marker Lane 1 (c control positive) *T. gondii* B1 gene PCR products (529 bp) were amplified by external primers. Lanes 2 is negative control and 3, 4, 5, 6, 9 is positive clinical samples for *T. gondii* B1 gene, b, 8 is negative clinical samples.

Table 1. Infection rate of *T. gondii* in examined sheep using PCR, ELISA

<table>
<thead>
<tr>
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<th>ELISA</th>
<th>PCR</th>
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<tbody>
<tr>
<td>Positive</td>
<td>22(22.91%)</td>
<td>23(23.9%)</td>
</tr>
<tr>
<td>Negative</td>
<td>74(77%)</td>
<td>73(76%)</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>96</td>
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</tbody>
</table>

Compared with ELISA The sensitivity and specificity of PCR for detecting Toxoplasmosis measured according to use ELISA test as reference tools The sensitivity and specificity were 95.45% , 97.29 respectively (Table 2).

Based upon the Kappa statistical test, a good correlation 0.913(perfect agreement) between the results of ELISA and PCR. Strength of agreement based on $\hat{c}$ was judged according to the following guidelines: $<0.2$=slight; 0.2–0.4=fair; 0.4–0.6=moderate; 0.6–0.8=good; >0.8=very good, and 0.8-1= perfect agreement.

Table 2. Relative sensitivity and specificity of PCR in comparison with standard ELISA

<table>
<thead>
<tr>
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<th>ELISA</th>
<th>PCR</th>
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<tbody>
<tr>
<td>Positive</td>
<td>21(a)</td>
<td>2(b)</td>
</tr>
<tr>
<td>Negative</td>
<td>1(c)</td>
<td>72(d)</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>74</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>96</td>
</tr>
</tbody>
</table>

Sensitivity = $a/(a + c); 95.45$
Specificity = $d/(b + d) \quad 97.29$
Kappa agreement $*= 0.913$

*When two measurements agree by chance only, kappa = 0. When the two measurements agree perfectly, kappa = 1

5. Discussion:

Sheep represent an important source of meat, milk and wool for humans in many countries, and toxoplasmosis causes great economic losses to sheep industry worldwide (Buxton et al., 2007) The prevalence of *T. gondii* infection in sheep and goats may be due to sheep free range livestock associated with *T. gondii* infection due to contamination of environment with oocytes. The frequent presence of stray cats in a humid rainy climate favor the survival of oocytes which contributed to the Toxoplasma prevalence in Kurdistan. The primary goal of this study was to assess the risk of *T. gondii* infection to sheep in Kurdistan and to use different tools for the diagnosis of the parasite from aborted ewe. Out of 96 samples 22(22.91%) were positive by using ELISA while the 23(23.9%) samples were positive with B1 gen using PCR two.

Two samples were positive with PCR but negative with ELISA assay. The explanation is that there are cases of infection with *T. gondii* where serum levels of antibody are still not enough to trigger ELISA-sensitive reactions, ELISA is unable to detect the infection at early stages of infection. Again, in acute phase of infection *T. gondii* when death can happen in only few days, the disease progression is too fast to let immune system of animal to produce ELISA-detectable levels of antibody (Nguyen et al., 1996). One sample was seropositive but PCR negative results can be explained by the fact that antibodies may be present in the absent of parasite or may be false positive reaction due to the cross-reactivity, since several studies suggested a closer relationship between *T. gondii*
Detection of *T. gondii* DNA using PCR minimizes the problems which the researcher may face when using serological methods only and facilitates the diagnosis in complex cases. Compared to other countries, it has been found that *T. gondii* seroprevalence in Duhok was higher than that in Rahim Yar Khan (Punjab), Pakistan (11.2%) and India (3.8%) (Ramzan *et al.*, 2009; Sharma *et al.*, 2008), but lower than the prevalence rate of toxoplasmosis in Turkey and Iran which were (31%) for both (Taraneh *et al.*, 2006 and Sharif *et al.*, 2014 respectively). The differences could be related to differences in ecological and geographical factors such as temperature, rainfall or landscape differences. The study area had overall low temperatures and it generally thought that the prevalence and risk of *T. gondii* infection decrease with decreasing temperature because it affect the survival of oocytes in the environment such as pastures.

In Iraq, the prevalence of the *T. gondii* in sheep is higher in south than that of the north of the country (Ali Akber *et al.*, 2014) study showed that the province of Babylon is the highest in its seropositivity percentage in sheep 39.5%, followed by the province of Al-Anbar (34.4%) and the province of Nineveh (30%) as compared to the study of other Provinces. The present study showed that *T. gondii* seroprevalence in Duhok was lower than Babylon, Al-Anbar and Nineveh respectively. The differences could be due to the hot and humid environment in south, higher prevalence rate of toxoplasmosis in warm, moist areas compared to those with colder and dry is attributed to the longer viability of *T. gondii* oocytes in moist or humid environments. The study area had overall low temperatures and it generally thought that the prevalence and risk of *T. gondii* infection decrease with decreasing temperature because it affect the survival of oocytes in the environment such as pastures. PCR provides a simple and safe method for accurate and early diagnosis of toxoplasmosis (Tavassoli *et al.*, 2009; Moazen Jula *et al.*, 2013).

In General many study has been performed using the serological and molecularare diagnosis of *T. gondii* such as (Burg *et al.*, 1989; Al-Sanjary and Hussein, 2012; El-Madawy and Metawea 2013; Al-Abady *et al.*, 2014), all those studies agree with our conclusion that molecular (PCR) is more sensitive and specific tools than the serological tools for the detection of *T. gondii* parasite. The sensitivity and the specificity of PCR for detecting Toxoplasmosis was measured according to ELISA test as a reference test.

**References**


دبیسیشانکرنا نسخه کتابی کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابا کتابa
تشخيص داء المقوسات في الأغنام باستخدام فحص المصلي (اللبلاب) وتقنية الجزيئية في محافظة دهوك - إقليم كوردستان

الخلاصة:

داء المقوسات يعتبر من الأمراض المهمة المشتركة بين الحيوانات والبشر. الإصابة بهذا المرض يتسبب عن طريق Toxoplasma gondii العدوى بطيفي من الأغام والكثير الأنوائي. يعتبر هذا المرض من الأمراض المهمة من الناحية الاقتصادية في الثروة الحيوانية خاصة في الأغنام والماعز. أجريت الدراسة الحالية لتشخيص المرض في الأغنام بواسطة فحص المصلي (ELISA) والتقنية الجزيئية (PCR) وكذلك مقارنة النتائج في كل الفحصين لتحديد نسبة الحساسية والدقة التقنية الجزيئية.

أجرت الدراسة في مركز دهوك للبحوث البيطرية في جامعة دهوك. تم جمع ستة وتسعة عينات دم من العناجر التي أُجهضت خلال فائدة الحمل في محافظة دهوك من الفترة من شهر أيلول 2013 وليومأيلول 2014. تم قياس العينات من عدة مناطق في المحافظة وتشمل منطقة عقيرة، مركز مدينة دهوك، منطقة شيخان، ومنطقة زاخور.

بينت الفحوصات المصلية باستخدام فحص الألبوموزينات وعرضه 99.24% نسبة نتائج الإيجابية، في حين أظهرت الفحوصات الجزيئية باستخدام تقنية PCR نتائج إيجابية لثلاثة عشرون عينة (93.22%). الفحص الجزيئي باستخدام تقنية PCR أجريت على جميع العينات بعد استخلاص المادة الحمض النووي من الدم مباشرة لفحص تضخم جين B1. لم تكن هناك أي اختلافات في دالة PCR و ELISA.

أظهرت هذه الدراسة علاقة جيدة بين نتائج فحص الجزيئي والفحص المصلي. تستطيع أن تُثبت هذه الدراسة بأن استخدام فحص PCR الطريقة الأمثل لتشخيص المرض بصورة دقيقة ولكن بالرغم من ذلك تبقى تقنية ELISA أكثر دقةً لتحري عن Toxoplasma gondii. مع نسبة الحساسية تصل إلى (95.45%) ونسبة الدقة تصل إلى (97.29%). عندما تأخذ فحص PCR كفحص مؤqué، نتائج تقنية (ELISA) أظهرت أهميتها في التشخيص في حالات المزمنة أكثر من نتائج ELISA.

(ELISA)
BIOASSAY AND PATHOGENICITY OF WHEAT SEED GALL NEMATODE
ANGUINA TRITICI

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Abstract:
Highest infection percentage recorded in Abo-graib and wahie CVs. (83.3%) followed by Smeto (80.3%) while less infection percentage was in Rezg are and Al-Aez CVs. (33.3%). more galls (14 galls/spike) formed in Sham-2 C.V. followed by Abo-graib, Smeto, Tammoz-2, Maxipak and Al-Aez respectively. highest number of nematode (12773) occurred in large galls, while the lowest (8145) in small galls. A. tritici population density increased dramatically with increase in galls size reached a peak (15112 second stage juveniles/gall) in big size gall. Results revealed that the vitality of second stage juveniles reached its maximum level (8.87%) at 12 Cº with no significant differences with the validity percentage at 6 Cº while it reached its minimum level (1%) at 20 Cº.

KEYWORDS: Bioassay, pathogenicity, Wheat, Anguina tritici.

INTRODUCTION
Wheat (Triticum aestivum L.) is the most economical important crops in the world. 65% of produced wheat is consumed by human, whereas 21% - 14% for animal feeding and industry (Gooding and Davies, 1977). According to USAID (2006), Iraq currently consumes nearly 4 million tons of wheat annually, yet only produces 500,000 tons of qualified wheat. Thus more than 85% of consumed wheat in Iraq imported, the current average is 800 Kg/h. The Iraqi Kurdistan Region produces about 50% of the wheat produced in Iraq.

According to Bhatti, et al., (1978) Ear- cockle was the oldest reported disease of wheat which was caused by wheat seed gall nematode Anguina tritici. It is one of the major aerial diseases and causes sustainable losses in wheat crop of tropical and sub-tropical countries (Kort, 1972). It is present wherever wheat is grown and this pest still common in Eastern Europe and in part of Asia and Africa (Agrios, 2005). From the first record (1921) of this nematode in Iraq (Rao, 1921), A. tritici remain the major pest in Iraq occurred in the most wheat growing areas by 22.9 to 45% on mexipac c.v. of Wheat (Al-Beldaw; et al, 1974) increased to 75% on the same cultivar in Duhok Province in 1989 (Stephan and Antoon, 1990), Ami, et.al, (2004) reported that the percentage of infestation by galls reached its maximum value (50%) in bread wheat in Bashika – Kurdistan region of Iraq.

Ear- cockle disease reduces market price and human consumption of wheat (Paruthi and Bhatti, 1988), with significant reduction in the protein and gluten contents of the flour product of infested wheat with seed galls (Mustafa, 2009). This study aimed to test vitality of 2nd stage juveniles of A. tritici under different temperature and it’s pathogenicity toward some common wheat cultivars in addition to its effect on quality of wheat flour.

Materials & Methods:
Seed galls (ear-cockle) collection: Galls used in this experiment were collected handmade from infested bread wheat samples brought from Faydia Silo.

Soil sterilization:
Soil was sterilized by Formalin 1% (1 liter/m-3) after sieving to discard stones and soil lumps, and then placed on thick layer of polyethylene. Formalin was mixed thoroughly with soil which was covered by other pieces of polyethylene and closed tightly to prevent any escape of formalin, after 48 hours soil aerated for a week to remove residual of Formalin (Mustafee and Chattopadhyay, 1981).

Physical and chemical analysis of soil:
a- Soil texture: it was determined by hydrometer method to evaluate percentage contents of clay, silt and sand (Jaiswal, 2003).
b- Electrical conductivity (E.C) was estimated by using Conductivity Bridge of soil extract of 1:1. (Dipak and Haldar, 2005).
c- Percentage of organic matter: was measured in the soil by wet digestion method with
concentrated sulphuric acid according to (Black, 1965).

- Soil pH by pH meter in the soil extract of 1:1 (Dipak and Haldar, 2005).

**Susceptibility of wheat cultivar:** Nine wheat cultivars (Abo-qraib, Aba 95, Tammoz-2, Maxipak, Rezgare, Al-Aez, Wahe, Smeto, and Sham-2) were sown at the rate of 5 seeds/pots (20 cm) in diameter containing sterilized soil which was infested directly with five galls, in 1cm deep holes between seeds at the rate of one gall/hole, Pots were plunged randomly in the trenches. This experiment consisted of 18 treatments (9 wheat C.V. X 2 levels of infestation) performed as factorial experiment in complete Randomized design (CRD) with 3 replication for each treatment, carried out in 2014 growing season, in one fields of the College of Agriculture, University of Duhok, Pots were irrigated when needed and the following infection criteria were calculated:

1- Infection percentage according to the following equation:-

\[
\text{Infection percentage} = \frac{\text{Number of Infected plants}}{\text{Total number of plants in each pot}} \times 100
\]

2- Number of galls/spike.
3- Number of seeds/spike.
4- Reduction percentage of seed number /infected spike according to the following:-

\[
\text{Seed number/spike in control treatment} - \text{seed number/spike in infested soil} \times 100
\]

**Effect of size and weight of galls on nematode population:** Galls were divided according to their size to (Small, Moderate and large) and weight [18.3 milligram (19.6-16.5), 11.5mg (12.7-11.3) and 3.6mg (4,1-3.1)]. Each galls was opened in 6.5 Cm diameter Petri-dishes filled with 2ml of distilled water (D.W) with the aid of two needles then transferred to a 250 ml beaker, volume was adjusted by adding D.W water and number of juveniles were calculated by the aid of counting dish and stereomicroscope.

**Effect of different temperature on vitality of second stage juveniles:** Nine Petri dishes were divided into 3 groups and 2ml of nematode suspension (200 second stage juveniles) was transferred into each Petri dish and after covering 1st and 2nd group placed in refrigerator at 6, 12°C respectively while the third group left at laboratory conditions (20°C). Percentage of nematode vitality was calculated according to the following equation:-

\[
\text{Percentage of nematode vitality} = \frac{\text{number of moving nematodes in each replicate}}{\text{Total number of nematode in the same replication}} \times 100
\]

This experiment consists of 5 treatment and run out as CRD.

**Effect of different temperature on the emergence of 2nd stage juveniles from galls:** Four different temperatures including (5, 10, 15 and 20°C) were selected to test their effect on juveniles emergence from their galls which were placed in Petri dish contained a small amount of moist soil at the rate of one gall / petri dish. Examining of galls was done after each 48 hrs. for the period of 20 days to determine the emergence of juveniles.
Results and Discussion:

1- Physical and chemical analysis of soil:

Results of soil analysis (Table 1) revealed that the texture was sandy loam which is suitable for nematode movement as well as chemical characters involving soil salinity, organic matter and soil pH were suitable for most plant parasitic nematode (Ismail, 1998).

Table (1): Physical and chemical analysis of soil.

<table>
<thead>
<tr>
<th>Clay %</th>
<th>Silt %</th>
<th>Sand %</th>
<th>Texture</th>
<th>pH</th>
<th>Organic matter</th>
<th>Electric conductivity (salinity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.13</td>
<td>23.23</td>
<td>59.65</td>
<td>Sandy loam</td>
<td>8.13</td>
<td>1.38</td>
<td>0.52</td>
</tr>
</tbody>
</table>

2- Susceptibility of wheat cultivars:

All wheat cultivars used in this trial were infected by *Anguina tritici* with some significant differences in their susceptibility. Highest infection percentage recorded in each of Abo-ghraib and wahe CVs. (83.3%) followed by Smeto (80.3%) while less infection percentage was in Rezgare and Al-Aez CVs. (33.3%) (Fig. 1) which means there were differences in susceptibility of those cultivars to ear-Cockle disease and also in ability of *Anguina tritici* to infect those cultivars.

![Figure 1](link)

**Figure (1):** Infection percentage *Anguina tritici* with on different wheat cultivars.

Figure 2 illustrates that more galls (14 galls/ spike) formed in Sham-2 C.V. followed by Abo-ghraib, Smeto, Tammoz-2, Maxipak and Al-Aez respectively with significant differences between same cvs. No galls appeared on Abae 95 and Rezgare while both showed symptom of infection, this phenomenon indicated that *A. tritici* can’t completed its life cycle after climbing of seedlings, which might be attributed that cultivar failed to supply adequate essential nutrients for nematode juveniles (Taher, 2012).

![Figure 2](link)

**Figure (2):** Number of galls / spike of different wheat cultivars
Differences in gall number/plant are also due to host genotypes and their susceptibility to *A. tritici* (Hamood and Fattah, 1989). Ear-cockle disease caused reduction in seed production in all tested cultivars. More reduction percentage which were 93.23, 86.93 and 86.93% was recorded from Rezgare, Abae 95 and Tammoz-2 respectively while the lowest were 36.67 and 38.87% recorded from Smeto and Al-Aez, the same results were recorded in different wheat cultivars according to the extent of their susceptibility (Al-Beldawi *et al.*, 1977; Fattah, 1988; Saleh and Fattah, 1990; Stephan *et al.*, 2000; Mustafa 2009; Taher, 2012).

**Figure (3):** Reduction percentage of seeds/spike

3- **Effect of gall size and weight on nematode population density:**

Results indicate significant effect of gall size on the nematode population density, thus highest number of nematode(12773) occurred in large galls, while the lowest (8145) in small galls (Table 2). This result consistent with finding of Ami, *et al*, (2004); Esser *et al*., 1991 and Taher, (2012).

**Table (2):** Effect of galls size on the population density of *Anguina tritici*.

<table>
<thead>
<tr>
<th>Galls size</th>
<th>Nematode population density (Nematode/ gall)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large</td>
<td>12773 a (10114 –13881)</td>
</tr>
<tr>
<td>Moderate</td>
<td>11919 a (9766 – 12088)</td>
</tr>
<tr>
<td>Small</td>
<td>8145 b (7255 – 9617)</td>
</tr>
</tbody>
</table>

Number of juveniles/gall depend on many factors such as wheat cultivar, gall size, environment conditions and soil biosphere characters in addition to their behavior in different geographical regions (Taher, 2012). Weight of galls also has noticeable effect on *A. tritici* population density which increasing dramatically with increase in galls size and reached a peak (15112 nematodes/gall) in big size gall, while number decreased to lowest level (3978 nematode/gall) in small size (table, 3) which means reduction in nematode population by 73.7%.
Regression analysis and correlation coefficient (Fig. 4) demonstrate positive relation between galls weight and nematode population density. From regression equation it was found that an increase of one unit (one mg of galls) in used galls followed by increasing in nematode population density by 803.19 with high determining factor, meaning that it can rely on this equation by 90.7%. Increase in nematode population density in big size galls and heavier ones might be due to the presence of more space in large galls to contain more juveniles during penetration of ovary wall (Ami, et al, 2004) on the other hand more weight and size of newly formed embryo supply more nutrient for feeding of juvenile and increase the ability of female to laying more eggs, otherwise big and heavy galls may belong to durum wheat Cultivars which are always bigger and have more protein contain that have effect in female fertility as we mentioned before.

![Figure (4): Linear relation, regression equation and correction coefficient between gall weight and nematode population density.](image)

**Table (3):** Effect of galls weight on the population density of *Anguine tritici*.

<table>
<thead>
<tr>
<th>Galls weight</th>
<th>Nematode population density (Nematode/ gall)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.3(19.6-16.5)</td>
<td>15112 a</td>
</tr>
<tr>
<td>11.5 (12.7-11.3)</td>
<td>9440 b</td>
</tr>
<tr>
<td>3.6 (4.1-3.1)</td>
<td>3978 c</td>
</tr>
</tbody>
</table>

**Figure (4):** Linear relation, regression equation and correction coefficient between gall weight and nematode population density.

4- Effect of different temperature on vitality of second stage juveniles:

The results of revealed that the vitality of second stage juveniles reached its maximum level (8.87%) at 12 Cº with no significant differences with the validity percentage at 6 Cº while it reached its minimum level (1%) at 20 Cº as well as the vitality percentage increased slowly by the time and stretch 8.33% after 12 days from the time of gall opening with no significant differences with vitality percentage after 9 days (Table 4). On the other hand the interaction between temperature degrees and examining time showed its noticeable effect on vitality. In general maximum level of vitality was (13%) recorded after 12 days at 12 Cº with no significant differences with vitality after 9 days at the same temperature and with that after 9 and 12 at 6Cº while none of the juveniles moved after 3 days at 20 Cº. There are many studies on effect of planting date on infection and all are found that there are differences in infection behaviors of juveniles (Mustafa,2009; Radjender et al., 2009; Taher, 2012) and most important factor in delay or early planting are temperature that influence activity of juveniles. The sudden high temperature may not be a catalyst to re-vitality of juveniles as they were in dormancy stage or cryptobiosis, in fact this study needs further confirmation and granting juveniles more time to check their vitality,
Table 4: Effect of different temperature degrees on the percentage of vitality of second stage juveniles of *A. tritici*.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>1st Reading</th>
<th>2nd Reading</th>
<th>3rd Reading</th>
<th>4th Reading</th>
<th>Effect of temperature degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Cº</td>
<td>1.5 c</td>
<td>3 c</td>
<td>10 a</td>
<td>10 a</td>
<td>8.63 a</td>
</tr>
<tr>
<td>12 Cº</td>
<td>4c</td>
<td>7.5 bc</td>
<td>11 a</td>
<td>13 a</td>
<td>8.87 a</td>
</tr>
<tr>
<td>20 Cº</td>
<td>0.0 c</td>
<td>0.5 c</td>
<td>1.5 c</td>
<td>2 c</td>
<td>1 b</td>
</tr>
</tbody>
</table>

Effect of reading 1.83 b 3.67 b 7.5 a 8.33 a

-which indicates that the juveniles need a longer period of time for emergence at low temperature that means their vitality increased with increasing of temperature that stimulated juveniles for getting out from galls. That means that the temperature plays an important role in emergence of second stage juveniles.

5- Effect of different temperature on the emergence of 2nd stage juveniles from galls: Results illustrated that the juveniles emerged from galls after 20, 16, and 14 days at 5, 10, 15 and 20 Cº respectively with the presence of moisture (Table 5)

Table 5: Effect of different temperature on the emergence of 2nd stage juveniles of *A. tritici* from the galls.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Period of time in days after which juveniles emergence were examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = period after which juvenile exited from galls. - = juveniles were not exited

References


**Anguina tritici**

**اخلاصه:**

ملخص أعلى نسبة الإصابة في صنفي أبو غريب و الواحة (33.33 %) ، بينما كانت أقل نسبة الإصابة في صنفي ريزكاري والغر (8.33 %) .

**کوئتی:**

در برترین ریز انتخابه همانه تومارکان ل هرمزدو جزرین گهشته ای ابو غرب و الواحة (83.33 %) و کمترین ریز انتخابه ل هرمزدو جزرین رژگاری و علر (40 %) .

مکسبیک زئی در دوستون گنگفا رهمو 14 (٪)

Anguina گوگنگی رهمو یوند و کمترین هزرام د گنگفا رهموی بجک (145) هاته تومارکان. هریا هزرامین زئی گوگنگی رهمو گنگفا و کمترین ناست (٪) 15112 (٪)

Anguina tritici

گوگنگی و هرمزدو در نکات هزیکین به هدیه یکی چالاکی و لفنیا نیماتودا گهشته بلندترین ناست (٪) 77.8 (٪) در گرما 7 (٪) و هرمزدو چاردیا به کاره د چالاکی و لفنیا نیماتودا لپی گدرما 6 (٪) در C0 و گهشته کمترین ریزه (1%) لپی گدرما C0.

کوئتی:

بیماری در این مطالعه در صنفی ای ای ال سی بزرگ و الواحة (83.33 %) و کمترین درصد بزرگ و الواحة (83.33 %) و درصد بزرگ و الواحة (40 %) .

مکسبیک زئی در دوستون گنگفا رهمو 14 (٪)

Anguina گوگنگی رهمو یوند و کمترین هزرام د گنگفا رهموی بجک (145) هاته تومارکان. هریا هزرامین زئی گوگنگی رهمو گنگفا و کمترین ناست (٪) 15112 (٪)

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C0.
Characterization of Five Microsatellite Markers for Genetic Diversity Structure Analysis of Walnut (*Juglans regia* L.) in Five Village in Duhok Province

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Abstract:

Five microsatellites markers (WGA202, WGA009, WGA332, WGA225 and WGA069) were used to characterize Persian walnuts (*Juglans regia* L.) populations in five villages (Sharanesh, Bedohe, Kanizarke (Akre), Kashane and Kuzo) of Duhok province. The microsatellites amplified (PCR products) a total of 186 alleles across all populations. The number of alleles per locus ranged from 4 alleles in Sharanesh and Kashane populations to 10 alleles also in Sharanesh and Kashane populations, with an average of 7.4. The molecular sizes of the amplified bands ranged from 158 bp to 289 bp in all populations. The observed heterozygosity (Ho) within populations ranged from 0.59 at WGA225 to 0.65 in the locus WGA202 with an average of 0.61. The PIC value (0.88) indicated that all markers were highly informative and useful for genetic diversity studies in these populations. The proportion of genetic variation presented among populations accounted for 8.4% of the total genetic diversity so it indicated a moderate level of genetic diversity between populations. The Fis average 0.24 indicated that, there was a regular tendency toward heterozygote deficiency and indicated the presence of inbreeding within the populations. The phylogenetic analysis or unrooted neighbor-joining tree highlighted the genetic distance among those five populations and separated them into two main groups placing each population according to its genetic background. The first group consists of populations Bedohe and Sharanesh in one subgroup and Kashane in second subgroup. The second group consists of populations Akre and Kuzo.

**Keywords**: genetic diversity, Duhok, microsatellites ,walnut and population.

**Introduction**

The genus *Juglans* L. is made up of 21 species of long-lived deciduous trees that produce large woody-shelled nuts. Juglans has been divided into four taxonomic sections as described by Manning (1978): (i) section Cardiocaryon Dode (the east Asian heartnuts) with three species, *J. ailantifolia* Carr., *J. mandshurica* Maxim., and *J. cathayensis* Dode; (ii) section Rhysocaryon Dode (black walnuts) of North, Central, and South America and the West Indies with 16 species; (iii) section Dioscaryon Dode with one species, *J. regia* L. (Persian walnut) distributed from southeastern Europe to the Himalayan mountains; and (iv) section Trachycaryon Dode ex Mann. with one species, *J. cinerea* L. (butternut) in eastern North America. *Juglans regia* L., has an exceptionally wide natural distribution, it occurs from the Carpathian Mountains of Eastern Europe, all through Western Asia, the Himalayan regions of Pakistan, India, Nepal, Bhutan and east into China. Most of walnut populations in Kurdistan including Duhok province have had very little attention from scientists, therefore they are not known if they are native trees from open-pollinated seedlings or new varieties introduced to the area by the local people for many years. Unfortunately, the traditional methods for characterization and assessment of genetic variability in perennial fruit crop species, based on morphological, physiological and biochemical studies are both time consuming and affected by the environment. The introduction of molecular biology techniques, such as DNA based markers, provides an opportunity for genetic characterization that allows direct comparison of different genetic material independent of environmental influences (Weising *et al.* 1995). Previously, in addition to morphological identification (Zenelli *et al.* 2005), various biochemical and molecular markers have been used for genetic characterization of walnut genotypes. These included isozymes (Ninot and Aleta 2003; Vyas *et al.* 2003; Foroni *et al.* 2001; Busov *et al.* 2002), restriction fragment length polymorphisms (RFLPs) (Fjellstrom and Parfitt 1995), randomly amplified polymorphic DNAs (RAPDs) (Nicese *et al.*1998; Yan-Min *et al.* 2000; Li *et al.* 2007), intersimple sequence repeats ISSRs) (Potter *et al.* 2002), simple sequence repeats (SSRs) (Woeste *et al.* 2002; Dangl *et al.* 2005; Foroni *et al.* 2005; Victory *et al.* 2006; Robichaud *et al.* 2006; Karimi *et al.* 2010), amplified fragment length
polymorphisms (Kafkas et al. 2005; Bayazit et al. 2007) and SNPs (Ciarmiello et al. 2011).

Simple sequence repeat (SSR), an array of short motifs of 1–6 bp in length, are hypervariable and widely spread in both coding and non-coding regions of plant and animal genomes (Kota et al., 2003). The reproducibility, multiallelicism, codominance, relatively abundance, and good genome coverage of SSR markers have made them one of the most useful tools for integration of the genetic, physiological, and sequence-based physical maps in plant species (Powell et al. 1996 and Kota et al. 2003).

Materials and Methods

Sample collection: Samples (fresh leaves) from five populations of J. regia L in the Duhok province were collected from Bedohe (Kanimase), kanizarke (Akre), kuzo (Zawita), Kashane (Batifa) and Sharaneshe (Darkare). Figure (1). Samples were collected according to procedure described by Karimi et al. (2010). Plants within 10 km from each other were considered as belonging to the same population, we limited our sampling to 8 trees per population and each tree separated by a distance of 100m from the other one. The leaves were sampled in the early morning hours and stored at −80°C until their use.

Figure (1): Names and location of five populations of J. regia L in the Duhok province. The red circle represents location of population.

Isolation of plant genomic DNA: The DNA was isolated from the plant (leaves) according to the method Dolye and Dolye (1987) with some modification. Three grams of fresh tissue (leaves) were homogenized to powder and added to 10 ml of Extraction buffer (2x CTAB preheated at 65°C for 15 min) [2% cetyl trimethyl ammonium bromide, 50 mM 1,4-dithiothreitol, 0.3% b-mercaptoethanol, 1.4 M NaCl, 100mM Tris, 20 mM ethylenediaminetetraaceticacid (EDTA), pH 8.0] and incubated at 65°C for 40 min. The aqueous solution was extracted with 10 mL (24:1) of chloroform–isoamyl alcohol and centrifuged at 5°C for 10min at 10,000 r/min, and the aqueous layer retained; 2/3 volumes of the aqueous layer of 95% ethanol were added (at −20°C) to precipitate the nucleic acids. The precipitate was washed with 0.2 M ammonium acetate in 75% ethanol and air-dried for 5 min. The DNA was precipitated, washed, dried, and dissolved in TE buffer.

Polymerase chain reaction amplification and electrophoresis. Samples (leaves) were obtained from five walnut populations of Duhok province. Each population contained eight individuals walnut tree leaves. Five primer pairs introduced by Woeste et al., 2002 were used to amplify genomic
DNA. The samples were prepared for PCR amplification in the form of single-individual genotypes for detection genetic diversity within populations.

The SSR reaction protocol was based on the protocol of Dangle et al. (2005). Amplification reactions were performed in a volume of 15 mL containing 1 x polymerase chain reaction (PCR) buffer, 25 ng genomic DNA, 200 mM dNTPs, 0.2 mM of each primer, 2 mM MgCl2, and 0.5 unit of Taq DNA polymerase. For DNA amplifications, an Eppendorf thermocycler was programmed according to the following profile: 94 °C for 5 min, 30 cycles at 94 °C for 30 sec, and a final elongation at 72 °C for 2 min, one cycle. After amplification, 5 mL of each sample was loaded and electrophoresed on a 2% horizontal agarose gel to control for positive amplification and to determine the approximate amount of product. Then, 3 μL of each sample was electrophoresed on a 6% polyacrylamide gel containing 1x TBE buffer. After electrophoresis, the gel was silver stained using the procedure of Bassam et al. (1991). In all cases, PCR reactions were performed at least twice to ensure that allele sizes were consistent. Allele sizing and scoring was done using a 100 bp DNA ladder (MBI-Fermentas, Vilnius, Lithuania) as the length reference.

Table (1) Five walnut microsatellite primers

<table>
<thead>
<tr>
<th>Primers name</th>
<th>Annealing temperatures</th>
<th>Amplicon size range (bp)</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 WGA202</td>
<td>58 °C</td>
<td>246-289 bp</td>
<td>F. CCCATCTACCGTTGCACTTT GCTGGTGTTCTATCATCTG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R. GCCACAGGAACGAGTGCT</td>
</tr>
<tr>
<td>2 WGA009</td>
<td>50 °C</td>
<td>242-258 bp</td>
<td>F. CATCAAAGCAAGCAATGGG CCATTGCTCTGTGATTGGG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R.</td>
</tr>
<tr>
<td>3 WGA332</td>
<td>59 °C</td>
<td>212-230 bp</td>
<td>F. ACCTCGGTCGACTCTCTCT GGCACAGGAACGAGTGCT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R.</td>
</tr>
<tr>
<td>4 WGA225</td>
<td>59 °C</td>
<td>198-206 bp</td>
<td>F. AATCCCTCTCCTGGGCAAG TGTCCTACTGACCACCT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R.</td>
</tr>
<tr>
<td>5 WGA069</td>
<td>45 °C</td>
<td>158-182 bp</td>
<td>F. TTAGTTAGCAAAACCCACCG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R. AGATGCACAGACCAACCCCTC</td>
</tr>
</tbody>
</table>

Data analysis. The allele's data were entered in the form of single-individual genotypes. The following parameters of genetic variation were assessed for each population: number of observed alleles (No), observed heterozygosity (Ho), and expected heterozygosity (He) (Nei, 1987), inbreeding coefficient (F-statistic) Fst and Fis (Nei, 1987) were calculated at each locus and over all loci using POPGENE version 1.32 software. Polymorphic information content (PIC), availability (A), and genetic distance (Nei, 1972) were calculated by using Power Marker version 3.25 software. Finally, in order to visualize the relationships between provenances, and obtained tree with neighbor-joining method based on (Nei, 1972). The phylogenetic tree was constructed by using Power Marker version 3.25 software. The tree was then viewed by using the TREEVEIW version 1.66 software.

Results and Discussion

The total number of alleles scored in five populations was 186 alleles with the sizes between 158 bp to 289 bp. The number of alleles per locus varied from 4 alleles in both Sharanesh and Kashane populations at locus (WGA225) to 10 alleles in Sharanesh population at locus (WGA202) and Kashane population at locus (WGA069), with an average of 7.4 alleles per locus. Table (2)

The average number of alleles per locus obtained (7.4) showed to be much higher than some other reported studies on *Juglans regia* using different DNA markers. In a very recent report using SSR markers this value were found to be 5.5 and 6.2 in (Foroni et al., 2005 and Pollegioni et al., 2011) respectively. In other studies, however, the average of allelic variation was higher than the average obtained in this study. For example: Victory et al (2006) using SSR marker reported the allelic variation value as 9.13, whereas in another study using SSR marker, the average of allelic variation was 12.9 (Pollegioni et al., 2009).
Table (2): A summary of the number of alleles per each population revealed by five *Juglans* microsatellite loci

<table>
<thead>
<tr>
<th>Primers</th>
<th>Number of observed alleles (No) in each population</th>
<th>Average number of allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sharanesh</td>
<td>Bedohe</td>
</tr>
<tr>
<td>WGA202</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>WGA009</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>WGA332</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>WGA225</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>WGA069</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Sub total</td>
<td>38</td>
<td>35</td>
</tr>
<tr>
<td>Average</td>
<td>7.6</td>
<td>7.0</td>
</tr>
<tr>
<td>Total</td>
<td>186</td>
<td></td>
</tr>
</tbody>
</table>

In order to obtain reliable data analysis, the value of availability (A, number of observed alleles per number of individuals sampled) was determined. This value was found to be high in all populations with an average 0.91. This average indicated that the number of null allele (not amplified) was low in all populations with an average 0.19 Table (3).

The observed heterozygosity (*Ho*) value per each population ranged from 0.58 in Sharanesh population to 0.628 in Akre population with an average 0.61(Table 3). The (*Ho*) of a given locus ranged from 0.59 (WGA225) to 0.65 (WGA202), with an average over all eight loci of 0.61 (Table 4). The average expected heterozygosity (*He*) was 0.87 per each population and ranged from 0.84 to 0.889 in Sharanesh and Kuzo respectively (Table 3). The value of (*He*) per locus also varied from 0.81 at WGA225 to 0.96 at WGA202 with an average 0.90 (Table 4).

Table (3) Summary of Genic Variation Statistics for five *Juglans* population (Sharanesh, Bedohe, Akre, Kashane and Kuzo). Availability (A), expected heterozygosity (He), observed heterozygosity (Ho), and Polymorphic information content (PIC).

<table>
<thead>
<tr>
<th>Populations</th>
<th>A</th>
<th>Ho</th>
<th>He</th>
<th>PIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sharanesh</td>
<td>0.925</td>
<td>0.5893</td>
<td>0.8463</td>
<td>0.766</td>
</tr>
<tr>
<td>Bedohe</td>
<td>0.925</td>
<td>0.6214</td>
<td>0.8862</td>
<td>0.802</td>
</tr>
<tr>
<td>Akre</td>
<td>0.900</td>
<td>0.6286</td>
<td>0.8872</td>
<td>0.803</td>
</tr>
<tr>
<td>Kashane</td>
<td>0.825</td>
<td>0.6048</td>
<td>0.8722</td>
<td>0.779</td>
</tr>
<tr>
<td>Kuzo</td>
<td>0.975</td>
<td>0.6179</td>
<td>0.8897</td>
<td>0.811</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>0.910</strong></td>
<td><strong>0.6124</strong></td>
<td><strong>0.8763</strong></td>
<td><strong>0.792</strong></td>
</tr>
</tbody>
</table>

The *Ho* in *J. regia* per each locus in this study was found to be higher than the average *Ho* (0.54) reported in some population in the Veneto region in Northern Italy (Pollegioni *et al.*, 2009), whereas this value were close to the average *Ho* (0.69) reported in five population in Dohuk providence of Kurdistan (Abbas and Jubrael., 2013), but lower than those reported (0.72) in the four provinces of Iran populations (Ebrahimi *et al.*, 2011). From this result, it may be noticed that, the observed heterozygosity value was lower than expected, this may suggest that, there is a regular tendency toward heterozygote deficiency, and this may also indicate the presence of inbreeding within the populations (Coulson *et al.*,1998).
Table (4) Summary of Genic Variation Statistics for five *Juglans* microsatellite Loci. Availability (A), expected heterozygosity (He), observed heterozygosity (Ho) and Polymorphic information content (PIC).

<table>
<thead>
<tr>
<th>Marker</th>
<th>A</th>
<th>Ho</th>
<th>He</th>
<th>PIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>WGA202</td>
<td>0.95</td>
<td>0.6579</td>
<td>0.9618</td>
<td>0.946</td>
</tr>
<tr>
<td>WGA009</td>
<td>0.85</td>
<td>0.6176</td>
<td>0.8973</td>
<td>0.873</td>
</tr>
<tr>
<td>WGA332</td>
<td>0.95</td>
<td>0.6053</td>
<td>0.9098</td>
<td>0.889</td>
</tr>
<tr>
<td>WGA225</td>
<td>0.925</td>
<td>0.5946</td>
<td>0.8153</td>
<td>0.779</td>
</tr>
<tr>
<td>WGA069</td>
<td>0.875</td>
<td>0.6000</td>
<td>0.9358</td>
<td>0.915</td>
</tr>
</tbody>
</table>

Average 0.91 0.6151 0.9040 0.881

The highest polymorphic information content (PIC) value (0.81) was observed in Kuzo population and the lowest value (0.77) in Sharanesh population with an average 0.79 in each population. Tables (3) The highest PIC value per locus was (0.94) for the primer WGA202 and the lowest value was 0.779 for the primer WGA225 with an average 0.88 Table (4). This may suggest that all markers were highly informative: because the (PIC) value was higher than 0.50 in all loci, thus, they could be considered as useful markers for genetic diversity studies for *J. regia* populations grown in this region (Botstein et al., 1980).

The average PIC value per locus in this study was found to be higher than values (0.68, and 0.57) reported in (Karimi et al., 2010 and Pollegioni et al., 2011) respectively.

The Fst ranged from 0.05 for locus WGA069 to 0.11 in locus WGA225 with an average value of 0.084 Tables (5). This may suggest that the genetic differentiation in this study was a moderate one because the Fst value was higher than 0.05 and lower than 0.15 (Weir and Cockerham, 1984).

Table (5) Summary of (Fst and Fis) for five *Juglans* microsatellite Loci

<table>
<thead>
<tr>
<th>Locus</th>
<th>Fst</th>
<th>Fis</th>
</tr>
</thead>
<tbody>
<tr>
<td>WGA202</td>
<td>0.1095</td>
<td>0.2219</td>
</tr>
<tr>
<td>WGA009</td>
<td>0.0660</td>
<td>0.2560</td>
</tr>
<tr>
<td>WGA332</td>
<td>0.0788</td>
<td>0.2693</td>
</tr>
<tr>
<td>WGA225</td>
<td>0.1103</td>
<td>0.1675</td>
</tr>
<tr>
<td>WGA069</td>
<td>0.0579</td>
<td>0.3172</td>
</tr>
</tbody>
</table>

Average 0.0842 0.2492

This average value was found to be higher than the genetic differentiation among populations reported in other studies for *J. regia* based on microsatellite markers. For example, (Pollegioni et al. 2011) reported the value of Fst (0.053) in Veneto region at Northern Italy. But this average was lower than Fst (0.122) reported in some population in western Iran ( Karimi et al.,2010) and Fst (0.106) reported by Fornari et al (2001) who had analyzed different population of *J. regia* from Europe and USA .The average of Fst obtained here 0.084, indicated that 8.4% of the total genetic diversity existed among populations and 91.6% within populations.

The estimation of genetic structure in this study was indicated that the moderate level of genetic differentiation was observed among these five populations according to Fst values. This may be due to that forest trees generally display high within-population diversity and low differentiation among populations. Deforestation and/or progressive selection of valuable genotypes for nut production and removal of vigorous trees with high wood quality leading to the negatively affecting genetic variability (Hamrick et al. 1992 and Müller-Starck et al. 1992).

The Fis value in this study ranged from (0.167) for locus WGA225 to (0.317) in locus WGA069 with an average (0.249) per each locus. These results indicated that all five loci have the tendency toward heterozygote deficiency.

The average of Fis value obtained was higher than (0.199) reported in some populations in Central and Southwestern China (Wang et al.,2008) and (0.021) reported by Pollegioni et al (2011). The value of Fis indicated that, there was a regular tendency toward heterozygosity deficiency, this indicating that the presence of inbreeding within the populations. The genetic structure information that obtained in this study was essential for conservation and management of walnut tree.

Genetic distances were calculated for each pair of populations to estimate the extent of their divergence Table (6). The lowest genetic
distance (0.55) was found between populations Bedohe and Sharanesh and the highest genetic distance (1.02) was found between populations Sharanesh and Kuzo. In the five populations, the average genetic distance among populations equaled 0.87.

The values of genetic distances among populations as well as between individuals are vary for plant breeding programs, for example plant breeders usually selects two varieties with a high genetic distance between them in order to obtain the widest possible crosses. However in other cases if the breeder wishes to introduce a certain character which is controlled by a gene or a group of genes for specific variety without wide variation in the genetic material of this variety which contains desired characters. In this case the breeder selects close variety that has this character within a studied variety because it is very difficult to find genetic variation between varieties depending on morphological characters (Smith, 1992).

Table (6) genetic distance between five *Juglans regia* populations (Sharanesh, Bedohe, Akre, Kashane and Kuzo)

<table>
<thead>
<tr>
<th>Populations</th>
<th>Sharanesh</th>
<th>Beduhe</th>
<th>Akre</th>
<th>Kashane</th>
<th>Kuzo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sharanesh</td>
<td>00000</td>
<td>0.6178</td>
<td>0.5970</td>
<td>1.0234</td>
<td>0.5556</td>
</tr>
<tr>
<td>Beduhe</td>
<td></td>
<td>00000</td>
<td>0.7045</td>
<td>0.6652</td>
<td>0.7587</td>
</tr>
<tr>
<td>Akre</td>
<td>0.6178</td>
<td>0.7508</td>
<td>00000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kashane</td>
<td>0.5970</td>
<td>0.8007</td>
<td>0.6923</td>
<td>00000</td>
<td></td>
</tr>
<tr>
<td>Kuzo</td>
<td>1.0234</td>
<td>0.7045</td>
<td>0.6652</td>
<td>0.7587</td>
<td>00000</td>
</tr>
</tbody>
</table>

The dendrogram separated the five populations into two main groups. The first group consists of populations Bedohe, Sharanesh and Kashane. The second group consists of populations Akre and Kuzo. The first group divided into two subgroup, Beduhe and Sharanesh were in one subgroup and Kashane in another subgroup. In the first cluster or group the Sharanesh and Bedohe populations had closest genetic relationship together then the second subgroup consist of Kashane population as detected in Figure (2). Also in second group Akre and Kuzo had close genetic relationship together. The phylogenetic analysis highlighted the genetic distance among the populations, placing each according to its genetic background. These results confirmed the power of microsatellites to diversify *Juglans regia* in five populations.

The unrooted tree determined in this study observed that the populations separated one from another according to their genetic differentiation that present among them and not according to the geographical distribution. This may be attributed to the fact that all populations belong to Duhok province and the area or geographical distance between populations is not very wide to produce high amount of genetic differentiation among populations. In addition, the habitat of the populations are approximately similar including climate, temperature, rainfall, landform whereas suitable for growth of walnut tree in this region. (Abbas and Jubrael, 2013)
References


دراسة عصائفل حزمة مؤشرات (microsatellite) في جنس قرى قبعة Juglans regia L (Juglans regia L.)

الخلفية:

تم استخدام كائنات صغيرة وكتراكت تسلسل بسيط (WGA009, WGA202, WGA069, WGA225, WGA33) لوصف تجمعات الجوائز الفاضلي (Juglans regia L.) في حس قرى من محافظة هركوب (شراش، ميدوهي، كناني زركي، عقيري). ضعف المراقبات (تكراكات تسلسل بسيط) من مجموعة 186 أليل السنجات. عدد الأليلات لكل موقع رتب من أربعة أليلات في تجمع سنجات كشاك لن معسكر ستة قاعدة من 150 إل 289. في موقع WGA225، تم فحص الأليلات لجين Gm-ha18 في النتائج الملاحظة. الاختلافات في النتائج تشير إلى أن جنس قرى كانت متنوعة بشكل عالي. الدراسة انتشرت في دراسة التحاليل الوراثي في هذه التجمعات.

كانت نسبة التحاليل الوراثي من التجمعات هي 8.4% من مجموعة التحاليل الوراثي ولذا فهو يُعد على مستوى متعدد من التحاليل الوراثي بين التجمعات. اشار معدل و المبلغ 20.4% إلى أن هناك سندات بالجاد نفسه التحاليل المتناوبة و اشار أيضا إلى وجود تواجد داخلي (زوج الأليلات) بين التحاليل.

خلال تطور التحاليل (الشيوخ والطور) اشارت النتائج إلى أن النشاط الوراثي متواجد في الجين السنجات الحزمة وفصلها إلى مجموعتين رئيسيتين. وضع لكل جين على أساس الخلفية الوراثية. المجموعة الأولى تتألف من مجموعتين ثانويتين الأولى: بيدهو وشراش و النادية: كناني. المجموعة الثانية تتألف من تجمع أكير و كناني.
IN VITRO MICROPROPAGATION OF VITIS VINIFERA L. IN KURDISTAN REGION OF IRAQ

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Abstract
This investigation was carried out at plant biotechnology laboratories of Scientific Research Center in Faculty of Science, University of Duhok, Kurdistan Region of Iraq, during the period from April 2013 to January 2014. The objectives of the study were to investigate the influence of different plant growth regulators in micropropagation on two local cultivars of grapes Vitis vinifera L. The most effective explant disinfestations were obtained by using NaOCl in a ratio of 1:1 water for 10 min which was very effective in reducing contamination in the established cultures resulting in a higher percentage of contaminations free cultures. At initiation stage, the highest initiation rate was achieved by using specific concentrations of BA on basal MS media. At multiplication stage, BA alone and BA combined with GA₃ which was showed different responses in the measured parameters. At rooting formation stage two auxins were tested including IAA and NAA to estimate their ability for root formation. The in vitro propagated of grape plantlets were gradually transferred in successful way from lab. To field conditions, which didn’t showed any morphological abnormalities.

Keywords: In vitro, Micropropagation, Vitis vinifera L.

Introduction

Grapevine "Vitis vinifera L." belongs to the family Vitaceae. Vitaceae comprise woody climbers, vines, trees, shrubs and succulent’s trees (Timmons et al., 2007; Chen and Wen, 2007) which are important food sources. Vitaceae family is valuable raw material for the production of wine, medicine and perfumery (Gashkova, 2009). Different varieties produce different types of dried fruit; the dried fruits are the raisins (Kishmish), Currants (Zabib, and the small seedless variety of grape also known as Kishmish). The fruit juice can be concentrated and used as a sweetener. Leaves are cooked. Vitis vinifera is not found wild in Kurdistan Region of Iraq although it is very near to the region where it is native to it, i.e. the area between Taurus range and the southern shores of Caspian Sea. But it is extensively cultivated throughout Kurdistan, especially in the mountains where grape yards are usually not irrigated, often on mountainsides of gentle slope, open oak forest, and small moist plains.

Micropropagation of grape through tissue culture provides a valuable tool for a more efficient and rapid multiplication of plant. Plant cell and tissue culture offers means not only for rapid mass multiplication of existing stocks but also for the conservation of important, elite and rare plants. Micropropagation of selected vitis genotypes can be carried out, among others, by the culture of intact or fragmented shoot apical meristems, axillary-bud, microcuttings or through adventitious bud formation (Heloir et al., 1997). However, most efficient protocols have been reported for muscadine or other than V. vinifera grapes (This and Graves, 1992; Qiu et al., 2004), while studies with cultivars of V. vinifera L. have met with less success (Chee and pool, 1983; Zatiko and Molnar, 1985).

The use of apices and axillary buds for the in vitro propagation of various species and cultivars of Vitis is documented (Gray and Fischer, 1985). Working on tendril explants of cultivars of grape, reported somatic embryogenesis and low frequency conversion of embryos to plants. Many authors have found that the use of MS medium supplemented with BA at (1-3 mg l⁻¹). Apical meristem explants were successfully taken from cultures grown on MS medium with 5 µM BA. Cytokinins at different concentrations were tested: 5, 10 and 20 µM BA; 10, 20 and 40 µM kin; 0.5, 1 and 5 µM TDZ (Gray and Benton, 1991). It has been shown that MS medium was the most suitable medium for multiplication stage and BA was the best cytokinin to obtain higher proliferation rate. The range of BA that was used in MS medium ranged between 0.5-2 mg l⁻¹ showed a better response. Generally, micropropagated plants are difficult to transplant
for two primary reasons: a heterotrophic mode of nutrition and poor control of water loss (Trigiano and Gray, 1996). This investigation was carried out to test the effects of different plant growth regulators on shoot multiplication to establish a micropropagation protocol of grapes cultivars via (shoot tips and axillary buds segments).

**Materials and Methods**

Several experiments were carried out during the period from April 2013 to January 2014, in plant biotechnology laboratories of Scientific Research Center, Faculty of Science, Duhok University, Kurdistan Region of Iraq to study the effects of growth regulators on *in vitro* propagation of *Vitis vinifera* L.

All culture glasswares were carefully washed with detergent, and distilled water. Before culturing and using the laminar air flow cabinet, the UV light of the laminar was switched on for 24 hours for sterilizing before culturing. Murashige and Skooge (1962) medium was used as basal medium. Stock solutions, for macronutrients, micronutrients, vitamins and growth regulators at (100 ppm) were prepared and used for the preparation of the media. Also, the media was supplemented with 100 mg l⁻¹ myo-Inositol, 30 g sucrose and 7 g Agar. The medium was dispensed in equal sizes (25 ml) into culture vessels. Finally, they were autoclaved at 121°C and 1.04 kg/ m² and then left at room temperature for solidifying to be ready for culture after 24 h (Karim, 2008).

Two kinds of explants (apical shoot tips and lateral buds) of 1.5-2 cm long were taken from *Vitis vinifera* L. field plants, the explants were rinsed under running tap water for 30 mins and drops at dishwashing liquid detergent for 5 min then the explants were transferred to the laminar air flow cabinet to complete the following combinations:

A. 5% NaOCl and distilled water (2:1) + drops of tween-20 for 5 min.
B. 5% NaOCl and distilled water (1:1) + drops of tween-20 for 10 min.
C. 5% NaOCl and distilled water (1:2) + drops of tween-20 for 15 min.

The explants were then rinsed with sterilized distilled water three times, for 5 min, followed by removing the ends of explants that exposed to sterilization. The explants were cultured in jars containing 25 ml of MS medium alone and supplemented with (1 mg l⁻¹) BA as an initiation media according to Deniel, (2009).

To determine the suitable combination of plant growth regulators for explants (lateral buds) establishments, the sterilized explants were cultured on MS medium supplemented with BA (0.0 and 1.0 mg l⁻¹). Because of the limited numbers of grape vine apical shoot tips, the *in vitro* growing shoot segments with two nodes (1-3 cm) were taken as lateral buds explants. Measurements were recorded after 4 weeks including shoot number / explants and mean length of shoot. After 4 weeks of culture, the shoots were cut into segments of shoot tips and two nodes (≥ 1 cm long) and were cultured on multiplication MS medium. Multiplication stage was divided into two experiments, at the first experiment the explants were cultured on MS medium supplemented with BA alone at different concentrations (0.0, 0.5, 1.0, 1.5 and 2.0 mg l⁻¹) and same BA protocols combined with GA₃ (0.25 mg l⁻¹). For the second experiment to determine the effect of these combinations on the studied parameters.

After 4 weeks of incubation in multiplication medium, the plantlet microshoots were transferred to rooting media. To determine the most suitable auxins concentrations and salt strength of MS media for rooting, microshoots were cultured on:

A. MS medium supplemented with different concentrations of NAA (0.0, 0.5, and 1.0 mg l⁻¹).
B. MS medium supplemented with different concentrations of IBA (0.0, 0.5, and 1.0 mg l⁻¹).

After 4 weeks, data were recorded including rooting percentage, number of root/explant and mean length of roots. For all the treatments, the cultures were incubated in the growth room at 24±1°C under 16 h photoperiod by white fluorescent tubes under light intensity of 1000 lux. Three replicates were cultured in jars containing 25 ml of MS medium.

The rooted plantlets were taken from rooting media and washed thoroughly with water to remove adhering media, immersed in a beaker containing 1 g ml⁻¹ Benlet fungicide for 10 mins. and washed with distilled water then the rooted plantlet were transferred to pots containing autoclaved peatmoss, loam and Styrofoam (1:1:0.5) (v:v:v). The pots were placed in sterilized box and covered by polyethylene cover during the first week. The post were enclosed in polyethylene bags, which were closed and placed in a shaded area of temperature – controlled greenhouse set at 23-25°C. The plants
were irrigated with a nutrient solution containing 1/4 strength of MS salts. After 8 to 10 days, the bags were opened and after another 8 to 10 days, the bags were removed and plants were grown under regular greenhouse condition (Toma, 2009).

The experiments were arranged according to Complete Randomized Design (C.R.D) using three replicates for each treatment. Data scored in percentage were subjected to arcsine transformation before statistical analysis and then converted back to percentages for presentation. Data were analyzed and means were compared with each other using Duncan’s multiple range test to evaluate the significant differences between the means.

Results and Discussion

The results showed that the combination (B) which have been used in surface sterilization of both types of explants gave the highest percentage of healthy - uncontaminated- explants which was achieved 70 % in Be-dandk cultivar, while was reached 66 % in apical shoot tips of Des-alaneez cultivar. On the other hand, healthy lateral bud explants showed a higher initiation response (95 %) in Be-dandk cultivar, followed by (90 %) in Des-alaneez lateral buds cultivar. Therefore, the second treatment was selected for disinfesting the explants for later experiments.

As it is known that the minimum concentration and duration of sterilization treatment is more preferable to insure not damaging the treated explant (Razdan, 2003). Sodium hypochlorite proved to be the best sterilant ever being effective not only as decontaminant but also easy to remove from explants in a minimal damage to the explant tissue. And the sterilization was efficient in eliminating various pathogens such as bacteria and fungi, as well as prevention of browning of explant in grapevine tissue culture (Dalal et al., 1991). The higher initiation response in lateral buds it might be due to the higher content of endogenous hormones. These results are seems to be similar to those obtained by Razdan (2003) and Amin (2007).

At multiplication stage, microshoots produced at initiation stage were used as explants sources for shoot multiplication stage. The ability of different concentrations of BA (0.0, 0.5, 1.0, 1.5, and 2.0 mg l⁻¹) alone and same levels of BA supplemented with (0.25 mg l⁻¹) GA₃ were used to induce Vitis vinifera L. shoot multiplication.

Table (1): Effect of different BA concentrations and BA+ GA₃ combination on highest shoot length (cm) of Grapevine plants after four weeks in culture.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Hormones</th>
<th>Hormone concentration mg l⁻¹</th>
<th>Effect of cultivar × hormone</th>
<th>Effect of cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Be-dandek</td>
<td>BA</td>
<td>1.43c</td>
<td>2.27ab</td>
<td>2.20ab</td>
</tr>
<tr>
<td></td>
<td>BA + GA₃</td>
<td>0.70c</td>
<td>1.18ac</td>
<td>1.48a</td>
</tr>
<tr>
<td>Des-alaneez</td>
<td>BA</td>
<td>1.15b</td>
<td>1.57ab</td>
<td>1.93a</td>
</tr>
<tr>
<td></td>
<td>BA + GA₃</td>
<td>1.37a</td>
<td>1.67a</td>
<td>1.23a</td>
</tr>
</tbody>
</table>

Effect of cultivar × concentration

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Hormones</th>
<th>Hormone concentration mg l⁻¹</th>
<th>Effect of hormone × Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Be-dandek</td>
<td></td>
<td>1.07e</td>
<td>1.73bc</td>
</tr>
<tr>
<td>Des-alaneez</td>
<td></td>
<td>1.26ed</td>
<td>1.62bc</td>
</tr>
</tbody>
</table>

Different letters represent significant differences according to Duncan’s multiple range tests at 5% levels.
Table (1) shows the effects of BA levels and BA enriched with \((0.25 \text{ mg l}^{-1})\) \(\text{GA}_3\) after four weeks in culture on highest shoot length of grapevine plant.

The effect of the hormones clarify that the media supplemented with different concentration of BA increased significantly and reaches a highest length \((1.82 \text{ cm})\) when compared with the BA + \(\text{GA}_3\) \((1.24 \text{ cm})\).

Table (2) Generally, the data clarify that there is no significant differences between the two cultivars used in this investigation, whereas the effect of cytokinins induce significant increase in this parameter by using the combination of BA with \(\text{GA}_3\) \((1.09 \text{ cm})\) when compared with the medium supplemented with BA alone \((1.01 \text{ cm})\). In the same table, the result elucidate that the shoot length average reaches \((1.17 \text{ cm})\) in Be-dandek cultivar because of the interaction between cultivars and plant growth regulators, which was reduced significantly \((0.88 \text{ cm})\) by adding BA + \(\text{GA}_3\).

Contrastive response has been shown in Des-alaneez cultivar whereas the average shoot length increased significantly in the medium enriched with BA + \(\text{GA}_3\) \((1.31 \text{ cm})\) while achieve \((0.85 \text{ cm})\) in same cultivar cultured in different levels of BA alone.

**Table (2):** Effects of different BA concentrations and BA+\(\text{GA}_3\) combination on shoot length average \((\text{cm})\) of Grapevine plants after four weeks in culture.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Hormones</th>
<th>Hormone concentration mg/l$^{-1}$</th>
<th>Effect of cultivar × hormone</th>
<th>Effect of hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Be-dandek</td>
<td>BA</td>
<td>1.10bc 1.40ab 1.07bc 1.60a 0.70c</td>
<td>1.17a 1.02a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BA + (\text{GA}_3)</td>
<td>0.41b 0.86ab 1.02ab 1.13a 0.93ab</td>
<td>0.88b</td>
<td></td>
</tr>
<tr>
<td>Des-alaneez</td>
<td>BA</td>
<td>0.63bc 0.97ab 0.97ab 1.13a 0.57c</td>
<td>0.85b</td>
<td>1.08a</td>
</tr>
<tr>
<td></td>
<td>BA + (\text{GA}_3)</td>
<td>1.13a 1.30a 1.50a 1.28a 1.35a</td>
<td>1.31a</td>
<td></td>
</tr>
</tbody>
</table>

Effect of hormone × Concentration

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Hormones</th>
<th>Concentration effect</th>
<th>Effect of hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Be-dandek</td>
<td>BA</td>
<td>0.76c 1.14bac 1.04bac 1.37a 0.82bc</td>
<td></td>
</tr>
<tr>
<td>Des-alaneez</td>
<td>BA</td>
<td>0.88bc 1.13bac 1.23ba 1.21ba 0.96bac</td>
<td>0.82c 1.14ba 1.14ba 1.29a 0.89bc</td>
</tr>
<tr>
<td></td>
<td>BA + (\text{GA}_3)</td>
<td>0.77dc 1.09bac 1.26ba 1.21ba 1.14bac</td>
<td>1.09a</td>
</tr>
</tbody>
</table>

Different letters represent significant differences according to Duncan’s multiple range tests at 5% levels.

Table (3) declares that the addition of BA levels in general to MS multiplication media was valuable in case of number of shoots per explants after four weeks in culture. Since the highest number of shoots per explants \((2.9)\) in the medium enriched with different levels of BA which decreased significantly \((1.78 \text{ shoot / explant})\) in the same concentration of BA supplemented with \(0.25 \text{ mg l}^{-1}\) \(\text{GA}_3\) (Figure 1). On the same manner, both cultivars (Be-dandek and Des alaneez) recorded the highest significant shoot numbers per explants \((2.99 , 2.81)\) respectively when cultured in the media supplemented with BA whereas, achieved \((1.8 , 1.77)\) in the medium containing different concentrations of BA + \(\text{GA}_3\). Although that, the data didn’t shown any significant differences between the two cultivars.
Table 3: Effects of different BA concentrations and BA+GA3 combination on number of shoots/explant of grapevine plants after four weeks in culture.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Hormones</th>
<th>Hormone concentration mgl⁻¹</th>
<th>Effect of cultivar × hormone</th>
<th>Effect of cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Be-dandek</td>
<td>BA</td>
<td>1.50b</td>
<td>3.03cb</td>
<td>3.17ba</td>
</tr>
<tr>
<td></td>
<td>BA + GA3</td>
<td>1.83a</td>
<td>1.67a</td>
<td>2.00a</td>
</tr>
<tr>
<td>Des-alaneez</td>
<td>BA</td>
<td>1.10c</td>
<td>2.60bcd</td>
<td>2.73bcd</td>
</tr>
<tr>
<td></td>
<td>BA + GA3</td>
<td>1.53a</td>
<td>1.83a</td>
<td>1.90a</td>
</tr>
</tbody>
</table>

Different letters represent significant differences according to Duncan's multiple range tests at 5% levels.

Table 4: Effects of different BA concentrations and BA+GA3 combination on number of leaves/explant of grapevine plants after four weeks in culture.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Hormones</th>
<th>Hormone concentration mgl⁻¹</th>
<th>Effect of cultivar × hormone</th>
<th>Effect of cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Be-dandek</td>
<td>BA</td>
<td>4.17cb</td>
<td>11.53a</td>
<td>9.00ab</td>
</tr>
<tr>
<td></td>
<td>BA + GA3</td>
<td>3.33a</td>
<td>4.77a</td>
<td>4.83a</td>
</tr>
<tr>
<td>Des-alaneez</td>
<td>BA</td>
<td>2.47c</td>
<td>10.17a</td>
<td>10.33a</td>
</tr>
<tr>
<td></td>
<td>BA + GA3</td>
<td>3.43b</td>
<td>3.50b</td>
<td>3.77b</td>
</tr>
</tbody>
</table>

Different letters represent significant differences according to Duncan’s multiple range tests at 5% levels.
Shoot multiplication traits were influenced by addition of BA more than mixing it with GA3 and this could due to the number of double bonds on its molecule structure (Mohammed, 1985) which is provided with three bonds in its side chain and consequently increase the activity. In addition to presence of benzene ring on BA structure improve its efficiency and become the most effective cytokinin (Wasfy, 1995). Positive role have been seen on multiplication stage (shoot numbers, length and leaves numbers), may be due to two causes:

First, is that the cytokinin increase the enzymes and proteins and RNA synthesis in the cells which promote bud growth (Al-Rifae and Al-Shobaki, 2002). The second, releasing lateral buds from the dominance of terminal buds without the need of removing the apical bud by promoting formation of xylem tissues of buds which facilitate the transporting of water and nutrients. (Mohmmed and Younis, 1991).

One of the major physiological effects of the auxins is the stimulating of adventitious roots formation in both *in vitro* and *in vivo* cutting (Hartmann *et al.*, 2002). At rooting stage, *in vitro* shoots derived from multiplication stage were used and two kind of auxins (IBA and NAA) as followed: MS medium supplemented with different concentration of NAA (0.0, 0.5, and 1.0 mg⁻¹). MS medium supplemented with different concentration of IBA (0.0, 0.5, and 1.0 mg⁻¹). Roots were initiated at rooting formation stage, following 4 weeks of incubation on rooting media of above specific concentrations, thereafter the roots were adventitiously initiated at cut margins of the shoots.

The percentage of root formation was significantly affected by the different treatments tested on *Vitis vinifera* L.. Table (5). It is clear that increase the concentration of investigated auxins from 0.5 to 1.0 mg1⁻¹ caused significant increase (82.0% and 86.33%) in rooting percentage of grape plants compared with free media of auxins.

Concerning the differences in rooting abilities of grape, regarding the combination between types of hormones and concentration of auxins, 0.5 and 1.0 mg1⁻¹ NAA was nearly similar in both concentration which produced the highest significant rooting percentage (87.83% and 87.33%), respectively when compared with control treatment (68.61%). Whereas 1.0 mg1⁻¹ concentration of IBA produced the highest rooting percentage (85.33%) and decreased significantly in control treatment (58.33%). On the other hand, no significant difference was seen between cultivars.

**Table (5): Effects of different Auxin (NAA and IBA) concentrations on rooting percentage (%) of grapevine plants after four weeks in culture.**

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Hormones</th>
<th>Auxin concentration mg⁻¹</th>
<th>Effect of cultivar × hormone</th>
<th>Effect of cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Be- dandek</td>
<td>NAA</td>
<td>68.89bc</td>
<td>95.67a</td>
<td>88.00bc</td>
</tr>
<tr>
<td></td>
<td>IBA</td>
<td>53.33c</td>
<td>82.00bc</td>
<td>87.33bc</td>
</tr>
<tr>
<td>Des –alaneez</td>
<td>NAA</td>
<td>68.33bc</td>
<td>80.00bc</td>
<td>86.67bc</td>
</tr>
<tr>
<td></td>
<td>IBA</td>
<td>63.33bc</td>
<td>70.33a-c</td>
<td>83.33bc</td>
</tr>
<tr>
<td>Effect of cultivar × concentration</td>
<td>Bedandek</td>
<td>61.11b</td>
<td>88.83a</td>
<td>87.67a</td>
</tr>
<tr>
<td>Des alaneez</td>
<td>65.83b</td>
<td>75.17ab</td>
<td>85.00a</td>
<td>65.83</td>
</tr>
<tr>
<td>Effect of hormone × Conc.</td>
<td>NAA</td>
<td>68.61bc</td>
<td>87.83a</td>
<td>87.33a</td>
</tr>
<tr>
<td></td>
<td>IBA</td>
<td>58.33c</td>
<td>76.17ab</td>
<td>85.33a</td>
</tr>
<tr>
<td>Concentration effect</td>
<td>63.47b</td>
<td>82.00a</td>
<td>86.33a</td>
<td></td>
</tr>
</tbody>
</table>

Different letters represent significant differences according to Duncan’s multiple range tests at 5% levels.
Data in Table (6) clarify that there is a significant difference in the number of roots formed on explants of *Vitis vinifera* L. as a result of tested treatments. The highest number of roots/explant (14.44) was recorded in Bae-dandk cultivar compared with Des-alaneez (7.039 roots/explant). Regarding the effects of the auxins on this parameter it is obvious that the effect of NAA was more valuable of IBA which increase the roots significantly number to (13.33 roots / explant) compared with IBA (8.15 root/explant)(Fig 1).

Increasing the concentration of auxin from 0.5 to 1.0 mg l⁻¹ increase the roots number (14.13 root/explant) significantly according to control treatment (4.33 roots / explants).

Table (6): Effects of different Auxin (NAA and IBA) concentrations on root numbers/ explants of grapevine plants after four weeks in culture.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Hormones</th>
<th>Auxin concentration mg l⁻¹</th>
<th>Effect of cultivar × hormone</th>
<th>Effect of cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Bae-dandek</td>
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<td>2.83f</td>
<td>29.67a</td>
<td>26.17a</td>
</tr>
<tr>
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<td>IBA</td>
<td>2.83f</td>
<td>11.50bc</td>
<td>13.67b</td>
</tr>
<tr>
<td>Des-alaneez</td>
<td>NAA</td>
<td>4.00ef</td>
<td>8.50cd</td>
<td>8.83cd</td>
</tr>
<tr>
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<td>IBA</td>
<td>7.67c-e</td>
<td>5.40d-f</td>
<td>7.83c-e</td>
</tr>
</tbody>
</table>

Effect of cultivar × concentration

| Bedandek        | 2.83c | 20.58a | 19.92a |
| Des-alaneez     |      |        |        |

Effect of hormone × Conc.

| NAA             | 3.42c | 19.08a | 17.50a | 13.33a |
| IBA             | 5.25c | 8.45b | 10.75b | 8.15b |

| Concentration effect | 4.33b | 13.77a | 14.13a |

Different letters represent significant differences according to Duncan’s multiple range tests at 5% levels.

Valuable inhibition in the number of roots formation was shown in Des-alaneez cultivar. It is clear that both auxins (NAA, IBA) weren't too much effective to increase the roots numbers although of simple increase when compared with control treatments. Combined cultivars with type of auxin significantly increased the number of roots (19.50 roots/explants) which were formed on shootlets of Bae-dandek cultivar when cultured on MS supplemented with NAA compared with Des-alaneez when cultured on MS supplemented with NAA and IBA (7.11, 6.97 roots/explants) respectively.

Root length average was affected significantly as a result of adding investigated auxins to the medium. Table (7) clarify that there was significant increase in root length in Bae-dandek cultivar (4.6 cm) compared with Des-alaneez (2.27 cm). Hormone effect declares that the IBA induce more significant increase in root length average (5.17 cm) than NAA (1.69 cm).
Table (7): Effects of different Auxin (NAA and IBA) concentrations on root lengths average (cm) / explants of Grapevine plants after four weeks in culture.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Hormones</th>
<th>Auxin concentration mg/l</th>
<th>Effect of cultivar × hormone</th>
<th>Effect of cultivars</th>
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<td></td>
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<td>0</td>
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<td>1.0</td>
</tr>
<tr>
<td>Be-dandek</td>
<td>NAA</td>
<td>3.50bc</td>
<td>2.27b-e</td>
<td>2.00b-e</td>
</tr>
<tr>
<td></td>
<td>IBA</td>
<td>4.00b</td>
<td>7.90a</td>
<td>7.92a</td>
</tr>
<tr>
<td>Des-alaneez</td>
<td>NAA</td>
<td>0.93c-e</td>
<td>0.67e</td>
<td>0.80de</td>
</tr>
<tr>
<td></td>
<td>IBA</td>
<td>3.33b-d</td>
<td>4.37b</td>
<td>3.50bc</td>
</tr>
</tbody>
</table>

Effect of cultivar × concentration

<table>
<thead>
<tr>
<th></th>
<th>Bedandek</th>
<th>3.75ab</th>
<th>5.08a</th>
<th>4.96a</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Des alaneez</td>
<td>2.13b</td>
<td>2.52b</td>
<td>2.15b</td>
<td>Hormone Effects</td>
</tr>
</tbody>
</table>

Effect of hormone × Conc.

<table>
<thead>
<tr>
<th></th>
<th>NAA</th>
<th>2.22bc</th>
<th>1.47c</th>
<th>1.40c</th>
<th>1.69b</th>
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<tr>
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<td>IBA</td>
<td>3.67b</td>
<td>6.13a</td>
<td>5.71a</td>
<td>5.17a</td>
</tr>
</tbody>
</table>

Concentration effect

|                | 2.94a | 3.80a | 3.55a |

Different letters represent significant differences according to Duncan’s multiple range tests at 5% levels.

Finally the result of root formation stage, indicate that the auxins have an important role in promoting adventitious root establishment on shoot end cuttings (Abdul, 1978; Saleh, 1991).

Adventitious root formation is a complex process that is affected by multiple endogenous factors including phytohormones and environmental factors. Both auxins NAA and IBA varied in their effects on grapevine rooting whereas NAA was more effective in the case of rooting percentage and root number per explants while IBA was preferable in its effect in the stimulation of root length, these results was similar to finding of those of Danial et al., (2009). And confirm that the presence of these auxins had positive influence on rhizogenesis under in vitro conditions.
Figure (1):
A. Initiation shoots from lateral buds on MS medium enriched with 1.0 mg l⁻¹ of BA after 4 weeks in culture. (Bae dandek, Des alanez Cultivars respectively in all picture)
B. Multiplication stage: Effects of different BA concentrations and BA+ GA₃ combination on highest Shoot Length (cm) of grapevine plant
C. Multiplication stage: Effects of different BA concentrations and BA+ GA₃ combination on Shoot Length Average (cm).
D. Multiplication stage: Effects of different BA concentrations and BA+ GA₃ combination on number of Shoots / explant.
E. Multiplication stage: Effects of different BA concentrations and BA+ GA₃ combination on number of leaves / explant.
F. Rooting stage: Effect of different IBA and NAA on rooting percentage (%) of Grapevine plants.
G. Rooting stage: Effects of different IBA and NAA on number of roots of Grapevine plants.
H. Rooting stage: Effect of different IBA and NAA on roots length average of Grapevine plants.
I. Acclimatization stag
CONCLUSIONS

It can be concluded that treating the explants with Sodium hypochlorite + distilled water (1:1) was more effective in reducing explants contamination. At initiation stage, BA showed better results for establishing aseptic Vitis vinifera culture from lateral buds while in initiation stage, lateral bud explants were more effective in producing shoots than apical bud explants. Hence, shoot multiplication traits were influenced by addition of BA more than mixing it with GA3.

References


الاكثار الدقيق للنبات المحلي في أقليم كردستان العراق

الخلاصة:

أجريت هذه الدراسة في مختبر الزراعة في جامعة المستنصرية في كردستان العراق، خلال السنة الأكاديمية 2013-2014 من يبان ووهان. وكاننا نهدف من هذه الدراسة إلى دراسة التأثيرات المركبة للنماذج النمو على الاضثاث الدقيق للأصناف المحلية من العنب المزهر في أقليم كردستان العراق، وتحديد النسبية من مادة النمو BA (2،4-ÁÊ) و BA+GA و (GA3) على النباتات في مرحلة البدن المعدة العالية، وبحث تأثير التركيزات المحددة من BA و (BA+GA) و (GA3) و نفايات التربة في معدل النمو و نشير إلى تأثير من النسبة من BA و (BA+GA) في النباتات في مرحلة البدن المعدة العالية. و نظرًا لأن النتائج في التجربة في الأوزان التي تغطي التأثيرات الدقيقة، ونقوم بدراسة تأثير من الظروف التي تؤدي إلى نمو النباتات بشكل ناجح.

كوري:


USE OF WOOD MACERATED ELEMENTS FOR CLASSIFICATION OF
SALIX L. SPECIES IN KURDISTAN REGION- IRAQ

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Summary:
Characters of wood macerated elements were investigated for taxonomic application. Wood samples from 5 stems of each Salix acmophylla, S. alba, S. babylonica, S. aegyptiaca, and S. Purpurea were collected from different geographical regions of Kurdistan to clasps most possible variation. Wood of most species contains vessel elements described of primitiveness together with those descriptive of more or less advanced. Vessels of different species are similar in possessing the simple perforation plate. The differences are huge in vessel length and vessel width and hence in length/width ratio. Differences are also huge in vessel type or the end wall characters of vessel elements described of primitiveness and advancement, occurrence or absence of spiral thickening, cell walls pitting intensity and distribution within and between species. All these features are found to be important for providing taxonomic application. Libriform fibers are the only fibers present in the wood of this genus, no fiber trachieds occur. All species have fibers possessing dentition, almost exclusively at the distal parts, very rarely situated throughout the fiber length. Dentition morphological structure, distribution, size and orientation form important diagnostic characters.

Key words: Vessel elements, Libriform fibers, Salix acmophylla, Salix Alba, Salix babylonica, Salix aegyptiaca, Salix purpurea.

Introduction
There is about 526 species of Salix in the world (Fang-Zhen, 1987), most of which are distributed in the Northern hemisphere with only a few species in the Southern hemisphere. In Asia, according to the same researcher, there are about 375 species, making up 71.29 percent of the total Salix species in the world with the center of origin and development in the region between 20-40 ° N in East Asia. In Iraq, 6 species are described, they are S. alba, S. acophylla Boiss., S. babylonica L., S. purpurea L., S. aegyptiaca L., S. triandra L. All of Iraqi representative species occur in Kurdistan region with the exception of S. triandra which has a very limited area of distribution at Tigris river banks near Rashidia north of Mosul city. Salix species are moisture demanding plants, commonly found in the lower and middle forest zones, in the latitudinal range 439-1840m (Abdulrahman, 2011), at river banks, moist mountain valleys, in riparian thickets, occasional in the moist steppe region. Willow species S. alba and S. aegyptiaca and S. babylonica need high soil moisture content and lower mean annual temperature compared to similar requirements for S. acmophylla and S. Purpurea. In Kurdistan region, the species S. acmophylla is of especially high drought tolerance (Townsend and Guest, 1980). All willow species with the exception of S. Babylonia is found in the wilderness. Both S. alba and S. acmophylla are abundant and common in the region, while S. Purpurea and S. Aegyptiaca are rare and occasional, especially in the western and central mountain sectors, they may be absent from the eastern sector. Only male plants of S. Purpurea have been observed in Iraq.

According to Stace (1981) anatomical features are of particular value to scientists who need to identify small scraps of plant material. Some recent scientific works relate characteristics of the secondary xylem of Salix species to environmental conditions and pollution in Canada and Eastern Europe (Cooper and Cass 2001; Chavchavadze et al. 2002; Sizonenko and Chavchavadze 2002). On the other hand, Wagner (2009) studied the wood anatomy of young and adult samples of Salix × rubens, aiming to improve the knowledge about the most used species for basketry in South Brazil by relating wood anatomy characters of xylem quantity and ratio of pith/xylem to wood flexibility.

The vessel elements that originated in a precursor to the angiosperms may have been subsequently lost in some lineages like Amborellaceae, Tetracentraceae, Trochodendraceae, and Winteraceae. These
basal lineages were described by Cronquist (1981) as primitively vesselless. Moreover Cronquist considered the vessels of *Gnetum* to be converging with those of angiosperms. When data of vessel element characters of density, vessel lumen diameter, vessel length, and vessel clustering, in four endemic *Salix* taxa from the Lake Athabasca-Canada were compared by Cooper and Cass (2001) with the putative sister species for each endemic, it revealed similar vessel element densities to their associated sister species. In addition to that, the vessel element lumen diameter and length were significantly different in some of the species pairs. The authors indicated that the structural differences for these endemic willows appear to be related to their open sand habitat. The fiber dimorphism noticed in *Dubautia* and many other genera in which there are bands of thinner-walled fibers at intervals within a background of thick-walled fibers are probably a division of labor between fibers serving for photosynthate storage and fibers serving for mechanical strength (Sherwin, 1988). According to Sherwin (2014), most authors contrast wider, thinner-walled, shorter fibers with narrower, thicker-walled fibers that have narrower Lumina. For the same author, the evolutionary significance of fiber dimorphism in the form of few small changes in fiber structure can result in the achievement of different functions. Moreover, Sherwin (1984) indicated that the simple slit-like pits of libriform fibers cause minimal loss in mechanical strength compared to bordered pits.

Many researchers consider *Salix* as one of the most difficult genus for identification; there is still disagreement regarding the identity, number and distribution of species (Heywood et al. 2007; Mabberly 2008). The easy process of hybridization between species in the wild, together with their phenetic plasticity and different time of flowers and leaves development, made it difficult to observe all of these relevant characters on a single plant or specimen (Salih, T. et al. 2014). In many instances morphological characters are not sufficient to delimit closely related species of *Salix*. Therefore, in addition to morphological features, anatomical characters may provide valuable information for the classification of *Salix* (Arihan and Güvenç 2011).

The taxonomic relationships among the *Salix* species are not well understood and are still under debate. It is always felt for this genus that wood macerated elements should be viewed carefully in an attempt to provide useful taxonomic traits to identify taxa and demonstrate taxonomic relationships between species representative of Kurdistan-Iraq.

### Material and Methods

Wood samples from *Salix acmophylla*, *S. alba*, *S. babylonica*, *S. aegyptiaca*, and *S. Purpurea* were collected from different geographical regions to clasp most possible variation. Samples of 2-3mm thick and 2-4cm long were prepared from growth increments 4-5, starting from the pith of the 5 stems of each species, to avoid juvenile wood of it. The herbarium vouchers of them were deposited in the Biology department/Faculty of Science/University of Zakho.

Wood samples were macerated using Franklin solution (Franklin 1946). A sample of fibers was removed from the maceration and spread over a glass slide. 25 measurements of each of the following parameters were surveyed:

1. Libriform fiber length in mm.
2. Libriform fiber diameter (at the midpoint) in µm.
3. Libriform fiber wall thickness in µm.
4. Vessel element length in mm.
5. Vessel element diameter (at the midpoint) in µm.

### Results and Discussion

**Characteristics of the Genus:**

Vessel element and libriform fiber dimensions are given in table (1). The overall range of vessel length and vessel diameter is found to be 0.2-0.55mm and 95.2-209µm respectively. These values together with STDs indicate high variance in vessel dimensions. The vessel elements have simple perforation plates, inter-vessel pits are bordered pits, wood of most species contain vessel elements described of primitiveness together with those descriptive of more or less advanced.

Libriform fibers are the only fibers present in the wood of this genus, no fiber trachieds are to be observed. Fibers range 0.55-1.20mm in length, 13.2-30.8µm in diameter at the midpoint, 1.5-7.0µm in cell wall thickness; statistics given in table (1) indicate low variability in measured parameters of the fiber. All species have fibers possessing dentitions, almost exclusively at the distal parts, vary rarely situated throughout the fiber length.
Fibers of *S. alba* seems rather homogenous, mostly straight, thin-walled with small slit-like pits. Only distal dentitions occur on one side of some fibers, in most cases the dentitions of one end differ from dentitions of the other end, few fibers are with very smooth walls, containing no dentitions. Actually the overall fiber characters refer to a considerable modification and evolutionary process (fig. 1).

Vessels of *S. alba* exhibit high variability in shape and size. The wood tissue contains numerous vessel types at different level of advancement. According to Ghose (1984) and Klotz (1978) the vessel elements with simple perforation plate, horizontal end wall and least value of vessel length/vessel width are advanced, while those of scalariform perforation plate, sloping end wall and high vessel length/vessel width value are primitive. Considering these criteria, it is apparent that vessel type of *S. Alba* lay between the two extremes, none of them is more advanced or more primitive.

Number of lateral inter-vessel pitting areas differs from vessel to vessel, regardless of the level of advancement or primitiveness; it is a characteristic feature of this species.

**Table (1):** Mean, Standard Deviation (STD) and Range for Libriform Fibers and Vessel Elements.

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>S. alba</em></th>
<th><em>S. acmophylla</em></th>
<th><em>S. babylonica</em></th>
<th><em>S. aegyptiaca</em></th>
<th><em>S. purpurea</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Libriform fiber length (mm)</td>
<td>Mean 1.01</td>
<td>0.93</td>
<td>1.10</td>
<td>0.88</td>
<td>0.90</td>
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<tr>
<td></td>
<td>STD ± 0.11</td>
<td>± 0.14</td>
<td>± 0.12</td>
<td>± 0.1</td>
<td>± 0.13</td>
</tr>
<tr>
<td></td>
<td>Range 0.77-1.19</td>
<td>0.66-1.15</td>
<td>0.8-1.2</td>
<td>0.69-1.04</td>
<td>0.55-1.1</td>
</tr>
<tr>
<td>Libriform fiber diameter (μm)</td>
<td>Mean 20.93</td>
<td>19.70</td>
<td>23.22</td>
<td>20.8</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td>STD ±2.96</td>
<td>± 2.90</td>
<td>±3.11</td>
<td>± 2.35</td>
<td>± 2.93</td>
</tr>
<tr>
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<td>Range 15.4-25.3</td>
<td>13.2-24.2</td>
<td>19.5-30.8</td>
<td>16.5-24.0</td>
<td>15.2-26.0</td>
</tr>
<tr>
<td>Libriform fiber wall thickness (μm)</td>
<td>Mean 3.4</td>
<td>4.2</td>
<td>4.1</td>
<td>3.1</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>STD ± 0.93</td>
<td>± 1.08</td>
<td>± 1.35</td>
<td>± 0.80</td>
<td>± 1.45</td>
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<td>Mean 0.32</td>
<td>0.38</td>
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<td>0.37</td>
<td>0.31</td>
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<tr>
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<td>STD ± 0.09</td>
<td>± 0.07</td>
<td>± 0.06</td>
<td>± 0.11</td>
<td>± 0.07</td>
</tr>
<tr>
<td></td>
<td>Range 0.22-0.50</td>
<td>0.26-0.52</td>
<td>0.27-0.49</td>
<td>0.26-0.57</td>
<td>0.20-0.55</td>
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<tr>
<td>Vessel element diameter (μm)</td>
<td>Mean 135.9</td>
<td>143.6</td>
<td>140.4</td>
<td>142.9</td>
<td>153.7</td>
</tr>
<tr>
<td></td>
<td>STD ± 16.27</td>
<td>± 21.85</td>
<td>± 18.5</td>
<td>± 31.35</td>
<td>± 26.4</td>
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<tr>
<td></td>
<td>Range 115.5-187</td>
<td>110-180.5</td>
<td>99-165</td>
<td>95.2-220</td>
<td>108-209</td>
</tr>
</tbody>
</table>
Each value represents 25 replicate

*S. acmophylla*

The fibers of this species express a high range of variation in fiber length and fiber cell wall thickness (table 1). Wide differences occur in fiber overall shape, some of them are quite straight; others are winding, especially at their distal parts (figure 2). Differences also occur in dental number, shape and distribution; mostly concentrated at the two fiber ends while some fiber possesses dentition distributed along the fiber length, forming a significant diagnostic character. Like *S. alba*, dentitions occur only on one side of some fibers, moreover dentitions of one end often differ from the dentition of the other end. Fiber wall is relatively thin, but thicker than that of *S. alba*, with small slit-like pits.

As figure (2) shows, vessel members of *S. acmophylla* are highly variable. End wall slopes of vessel elements show a wide range of variations such as, both end walls transverse, both end walls oblique and mixed type, i.e., one end wall transverse and the other oblique, the length/width ratio differences are also high indicating differential evolutionary process. The same figure refers to vessel shapes of short and transverse end wall possessing apomorphous character states in common together with members exhibiting pliesiomorphous character states of both end walls oblique. Moreover, mixed vessels of one end wall transverse and the other oblique are also found in the mixture of the macerated elements.

*S. babylonica*

The fiber length and fiber diameter measurements of this species are found higher with moderately thick cell wall compared to other *Salix* species (table 1). Fibers are ranging between straight forms to winding or slightly curved or zigzag with highly tapering distal ends. Dentitions differ in shape, number and size. Only distal fiber ends have dentitions, middle portions possess none of them. Number of dentitions are usually few, often one of them is conspicuous, either oriented upward to the fiber tip or perpendicular to the surface (figure 3), it is worth mentioning that the presence of such distinct dentition provide a significant taxonomic application. Pits, like other species, are small slit-like.

Vessel members macerated from the wood of this species show a great resemblance to one another, only two types of vessels are distinct; one possessing transverse and oblique end wall, while the other has oblique ends similar to those of very primitive vessel members, but not to the foraminate which contains several round perforations. These vessel members have lateral inter-vessel pits, mostly located around the perforation plate.

*S. aegyptiaca*

Fiber length and the fiber cell wall thickness are inferior to the corresponding measurements of other species. Fibers are rather irregular in shape; few seem to be straight with no or with very small dentitions. One of the distinct diagnostic character is the sudden reduction in the fiber diameter at both ends (figure 4) leaving three portions, two of them are strongly tapering distal portions and one constituting the median portion, the two tapering distal part may or may not equal the mid portion.

Statistics given in table (1) refer to high variability in length and diameter of vessel members and the occurrence of the high rate of large sized vessel in the macerated mixture. Vessel shapes displayed in (figure 4) shows different evolutionary levels (Ghose and Das, 2001), ranging from most developed or advanced to the most primitive, but with higher ratios for the longest primitive members in the wood mixture. All vessel types of (i) both the end walls transverse (often typically quadrate) (ii) both the end walls oblique and (iii) mixed type are present. The occurrence of minute inter-vessel pitting and spiral thickening is a useful tool for identifying this species.

*S. purpurea*

The average fiber cell walls of this species are significantly thicker than cell wall of other species. Few fibers are found to exhibit straight forms; most are zigzag, curved or winding. Dentitions are restricted to the distal ends, ranging in number from 1to 6, on one side of the fiber. The two ends are often dissimilar in shape, number and size of dentitions. One of the diagnostic characters is the presence of course irregular of up to 6 dentitions on only one side of some fibers (figure 5).

The average length of vessel elements of *S. purpurea* is significantly lower than that of other species, but their average vessel diameter is significantly higher than other species (table 1). The advanced vessel type of both end walls transverse is common, one end wall transverse
and the other oblique is also common, while both the end walls with very slightly oblique is less common. The lower length/width ratio and the increased number of both end walls transverse are evidence of advancement of the wood tissue of this species more than any other Salix species.

From comparing different species, it reveals great differences in intensity of inter-vessel pitting; some members are even without lateral pitting. The former type of vessels which appear to be quadratic in cross section are very abundant, while the latter is rather rare and appear circular in cross section, a structure probably indicating the trend of wood tissue evolution. For the first type of the one end truncate and the other oblique, the length/width ratio is highly variable probably referring to the different environmental adaptation. According to Haarer (1952) the result may be influenced by parental gene tree and other environment condition effect.

Figure (1): *S. alba*. 1. Vessel element, both ends slightly oblique, inter-vessel near adjoining vessels, 2. Vessels of variable ends and areas of pitting, 3. libriform fibers, 4. Medium part of a libriform fiber showing cell wall thickness and cell lumen, 5. Distal part of a libriform fiber showing dentitions on one side of the fiber.
**Figure (2):** *S. acmophylla*: 1. Two ends by end connected vessel elements, both with two end transverse, 2. Two ends by end connected vessel elements, both with two ends oblique (140x), 3. Typically, straight-shaped libriform fiber, 4. Distal part of a fiber showing minute irregular dentitions, 5. Fiber cell wall and lumen with a fiber tip, 6. An irregular-shaped libriform fiber (below), Vessel member and numerous variously shaped libriform fibers (above).
Figure (3): *S. babylonica*. 1. Vessel element of mixed end walls with intensive lateral pitting, 2. A vessel element of mixed end walls with no lateral pitting, 3. High length/width vessel element with libriform fibers, 4. A primitive type of a vessel element with both ends steeply oblique, 5. Two ends by end connected vessel elements, both with two end transverse, 6. Libriforms of different length and shapes, 7. Fiber cell wall thickness and lumen, 8 and 9. Distal fiber portions showing variously oriented dentitions.
Figure (4): *S. aegyptiaca.* 1. Vessel member, very short with both ends transverse, 2 and 3. Vessel members of more or less transverse end walls, 4. Vessel member with one end wall transverse and the other oblique, 5. Vessel member with both ends oblique, 6. A very primitive vessel member with the both end walls steeply sloped, 7. Different types of libriform fibers, 8. Distal fiber portions showing different types of dentitions.
Figure (5): S. purpurea. 1. Vessel member with one end oblique and the other transverse, 2. Vessel member both end walls transverse, 3. Two vessel members, one (above) with mixed end walls, the other (below) with both end walls slightly oblique, 4. Libriform fibers, 5. Fiber cell wall thickness and cell lumen, 6 and 7. Two types of distal portions showing variable kinds of dentitions.
Important taxonomic applications:

A. Fiber dentition:
1. Dentitions of most fibers occur only on the distal ends: *S. alba*, *S. babylonica*, *S. aegyptiaca*, *S. purpurea*.
2. Dentitions of some fibers occur along the length of the fiber: *S. acmophylla*.

B. Dentition size and orientation:
1. One of dentitions on the distal portion is large and conspicuous, either oriented upward to the fiber tip or perpendicular to the surface: *S. babylonica*.
2. None of the dentitions are so large and conspicuous, little differences occur between them: *S. alba*, *S. acmophylla*, *S. aegyptiaca*, *S. purpurea*.

C. Fiber structure:
1. Sudden reduction in the fiber diameter at both ends, dividing the fiber into three portions, two at both ends, one median: *S. aegyptiaca*.
2. Gradual reduction in the fiber diameter towards ends: *S. alba*, *S. acmophylla*, *S. babylonica*, *S. purpurea*.

D. Vessel length:
1. Vessel element never exceeds 0.55mm in length: *S. alba*, *S. acmophylla*, *S. babylonica*, *S. purpurea*.
2. Vessel element exceeds 0.55mm and reaching 0.57mm in length: *S. aegyptiaca*.

E. Vessel morphology:
1. Vessels with minute inter-vessel pitting and spiral thickening: *S. aegyptiaca*.
2. Vessels with larger-sized inter-vessel pitting, with no spiral thickening; *S. alba*, *S. acmophylla*, *S. babylonica*, *S. purpurea*.

F. Vessel type:
1. Wood macerated mixture containing vessel type with both the end walls steeply oblique: *S. babylonica*, *S. aegyptiaca*.
2. Wood macerated mixture containing no vessel type of both end walls steeply oblique: *S. alba*, *S. acmophylla*, *S. purpurea*.

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Evolutionary Aspects of Dicotyledon Wood.
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استعمال العناصر الخشبية المفصلة في تصنيف أنواع جنس الصفصاف في منطقة كردستان العراق

**المخصر**

درست صفات العناصر الخشبية المفصلة لأجل التطبيقات التصنيفية إذا جمعت العينات من خمسة سفان لكل من (Salix acmophylla, S.alba, S.purpurea, S.aegyptiaca, S.babylonica) والتي اختبرت لمثل المناطق جغرافية مختلفة للحصول على أعلى تباين ممكن. أثبتت الدراسة بأن خشب معظم الأنواع يضم العناصر الخشبية البدائية فضلا عن تلك التي توصف بالاقل أو الأكثر تقدمًا. بينما النتائج تشابه مختلف الأنواع في امتلاكها متساوية بالنسبة فضلا عن وجود اختلافات كبيرة في طول وعرض الأوعية وبالتالي نسبة الطول/العرض وقد لوحظ أيضا وجود اختلافات كبيرة في نوع الوعاء الخشبي وصفات الجذور النهائية لعناصر الأوعية البدائية منها فضلا عن توزيع وغزارة النقر ضمن النوع الواحد وبين الأنواع المختلفة، و تعد جميع الصفات اعلاه ذات أهمية في التطبيقات التصنيفية. الألياف الطويلة ذات النهايات الضيقة هي الوجهة التي وجدت ضمن هذا الجنس فضلا عن وجود الألياف التسلسية كما وجد بأن جميع الأنواع قد اظهرت قدرة هدف قيئ في نهایات العبدة ونادرًا، ماظهرة على طول اللفيف بشكل الترتيب الشكلي لسنن، توزيعها، حجمها، واتجاهها صفات تشخيصية هامة.
OCCURRENCE OF TOMATO LEAF MINER TUTA ABSOLUTA MEYRIK (LEPIDOPTERA:GELECHIIDAE) IN Duhok REGION (A)

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Abstract:
The aim of this work is to investigate the population density and infestation percentage of Tuta absoluta on tomato crop Lycopersicum esculentum Mill under field conditions in three locations at Duhok province/ Kurdistan region/ northern of Iraq in 2012. The average number of mines/ leaflet, larvae/ leaf and larvae/ fruit during the study season were 2.33, 0.34 and 0.61 respectively. The results showed a significant differences in infestation percentage and number of male per trap among the three locations. The highest percentage of infestation was recorded in September as 74.00, 72.00 and 60.00 for Summel, Shekhan and Zawita respectively. The maximum number of males/ trap/ week was 1205.40 recorded on 26/8/2012 in Summel. Concerning the use of pheromone trap for pest monitoring, linear regression analysis results was significant between trap catches and the number of mines per leaf and the infestation rate of leaves.

Keywords: Tuta absoluta, population dynamics, Pheromone traps, Tomato.

1- Introduction

Tomato (Lycopersicon esculentum) is one of the economic important crops of Iraq. It is consumed as fresh table tomato and as an essential raw material for a variety of food processing industries. According to Food and Agriculture Organization 2010, tomato was planted in highland in the world reached to 1.2 million hectare and in Iraq the planted area reached to 842.296 hectare. In Duhok province, more than 12000 hectare of land was planted with tomato in 2012. Tomato production faces many problems from several causes such as seasonal weather, temperature, humidity, diseases and insect pests as thrips, whitefly, tomato fruitworm, leaf miner, leafhopper, aphid and mites.

The tomato borer, Tuta absoluta (Meyrik) (Lepidoptera:Gelechiidae) is one of the most destructive pest of tomato crops in South America (Epp,2010). After its first detection in eastern Spain in 2006, its rapidly invaded various other European countries and spread through the Mediterranean basin (Desneux et al.2010). In Iraq, tomato borer T. absoluta was first detected in Autumn 2010 from sex pheromone traps installed in Rabia region, Nineveh province (AbdulRazzak et. al.2010). Since that time, this pest spread quickly in all tomato growing areas, destroying entire open and protected fields. Ramireze et. al.(2010) reported that the damage by T. absoluta can reach to 100% in unprotect crops and was considered in its region distribution area as asignificant tomato insect pest (Leite et. al. 2008). It can develop on other Solanaceous plants, like potato (Pereyra and Sanchez,2006), eggplant and wild species (Garacia and Espul, 1982). Abbes and Chermiti, 2011 reported that the trap catches can be correlated with larval damage. In this way, the minimal amount of spraying required to control the pest population.

The aim of this work is to study the occurrence and infestation level of T. absoluta on tomato crops Lycopersicum esculentum Mill under open field conditions in three sites as Summel, Zawita and Shekhan at Duhok governorate during 2011-2012 crop season.
2- Material and methods

2-1: Study area

For general observations, initial survey was done before starting the project in order to put the hand on the predominant areas in which the tomato planted and settled in Duhok provinces. To carry out this work, three commercial fields located in three sites within Duhok governorate (36° 54’ N, 43° 8’ E) were chosen as Shekhan (483 m a.s.l and 34 km far from Duhok centre), Zawita (855 m a.s.l and 16 km far from Duhok centre) and Summel (473 m a.s.l and 16 km far from Duhok centre). The average degree of temperature and relative humidity was obtained from Metrological Station of Duhok Governorate.

2-2: Experiment procedure

To determine the tomato infestation by the tomato leaf miner, two methods were followed as:

1- leaves sampling for recording larvae and mines of *T. absoluta* :

To calculate the larvae and mines number of *T. absoluta*, fields were divided to five subareas approximately 0.2 ha. Each subarea with a similar number of plants to ensure that all the area represented in the samplings. Weekly sampling from May to September were carried out and in each field 25 plants were randomly selected being five in each subarea. At each selected plants five leaflets were collected and singly packed in labelled plastic box then transported to the laboratory.

At the laboratory, with aid of Binocular, each leaflet was examined and the number of mines and larvae per leaflet was registered (Leite et al. 2001). Also the number of larvae/fruit was recorded during this study.

2- Trapping methods for adult males harvesting :

This experiment was conducted in two fields, Summel and Shekhan site. The adults of *T. absoluta* were monitored by using pheromone lure TUA-500 and the TUTASAN® trap (TUTASAN® is a water trap specifically conceived to early detect and catch the tomato leaf miner males, each trap contains of one plate and one pheromone container (green basket with a lid)). The pheromone lure was put in the green basket, closed up with the lid and fixed at the top of the plate. The plate was filled with water till reaching the maximum level. The male insects are attracted by the sex pheromone lure from the top of the trap, and fall down into water. In each field six traps were well positioned and installed 0.5 m above ground level. Four traps at the tops of tomato plants (one in each direction) and two within the field centre. Sex pheromone lure were renewed every four weeks and the number of captured individuals was recorded every week during study period from May to September 2011-2012.

Note: Pest management strategies were applied by farmers and based largely on chemical applications (Insecticide) which targeted to controlling *T. absoluta* and other lepidopteran pests (Noctuidae: Heliothinae).

Data were statistically analysed as Factorial experiment using Randomize Complete Block Design (RCBD) with five replications and SAS program was used. The means were compared using Duncan's multiple range test (DMRT) at P ≤ 0.05. Also the relation between trap catches, the infestation rate and the number of mines per leaf was performed by a linear regression analysis.

3- Results and Discussion

3-1: Leaves sampling for recording larvae and mines of *T. absoluta*

The data represented in table (1) showed that the general mean number of *T. absoluta* mines/leaflet was 3.37, 2.46 and 1.2 for Shekhan, Summel and Zawita, respectively. *T. absoluta* mines resorted to concentrate on leaves on the middle and upper levels.

According to fig. (1), the number of leaf mines was initially low in May and June and increasing during August and September to reach a maximum of 24.33 on 26/8/2012. Allache and Demnati (2012) mentioned that in Biskra (Algeria), during the first phonologic stages of the crop, tomato plants are free from attack of *T. absoluta*.
Table 1: Mean mines, larvae number and the infestation percentage of leaves during study season.

<table>
<thead>
<tr>
<th>Sample sites</th>
<th>Average No. of mines/leaflet</th>
<th>Average No. of larvae/leaflet</th>
<th>Average No. of larvae/fruit</th>
<th>Leaves Infestation Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shekhan</td>
<td>3.37 a</td>
<td>0.36 b</td>
<td>0.54 c</td>
<td>10.67 B 67.20 a 69.00 A 72.00 a</td>
</tr>
<tr>
<td>Summel</td>
<td>2.46 b</td>
<td>0.46 a</td>
<td>0.68 a</td>
<td>34.00 A 68.00 a 73.33 A 74.00 a</td>
</tr>
<tr>
<td>Zawita</td>
<td>1.20 c</td>
<td>0.20 c</td>
<td>0.61 b</td>
<td>14.00 b 29.60 b 58.00 B 60.00 b</td>
</tr>
<tr>
<td>Average</td>
<td>2.33</td>
<td>0.34</td>
<td>0.61</td>
<td>19.56 54.93 66.78 65.33</td>
</tr>
</tbody>
</table>

Means followed by a common letter within the same Column are not significantly different at the 5% level by Duncan's Multiple Range Test (DMRT).

The maximum number of leaf mines was closely related to the density of *T. absoluta* larvae, that the damage caused by larvae on tomato leaves in three locations led to high density of mines rising up to an average of 7.20 mine/leaf starting from the beginning of July (fig. 1).

Harizanova et al. (2009) pointed that the leaves were the most heavily damaged plant parts with an average of 9.42 and 8.75 mines per leaflet on the middle and upper layer of the canopy respectively, followed by the fruits. Increase of the larvae number on leaves caused a high infestation rate reached to 73.33%, 69% and 58% in August for Summel, Shekhan and Zawita. The maximum number of mines/leaf recorded in September which was 31.4, 28.6 and 14.6 (fig. 2) in Shekhan, Summel and Zawita, respectively.

![Fig.1: Average number of *T. absoluta* mines on tomato leaves in three regions in 2012.](image-url)
Statistical analysis showed significant differences in leaf mines caused by *T. absoluta* larvae among the three locations. It could be concluded that in Zawita the mean number of mines (fig. 2), the infestation rate and the number of larvae per mine (tab. 1) was the lowest because it was located in mountainous location which was characterized by (855 m) above sea level and the low temperature degree.

![Graph showing the evolution of leaf mines per month and location in 2012.](image)

**Fig.2:** Evolution of the number of *T. absoluta* mines/month/location on tomatoleaves in three sites in 2012.

Lacordaire and Feuvrier (2010) reported that the number of *T. absoluta* was influenced perhaps by abiotic factors like temperature, insecticide application and by biotic factors i.e absence of natural enemies.

The average number of larvae/leaflet (tab. 1) was 0.46, 0.36 and 0.20 for Summel, Shekhan and Zawita, respectively. It was frequent according to Lacordaire and Feuvrier (2010) that the larvae of *T. absoluta* left their galleries and reinstalled in another leaflet or leaf as suggested by Torres et al. (2001), which also added that this low number might be due to insecticide applications which limited the development of the larvae.

3-2 Trapping methods for adult males harvesting:

According to fig. 3, the average number of adults male captured by pheromone traps was variable during study period in the two sites. *T. absoluta* was present from May until September in Summel, while in Shekhan it was noted from June to September (fig. 4). For the two sites, the adults captured increased towards the end of plant cycle. It was relatively low in June with an average of 10.9 adults/trap/week and their number became relatively high, as their attack became intense towards the end of crop cycle that reached to a maximum of 1055.40 adults/trap/week on 3/9/2012 (fig.3). An increase in temperature was detected at this time in the year. These results agreed with Nannini et.al. (2010), who mentioned that in Southern Sardinia (Italy), the highest number of moths caught in traps were in September-October. These results also matched with those found by Miranda et.al. (1998) and Lacordaire and Feuvrier (2010), who underlined the occurrence and increase in leaf miner captures during the crop season. During this gradual increase, each renewal of the capsules was often followed by an increase in the number of trapped adults.
The average number of captured males/week was respectively about 378.73 and 337.23 in Summel and Shekhan. Although the number of adults captured in Summel was more abundant than that in Shekhan and there was a significant differences between these two population, the figure of the evaluation of mass trapping in Summel field shows a similar shape of that in Shekhan (fig. 4). Indeed after 16.80 males caught on 19/6/2012, the number of trapped males continued to increase speedily to exceed 1205.40 on 26/8/2012.
To investigate that the sex pheromone trap reflects the real level of damage in tomato crops and the infestation rate of plants depending on the number of trapped males of *T. absoluta*, the relation between trap catches, the infestation rate and the number of mines per leaf was performed by a linear regression analysis.

Analysis showed a significant relationship between the number of mines per leaf and the number of trapped males in both Summel and Shekhan (fig. 5 A, B). However, linear regression between infestation rate and the number of catches in sex pheromone traps was also significant in two regions (fig. 6 A, B). These results agreed with Abbes and Chermiti (2011) who reported that the number of leaves and leaflets were differentially affected by the densities of *T. absoluta* infestation (*p* ≤ 0.05).

![Fig. 5: Linear regression between the number of caught males of *Tuta absoluta* and the number of mines per leaf in Summel (A) (*R^2* = 0.88) and Shekhan (B) (*R^2* = 0.53) in 2012.](image)
Fig. 6: Linear regression between the number of caught males of *Tuta absoluta* and the infestation rate of leaves in Summel (A) ($R^2 = 0.37$) and Shekhan (B) ($R^2 = 0.31$) in 2012.

Results of linear regression between the number of trapped males and the infestation level suggest the utilization of the sex pheromone traps as good indicators of the infestation rate of the crop. Some authors recommend 30 trapped males per trap per week to start chemical curative measures. Monitoring is very important in IPM programs; it allows the detection of the early infestations. Using of chemicals based on data from pheromones traps are usually more effective in controlling first attacks and avoiding the rapid colonization of the crop. This can significantly reduce the number of sprayed insecticides, and preserve natural enemies of the pest.

**References**


ل دفّارا دهوكٌ Tuta absolta Meyrick(Lepidoptera: Gelechiidae)

الخلاص:

أجريت هذه الدراسة لتحديد الكثافة العدوى و نسبة الإصابة بخطار اوراق الطماطة على Tuta absoluta. محصل الطماطة تحت ظروف الحقل المخطط في ثلاث مناطق تابعة لمحافظة دهوك (سييل، شيخان و زاوية) للكردستان العراق 2012. كان معدل عدد الاعتداء/ورقة عن المحافظات في جميع الإصابات وعدد الذكور لكل مصيدة بين المناطق الثلاثة. على نسبة الإصابة سجلت في إبلاول حيث وصلت إلى 74٪ و 26٪ في تأييل، شيخان و زاوية على التوالي. سجل أعلى عدد للذكور/مصيدة/اسبوع في سمٌيل بتاريخ 26/8/2012 حيث وصل إلى 120.5 ذكر. نتائج تحليل الخطي أظهرت وجود علاقة بين عدد الذكور المسروقة في المصائد وعدد الاعتداء/ورقة و أيضاً مع نسبة الإصابة الإلاراق.
EFFECT OF FOLIAR SPRAY OF KNO3, HUMIC ACID CULTIVARS AND THEIR INTERACTIONS ON LEAF NUTRIENTS OF OLIVE (OLEA EUROPAEA L.) CVS. KHITHARY AND I18 TRANSPLANTS

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Abstract:

This study was carried out during the growing season (2012) in Bakrajo Nursery Station/ Sulaimani, Kurdistan Region-Iraq. Uniform and healthy olive (Olea europaea L.) cvs. Khithary and I 18 transplants of (2) years old were used. Filled with river loamy soil to investigate the effect of three levels KNO3 (0, 100 and 200 mg.L⁻¹), three humic acid concentrations (0,150 and 300 mg.L⁻¹) and their interactions on leaf nutrients of Olive cvs. Khithary and I 18 transplants. The results are summarizing as follows: Khithary significantly dominated over cv. I 18 in total leaf chlorophyll, P, K, Zn. However cv. I 18 significantly increased N and Fe. The interactions between cv. Khithary with KNO₃ significantly increased P, K, Fe. While, cv. I 18 significantly increased N and Fe. The interactions between cv. I 18 +300ppm of humic acid significantly increased Fe. While Khithary with humic acid caused the highest values of N, P, K, Zn. The interactions between KNO₃, humic acid and cv. Khithery affected significantly on most of the leaf nutrients characteristics. While, 200 ppm KNO₃+0 humic acid with cv. Khithary increased all parameters except leaf Zn.

* Part of M.Sc thesis of the second author

Key words: KNO₃, Humic acid, Olive transplant

INTRODUCTION

Olive belongs to the botanical order, Ligusterales, family (Oleaceae), this family includes (30) genus including (Olea) which has (600) species. Olive is botanically called (Olea europaea L.). Commercial olives belong to the (Europaea) species, this species has two subspecies: oleaster and sativa (Bartolucci, and Dhakal 1999). World olive production performs an important role in the economy of many countries such as Spain, Italy, Greece, Turkey and Tunisia. Olive is an important perennial crop in many agricultural regions of the Mediterranean countries, as it is the most important olive growing region. The olive tree yield has two main products: oil and table olives (Sibbett, et al, 2005). In Iraq, olive trees growing in some areas of central North Iraq and Kurdistan Region, Nineveh is the governorate leading olive producer, its cultivation in Nineveh spreading in an area including villages of (Baashiqa, Bahzany, Fadiliya, Sheikh Uday, Dhecan, Sinjar), Diyala, Kirkuk, Baghdad, Erbil, Duhok, Aqrah, Bamarni followed by Babylon (Shaima, 2012).

The importance of olive fruit is due to heavy loading and dietic value, as the fruit is a good source of vitamins (A, B, C, D, E) and minerals like (K, Ca, Mg and P) Ibrahim, (2005). In addition, olive oil is filled with mono-unsaturated fatty acids and has many anti-oxidative properties as phenolic acid (Shaima, 2012). Potassium takes part in many important processes, regulating the opening and closing of stomata, the transport of organic and inorganic ions within the plant; promoting the maturity, yield, size and quality of the fruit. Sufficiency level of (K %) in olive leaves were (0.8-1.3), which sampled from the mid-length of current year's young shoots that do not bear fruit (Ashraf, et al., 2004). Organic fertilizers are natural materials and good medium for the interaction of micro-organisms and provide plant with nutrients as well as having an indirect role in nutrition by the activity of micro organisms. So using organic and bio-fertilizers instead of the chemical forms could be the way to produce the natural healthy fruits. In this respect, the organic fertilization improved vegetative growth and nutritional status (Farag, 2006).

This investigation aimed to:

Study the effect of KNO₃ and humic acid on leaf nutrients parameters of (Khithary and I 18) transplants in the climate at Kurdistan Region. Find out a fertilization program can replace the
mineral which will be beneficial for organic production of olives. Save human health and environment.

Impact of both olive cultivars which newly entered to the region on the vegetative growth of olive transplants. In addition to study the possibility of the production transplanting with proper size, in a short period of time.

**Materials and Methods:**

The study was carried out during (2012) in Bakrajo Nursery station/ Sulaimani, Kurdistan Region-Iraq, located on 15km southwestern of Sulaimani city. Uniform and healthy olive (cvs. Khithary and I 18) transplants of (2) years old were chosen, (Khithary is originated in Syria and I 18 is originated in Spain). The experiments were started in (March 15th 2012), as transplants were grown in pots each of (5 kg) weight. Three KNO3 concentrations (0, 100 and 200 mg.L⁻¹), KNO3 compound of 44% K₂O, 13% N and 37% K. (Restrep-Diaz et al.,. 2009). Humic acid (HA) concentrations (0,150 and 300 mg.L⁻¹), abo najmeh20, compound of %20 humic acid, %20 organic potassium and %10 organic carbon, Naser company for agrochemical-Syria), were sprayed at 15th April and repeated at same concentrations in 15th May.

**Experimental design and statistical analysis:**

The experiment was arranged in factorial experiment. The completely Randomized Block Design (R.C.B.D) was used, the experiment comprised of (18) treatments with three replicates, each replicate was presented by five pots each pot contained one transplant (Al-Rawi, and Khalafalla1980).

The obtained data were tabulated and statistically analyzed by computer using SAS system (1996). The differences among various treatment means were tested with Duncun multiple range test at (5%) level. SAS Institute (1996).

**Parameters:**

The following measurements were recorded on November 25th

1- Nitrogen concentration was determined with MicrokJeldahl.
2- Phosphorus concentration was determined with colorimetric methods using Spectrophotometer Pharmacia LKB method.
3- Potassium concentration was determined with using flamephotometer.
4- Iron and Zing concentration (ppm) was determined by Atomic Absorption Spectrophotometer.

**RESULTS AND DISCUSSION**

1- Leaf nitrogen%

Table (1) shows that the transplant when treated with 200 mg KNO₃.L⁻¹ gave the highest value of nitrogen content (1.56%) and the lowest value (1.44%) recorded from untreated transplants. Nitrogen content% in the leaves of transplants untreated with humic acid significantly increased leaf nitrogen contents compared with other treatments. Leaves nitrogen content% differed between the two cultivars, leaves of 'I 18' cultivar contained significantly higher nitrogen content (1.52%) compared with 'Khithary' leaves (1.46%). The interactions between KNO₃ and humic acid significantly influenced nitrogen content in transplants leaves that treated with 0 mg humic acid.L⁻¹ and 200 mg KNO₃.L⁻¹ which gave the highest value (1.76%) and the lowest value (1.26%) obtained with 200 mg KNO₃.L⁻¹ plus 300 mg humic acid.L⁻¹. KNO₃ and cultivar interactions displayed cv. 'I 18' transplants treated with 200 mg KNO₃.L⁻¹ gave the highest percentage of nitrogen (1.58%). The interactions between humic acid and cultivar significantly increased nitrogen content% untreated 'Khithary' transplant which gave the highest value (1.75%) and the lowest value (1.23%) was recorded from in 'Khithary'
Table (1): Effect of KNO₃, humic acid and their interactions on leaf nitrogen (%) content of olive transplants cvs. 'Khithary' and 'I 18'.

<table>
<thead>
<tr>
<th>Var.</th>
<th>HA (mg.L⁻¹)</th>
<th>KNO₃ (mg.L⁻¹)</th>
<th>Mean effect of Var.</th>
<th>Var*HA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>100</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Khithary</td>
<td>0</td>
<td>1.57 c-e</td>
<td>1.72 bc</td>
<td>1.95 a</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>1.32 g</td>
<td>1.32 d-f</td>
<td>1.66 d-f</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>1.39 e-g</td>
<td>1.16 h</td>
<td>1.46 b</td>
</tr>
<tr>
<td>I 18</td>
<td>0</td>
<td>1.45 e-g</td>
<td>1.48 d-g</td>
<td>1.57 c-e</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>1.53 d-f</td>
<td>1.64 b-d</td>
<td>1.79 b</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>1.35 g</td>
<td>1.53 d-f</td>
<td>1.37 fg</td>
</tr>
</tbody>
</table>

Means within a column, row and their interactions followed with the same letters are not significantly different from each other's according to Duncans multiple ranges test at 5% level.

Table (2) reveals that the transplant treated with 200 mg KNO₃.L⁻¹ gave the highest value of phosphorus content (0.47%) and the lowest value (0.39%) recorded in untreated transplants. Humic acid concentration decreased leaf phosphorus content, while untreated transplants gave the highest value of leaf p% compared with other treatments. Khithary cultivar leaves contained higher phosphorus content (0.45%) compared with leaves of 'I 18' (0.41%). The interactions between KNO₃ and humic acid significantly influenced phosphorus leaf content when treated 200 mg KNO₃.L⁻¹ which gave the highest value (0.66%) and the lowest value (0.37%) was recorded from 200 mg KNO₃.L⁻¹ plus 300 mg humic acid.L⁻¹. In the case of KNO₃ and cultivar interaction, it was found that 'Khithary' leaves treated with 200 mg KNO₃.L⁻¹ gave the highest percentage of phosphorus (0.53%) compared with other interactions. The interactions between humic acid and cultivar showed that phosphorus content % of untreated 'Khithary' transplants gave the highest value (0.56%) and the lowest value (0.39%) was recorded from 'I 18' transplants when treated by 150 mg humic acid.L⁻¹. Results of KNO₃, humic acid and cultivars interactions indicated that spraying 'Khithary' olive cultivar with 200 mg KNO₃.L⁻¹ plus 0 mg humic acid.L⁻¹ was the most potent treatment which gave (0.85%) phosphorus, while the lowest phosphorus content (0.33%) in Khithary transplant when treated with 0 mg KNO₃.L⁻¹ plus 300 mg humic acid.L⁻¹.
Table (2): Effect of KNO₃, humic acid and their interactions on leaf phosphorus (%) content of olive transplants cvs. 'Khithary' and 'I 18'.

<table>
<thead>
<tr>
<th>Var.</th>
<th>HA (mg/L⁻¹)</th>
<th>KNO₃ (mg/L⁻¹)</th>
<th>Var*HA</th>
<th>Mean effect of Var.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>100</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Khithary</td>
<td>0</td>
<td>0.35 ef</td>
<td>0.48 bc</td>
<td>0.56 a</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.41 b-f</td>
<td>0.43b-e</td>
<td>0.38d-f</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.33 f</td>
<td>0.49 b</td>
<td>0.37d-f</td>
</tr>
<tr>
<td>I 18</td>
<td>0</td>
<td>0.46 bd</td>
<td>0.36 ef</td>
<td>0.46 bd</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.39c-f</td>
<td>0.39c-f</td>
<td>0.38c-f</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.42b-f</td>
<td>0.41b-f</td>
<td>0.38d-f</td>
</tr>
</tbody>
</table>

Mean effect of KNO₃: 0.39 b 0.43 b 0.47 a

Mean effect of HA: 0.41 b

Means within a column, row and their interactions followed with the same letters are not significantly different from each other’s according to Duncans multiple ranges test at 5% level.

3- Leaf potassium (%)

Table (3) shows that spraying olive transplant with KNO₃ or humic acid at both levels not affect the leaf potassium content%. Leaves of 'Khithary' cultivar contained significantly higher potassium content (0.99%) when compared with leaves of 'I 18' (0.77%). The interactions between KNO₃ and humic acid significantly influenced potassium content in the leaves of transplants when untreated transplant gave the highest value (0.98%). KNO₃ and cultivar interactions showed that the leaves of 'Khithary' transplants treated with 100 mg/L⁻¹ KNO₃ contained the highest percentage of potassium (1.08%) compared with other interaction between KNO₃ and cultivar. The interactions between humic acid and cultivar non affecter on potassium content% of the 'Khithary' transplants when treated with 300 mg humic acid/L⁻¹ which gave the highest value (1.00%). Results of KNO₃, humic acid and cultivars interactions indicated that spraying 'Khithary' olive cultivar with 100 mg KNO₃/L⁻¹ plus 300 mg humic acid/L⁻¹ was the most potent treatment which gave the highest value (1.14%) potassium, while the lowest potassium content (0.62%) in 'I 18' transplant was found when treated with 100 mg KNO₃/L⁻¹ plus 0 mg humic acid/L⁻¹.
Table (3): Effect of KNO₃, humic acid and their interactions on leaf of potassium (%) content of olive transplants cvs. 'Khithary' and 'I 18'.

<table>
<thead>
<tr>
<th>Var.</th>
<th>HA (mg.L⁻¹)</th>
<th>KNO₃ (mg.L⁻¹)</th>
<th>Var*HA</th>
<th>Mean effect of Var.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>100</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Khithary</td>
<td>0</td>
<td>1.04 ab</td>
<td>1.00 ab</td>
<td>0.92 a-d</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.92 a-d</td>
<td>1.12 a</td>
<td>0.91 a-e</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.93 ab</td>
<td>1.14 a</td>
<td>0.92 a-d</td>
</tr>
<tr>
<td>I 18</td>
<td>0</td>
<td>0.92 a-c</td>
<td>0.62 e</td>
<td>0.92 a-d</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.89 a-e</td>
<td>0.77b-e</td>
<td>0.78 b-e</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.80 b-e</td>
<td>0.63 de</td>
<td>0.63 c-e</td>
</tr>
</tbody>
</table>

Mean effect of KNO₃: 0.92 a, 0.88 a, 0.85 a
Mean effect of HA

Means within a column, row and their interactions followed with the same letters are not significantly different from each others according to Duncans multiple ranges test at 5% level.

4- Leaf iron (ppm)

In table (4), iron content (ppm) in leaves differed significantly between the two cultivars, 'I 18' cultivar contained significantly higher iron content (82.70 ppm) when compared with 'Khithary' (78.69 ppm).

Table (4): Effect of KNO₃, humic acid and their interactions on leaf iron (ppm) concentrations of olive transplants cvs. 'Khithary' and 'I 18'.

<table>
<thead>
<tr>
<th>Var.</th>
<th>HA (mg.L⁻¹)</th>
<th>KNO₃ (mg.L⁻¹)</th>
<th>Var*HA</th>
<th>Mean effect of Var.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>100</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Khithary</td>
<td>0</td>
<td>34.68 i</td>
<td>60.28 h</td>
<td>101.40 a</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>68.91 f</td>
<td>95.69 b</td>
<td>99.79 a</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>86.61 c</td>
<td>75.23 e</td>
<td>85.63 c</td>
</tr>
<tr>
<td>I 18</td>
<td>0</td>
<td>63.36 g</td>
<td>93.97 b</td>
<td>77.79 d</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>95.11 b</td>
<td>66.43 f</td>
<td>68.01 f</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>84.96 c</td>
<td>95.08 b</td>
<td>99.59 a</td>
</tr>
</tbody>
</table>

Mean effect of KNO₃: 72.27c, 81.11 b, 88.70 a
Mean effect of HA

Means within a column, row and their interactions followed with the same letters are not significantly different from each others according to Duncans multiple ranges test at 5% level.
It was clear that treated transplants, with 200 mg KNO3 L-1 gave the highest value of iron content (88.70 ppm). Results of KNO3, humic acid and cultivars interactions indicated that spraying 'Khithary' olive cultivar with 200 mg KNO3 L-1 plus 0 mg humic acid L-1 was the most potent treatment which gave (101.40 ppm) iron. The interactions between KNO3 and humic acid significantly influenced iron content in leaf, when treated with 200 mg KNO3 L-1 plus 300 mg humic acid L-1 gave the highest value (92.61 ppm) and the lowest value (49.02 ppm) was recorded in untreated transplants. Regarding KNO3 and cultivar interactions, leaf of 'Khithary' transplants treated with 200 mg KNO3 L-1 contained the highest percentage of potassium (95.61 ppm) compared with other interaction between KNO3 and cultivar. The interactions between humic acid and cultivar had significantly increased in iron content (ppm) in cv. 'I 18' transplant when treated with 300 mg humic acid L-1 giving the highest value (93.21 ppm) and the lowest value (65.46 ppm) was recorded in untreated transplants of cv. 'Khithary'.

5- Leaf zinc (ppm).

In table (5), it was notice that the transplant when treated to 200 mg KNO3 L-1 gave the highest value of zinc content (17.02 ppm). Zinc content in leaf of transplants treated with 300 mg humic acid L-1 increased significantly. Zinc content in leaves differed significantly between the two cultivars, 'Khithary' leaves cultivar contained higher zinc content (13.48 ppm) when compared with 'I 18' (12.68 ppm). The interactions between KNO3 and humic acid significantly influenced zinc content in the leaves when treated with 200 mg KNO3 L-1 plus 0 mg humic acid L-1 by giving the highest value (20.75 ppm).

<table>
<thead>
<tr>
<th>Var.</th>
<th>HA (mg.L⁻¹)</th>
<th>KNO₃ (mg.L⁻¹)</th>
<th>Var*HA</th>
<th>Mean effect of Var.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>100</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Khithary</td>
<td>6.54 g</td>
<td>13.84 d</td>
<td>14.81 cd</td>
<td>11.73 cd</td>
</tr>
<tr>
<td></td>
<td>7.84 f</td>
<td>14.84 cd</td>
<td>14.25 cd</td>
<td>12.31 c</td>
</tr>
<tr>
<td></td>
<td>14.87 cd</td>
<td>15.24 c</td>
<td>19.12 b</td>
<td>16.41 a</td>
</tr>
<tr>
<td>I 18</td>
<td>6.53 g</td>
<td>11.85 e</td>
<td>26.68 a</td>
<td>15.02 b</td>
</tr>
<tr>
<td></td>
<td>15.11 c</td>
<td>7.42 fg</td>
<td>12.55 e</td>
<td>11.69 cd</td>
</tr>
<tr>
<td></td>
<td>7.85 f</td>
<td>11.43 e</td>
<td>14.67 cd</td>
<td>11.32 d</td>
</tr>
<tr>
<td>Mean effect of KNO₃</td>
<td>9.79 c</td>
<td>12.44 b</td>
<td>17.02 a</td>
<td></td>
</tr>
<tr>
<td>Var* KNO₃</td>
<td>Khithary</td>
<td>9.75 d</td>
<td>14.64 c</td>
<td>16.06 b</td>
</tr>
<tr>
<td></td>
<td>I 18</td>
<td>9.83 d</td>
<td>10.23 d</td>
<td>17.97 a</td>
</tr>
<tr>
<td>Mean effect of HA</td>
<td>9.38 b</td>
<td>13.38 b</td>
<td>17.86 a</td>
<td></td>
</tr>
<tr>
<td>HA KNO₃</td>
<td>0</td>
<td>6.53 e</td>
<td>12.85 c</td>
<td>20.75 a</td>
</tr>
<tr>
<td></td>
<td>11.47 d</td>
<td>11.13 d</td>
<td>13.40 c</td>
<td>12.00 c</td>
</tr>
<tr>
<td></td>
<td>11.36 d</td>
<td>13.34 c</td>
<td>16.90 b</td>
<td>13.86 a</td>
</tr>
</tbody>
</table>

Means within a column, row and their interactions followed with the same letters are not significantly different from each others according to Duncans multiple ranges test at 5% level.
Regarding KNO₃ and cultivar interactions, it was found that the leaves of 'I 18' transplants treated with 200 mg.L⁻¹ KNO₃ contained the highest percentage of zinc (17.97 ppm) compared with other interactions between KNO₃ and cultivar. The interactions between humic acid and cultivar had significantly increased zinc contents in 'Khithary' transplants when treated with 300 mg. humic acid.L⁻¹ giving the highest value (16.41ppm) and the lowest value (11.32 ppm) was recorded from the untreated transplants of cv. 'I 18'. Results of KNO₃, humic acid and cultivars interactions indicated that spraying 'I 18' olive cultivar with 200 mg KNO₃.L⁻¹ plus 0 mg humic acid.L⁻¹ was the most potent treatment which gave (26.68ppm) zinc, while the lowest zinc (6.53ppm) was recorded in untreated 'I 18' transplant.

**Discussions:**

1- KNO₃: It is clear from studied parameters that the effect of KNO₃ on nutrient composition characteristics significantly affected and improved all parameters, the results may be due to role of K and N in plants such as photosynthesis reactions, nucleic acid metabolism, protein and carbohydrate biosynthesis due to increased leaf mineral content. (Hafez, and El-Metwally 2007). Potassium takes part in many important processes, regulating the opening and closing of stomata, the transport of organic and inorganic ions within the plant, (Ibrahim, 2005) and (Elloumi, et al., 2009).

2- Humic acid: For the effect of humic acid, the same tables that show studied parameters indicates that leaf nutrient status gave the highest value. The reason for the positive effect of humic acid may be due to the role of (HA) to stimulate plant growth by acting on mechanisms involved in: cell respiration, photosynthesis, protein synthesis, water and nutrient uptake and enzyme activities (Nardi et al., 1996, Chen et al., 2004, and Ali, et al., 2007). Whereas direct effects are various biochemical actions exerted at the cell wall, membrane or cytoplasm and mainly of hormonal nature (Varanini and Pinton 2001 and Chen et al., 2004). The hormone like activities of HAs is well documented in various papers, in particular auxin, cytokinin and gibberellins like effects (Piccolo et al., 1992 and Pizzeghello, et al., 2002).

3- Cultivars: It's clear from most tables that the vegetative growth characteristics significantly differed between the two cultivars. The differences between the cultivars in leaves nutrient such as (N, P, K, Fe and Zinc, may be ascribed to the differences in genotype characteristics (Jordao, et al., 1999). In addition, the genetic integrity of the plant species might influence particular nutrient uptake efficiency (Popovic et al., 1999). Then, these differences in nutrient uptake efficiency between cultivars may cause differences in vegetation growth characteristics. Also, the differences in growth vigor between the two cultivars may be attributed to the response of different cultivars to the local environmental conditions according to the genetic variation between the cultivars (Gaafar and Saker 2006 and Khalifa 2007).

**References**


كاريتكرونا زبلي بيوضيس نايرفايت و ترشي هوميك و تيكة لكورا وان لسدار توجيت نال决战 لجامكنت زمبيوني

Khithary and 118

بوخته:

نذا فاكولهنا يهانه نندجاالان ل سال (2015) له نجامكنت زبلي لبيكدجز ل باتيرغها سليماني. هريما
كوردستانا غيرافي. نجامكنت ودل تيك و دورر ز نخوني ميلواراتني ز هرددور جوريي خزيري و 18 زدييون
(0.01، 0.02، 0.03، 0.05، 0.10، 0.20) ترشي هوميك (0.01، 0.05) ملغم. مختبر و كيسنكارتي ييتكا ها بلغا ز
مادى خاربي ين نجاما هرددور جوريي زدبيوو خزيريو 18 و KNO3 و

تشري هوميك: كليكةنا بلغا ب 3 و تشري هوميك زانيو بييسيوري دير بيزردوريي خزيرى ل همي خسالنتي گامش
کمک. تيكلكر كرن نايميرأ جوريي 18 دگرلا KNO3 و تشري هوميك زانيو بنيشيره كي تيكلكر گامش
ننني نامكنتي. دريزيي تيكتينينانت. تيكلكر كرن نايميرأ جوريي و 118 تشري هوميك زدبيوو كر بييسيوريي
پييسيوري ل تيرى قرمي. زمربى بلغا. روروبى بلغا. بنلى جوريي خزيرى دگر تشري هوميك هواكرىي هيديانا
ثيبيكي بلده ل نامكنتي. دريزيي تيكتينينانت. تيكلكر كرن نايميرأ زلبي و تشري هوميك
هواكرىي هيديانى ييتكا مدنننأ كيک بلده. تيكلكر كرنأ 4 و تشري هوميك و جوريي 18 اكرميرأ

تشري هوميك دگرل لحمشي كيکسي يو هواكرىي زدبيوو بلده نامكنتي. دريزيي تيكتينينانت.

(Olea europaea L.) وحاص الحيميك فى النمو وحمى أورا شلالات الزبون (KNO3 و RCBD
صنفي الحفوري و 118

الخلاصة:

تؤخذ هذه التجربة خلال موسم النمو 2012 في منطقة مشيل يكرو في محافظة السليمانية/إقليم كردستان/العراق.

بدأت التجربة في 15 آذار 2012 حيث تم زراعة الشتلات في آكياس النباتات مسحة (5 كجم) تم لملها بالرمل. لدراسة
تأثير ثلاثة تركيز من KNO3 (7.70، 10.00، 15.00 ملغم / الكرمة) وثلاث تركيز من حاص الحيميك (0.01، 0.20، 0.50)
وزراعة للدغة في خوداع تأثيرها على إحتوى الأوراق من العناصر لشتلات صنفي الزودي الاسبسي و 18 I تتبديد
التيتية عاملية بواقع ثلاث مكررات حسب تصميم القاطعات العشوائية الكاملة (RCBD) و ذلك بزراعة خمسة شلالات لكل
وحدة تجريبية بكم و الكموم الريش ننفس الزاكرى في 15 نيسان و كرت عملية الريش تباس KNO3 و حاص الحيميك في 15
و حاص الحيميك تمفا معينا في مجوع الأوراق من العناصر. صنف 18 I ساد KNO3 و حاص الحيميك تفا معايا معينا في مجوع الأوراق من العناصر. صنف 18 I و Zn، K، P معينا على صنف الحفوري في مجوع الأوراق من العناصر. الختام بين الصفن 18 0 مع
واجد معايا في تكرار KNO3 و حاص الحيميك للرش الورقي ب KNO3 و حاص الحيميك تفا معينا في مجوع الأوراق من العناصر. صنف 18 I و Zn، K، P معينا على صنف الحفوري في مجوع الأوراق من العناصر. الختام بين الصفن 18 I و Zn، K، P معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و Zn، K، P معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على صنف الحفوري في مجوع الأوراق من العناصر. صنف 18 I و KNO3 معينا على
EFFECT OF CULTIVARS, COMPOST, HUMIC ACID AND THEIR INTERACTIONS ON LEAF NUTRITIONAL STATES OF SWEET CHERRY (*PRUNUS AVIUM L.*)

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2 Department of Horticulture, Ministry of Agriculture and irrigation, Kurdistan Region – Iraq.

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Abstract:
The present study was carried out on sweet cherry (*Prunus avium* L.), during the growing season of (2012-2013) on private sweet cherry orchard, located in the Bibad village near Amadi town / Duhok governorate- Kurdistan region-Iraq. The field experiment was done in the orchard that contained two sweet cherry cultivars (“Nefertity” and “Berlit”). The application of compost was done in December 25th 2012, at (0, 2, 4 and 6 kg /tree), foliar spray of humic acid done at (0, 100, 200 and 300 ppm) and repeated after two weeks. The results are summarized as follows: Nefertity cultivar significantly dominated over Berlit cultivar in leaf N, P content, whereas Berlit cultivar dominated leaf (N, K, Ca). Compost specially at 4kg/tree has significantly improved leaf P, K, whereas, compost at 6 kg/tree leaf nutrient N. Humic acid specially at 300 ppm has significantly increased the leaf nutrients N, P, while, 200 ppm increased the leaf K, while, 100 ppm increased the leaf N, K. The interactions between cultivars, compost and humic acid has affected significantly most of the studied parameters. The more effective treatment interactions was Nefertity + 6 Kg /tree of compost plus 200 ppm of humic acid which increased significantly P,K, but Nefertity + 6 Kg /tree of compost plus 300ppm of humic acid treatment increased leaf N content.

*Part of M.Sc thesis of the second author

Key word: Cherry, Compost, Humic acid

INTRODUCTION:
The sweet cherry (*Prunus avium. L.*) belongs to the Rosaceae family, sub-family Prunidaecea (Rodrigues and Antunes, 2008). It is believed to be originated from the regions between the Black and Caspian Sea of Asia Minor. Seed spreading by birds carried it to Europe, where the earliest cultivation of sweet cherry was reported. Further spread to North America via English colonists occurred in the seventeenth century. (Webster and Looney, 1996). Sweet cherry is one of the most popular temperate fruits. According to (FAO, 2010), cherries produced worldwide, and an important horticultural crop - approximately 2.2 million tons of cherries were produced worldwide in 2009 (Anonymous, 2010). Compost is a resource of converting organic waste material, such as food waste, yard waste and manure, into a matter called humus, a nutrient-rich soil amendment.

Humus is an essential element in maintaining healthy soil and plant, making composting a useful tactic for nurturing productive agricultural fields, ornamental plants and grasses (Chiumenti, 2005). The compost has an important role in the agriculture sector because it contains a high amount of elements necessary for plant growth and soil improvement, the use of compost as a fertilizer for plant in Iraq and Kurdistan has a large space and this is backward in the field of agriculture when we compare with the developed countries. Thus, encouraging to use the compost as a first studies in Kurdistan and Iraq in order to encourage our farmer to use the compost as a plant fertilizer. The risks and problems posed by heavy metals in fertilizers and other soil inputs have increasingly drawn the attention of farmers, environmental organizations, consumers, and public policy makers.

This study evaluates a wide spectrum of soil amendments and fertilizers used in organic agriculture, including biosolids, major nutrient fertilizers, industrial wastes, composts, liming materials and micronutrient sources with a focus on inputs used in organic agricultural production in Iraq. Humic substances are no one single chemical recognized as humic acid, since the chemical makeup has never been completely defined. These materials are composed of complicated organic mixtures which are associated together in a random manner, resulting in extraordinarily complex materials. It has been suggested that no two molecules of humus are exactly the same (Mikkelsen, 2005). The cherry fruit is desirable in Kurdistan in
relations as far as consumption and also desirable by farmers to cultivate but the production is very little so far. Therfor, this study is as the first one on the nutrition of cherry fruits in order to improve the quality and increase their production.

This investigation aimed to study the response of two cultivars to local environmental condition, and their response to fertilized by organic matter (compost and humic acid). However, it also hopes to confirm the risk of heavy metal concentration in compost to find a fertilization program that can replace the minerals which will be beneficial for organic production of sweet cherry, since there are little or no studies in Kurdistan about the role organic fertilization in yield and quality of sweet cherry according the aims were to evaluate the interaction effect of cultivars, compost, humic acid on leaf nutrient statutes and some heavy metal concentration of two cultivars of sweet cherry.

MATERIALS AND METHODS

This study was carried out in the Bibad village near Amadi town/ Duhok governorate/ Kurdistan region of Iraq. The orchard is situated at latitude: 37.05°N, and longitude 43.29° E and at an altitude of 1202 m above the sea level. The experiment included two cultivar of sweet cherry "Nefertity" and "Berlit". Application of compost at different levels (0, 2, 4 and 6 kg /tree), and foliar spray of humic acid at concentrations (0, 100, 200 and 300 ppm). The compost were carried out is consisting from resides waste of Dohuk city, is produced in Kawsha manufacture of compost fertilizer. The orchard experiment of compost application was done in December 25th 2012, by working hole around the tree under brunch projection and mixed with the soil in order to investigate the effect of soil application of four levels of compost, (0, 2, 4 and 6 kg /tree). The humic acid is a liquid content analysis w/w, organic matter 5%, (K2O) 1%, total humic + fulvic acid 15%. The foliar spray of humic acid was done in April 15th 2013, after full bloom at four concentrations (0, 100, 200 and 300 ppm) and replicated the same concentrations after two weeks after the first spray.

Statistical analysis

All the obtained data were tabulated and statistically analyzed with computer using SAS system (SAS, 1996). The experiment followed Randomized Complete Block Design in factorial Experiment; the experiment comprised of 32 treatments with three replicates, each replicate was presented by one tree of each cultivar. The differences between various treatment means were tested with Duncun Multiple Range Test at 5% level, (Al- Rawi and Khalaf-Alla, 2000).

Measurements:
Leaf nutrients states
1- Total nitrogen (N %).
2- Total phosphorus (P%).
3- Total potassium (K %).
4-Total calcium (Ca%).
RESULTS

1- Leaf nitrogen content (%)

Figure (1) Effect of cultivar, compost and humic acid on leaf N content (%) of sweet cherry.

Table (1): interactions effect of cultivar, compost and humic acid on leaf N content (%) of sweet cherry.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Humic acid (ppm)</th>
<th>Compost (Kg/tree)</th>
<th>Cultivar × Humic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berlit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.13 h</td>
<td>1.37 gh</td>
<td>1.82 a-f</td>
</tr>
<tr>
<td>100</td>
<td>1.62 b-g</td>
<td>1.47 fg</td>
<td>2.03 a</td>
</tr>
<tr>
<td>200</td>
<td>1.86 a-e</td>
<td>1.51 e-g</td>
<td>1.92 a-c</td>
</tr>
<tr>
<td>300</td>
<td>1.98 ab</td>
<td>1.93 a-c</td>
<td>1.78 a-f</td>
</tr>
<tr>
<td>Nefertity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.54 d-g</td>
<td>1.74 a-f</td>
<td>2.04 a</td>
</tr>
<tr>
<td>100</td>
<td>1.61 c-g</td>
<td>1.83 a-f</td>
<td>1.88 a-d</td>
</tr>
<tr>
<td>200</td>
<td>1.31 gh</td>
<td>1.86 a-e</td>
<td>1.58 d-g</td>
</tr>
<tr>
<td>300</td>
<td>1.30 gh</td>
<td>1.90 a-d</td>
<td>1.57 c-g</td>
</tr>
</tbody>
</table>

Cultivar × Compost

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Humic acid × Compost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berlit</td>
<td></td>
</tr>
<tr>
<td>Nefertity</td>
<td>1.65 de</td>
</tr>
<tr>
<td></td>
<td>1.57 ef</td>
</tr>
<tr>
<td></td>
<td>1.89 a-c</td>
</tr>
<tr>
<td></td>
<td>1.93 ab</td>
</tr>
</tbody>
</table>

Humic acid × Compost

<table>
<thead>
<tr>
<th>Humic acid (ppm)</th>
<th>Cultivar × Compost</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.34 e</td>
</tr>
<tr>
<td>100</td>
<td>1.62 cd</td>
</tr>
<tr>
<td>200</td>
<td>1.59 d</td>
</tr>
<tr>
<td>300</td>
<td>1.64 cd</td>
</tr>
</tbody>
</table>

Means of each factor and their interaction followed by the same letter are not significantly different from each others according to duncans multiple ranges test at 5% level.
Figure (1) clearly shows that there are no significant differences between two cultivar on leaf nitrogen content. Soil application of compost at a level (6 kg/tree) had significantly effect which recorded (1.98 %), and the lowest value was recorded in control (1.55 %). Foliar spray of humic acid at a concentration (300 ppm) were significantly effective, which registered (1.82 %), while the lowest values were recorded in control (1.70 %). The interaction between cultivars and humic acid on leaf N indicate that cultivar Berlit with humic acid at a (300 ppm) was the best treatments when compared with other treatment. The results of interaction between application (6 kg/tree) of compost plus (200 ppm) of humic acid, produced the better treatment (2.059 %), while the lowest concentration was observed in control (1.464 %).

2. Leaf phosphorus content (%):

Figure (2) shows the phosphors concentration in cultivar Nefertity increased significantly compared with cultivar Berlit. Obviously soil application of compost at a (4 kg/tree) was better treatment when compared with other treatments which recorded (0.949%), and the lowest value was recorded in control (0.437%). The same figure illustrates that the humic acid at a concentrations (300 ppm) was suggestively better treatment which registered (0.823 %), but the lowest values were recorded in control (0.468 %).

However, the interaction between Nefertity plus (6 kg/tree) of compost gave the highest value (1.306 %) when compared with control. The interaction between cultivar Nefertity and (100 ppm) of humic acid gave the highest value compared with other treatment.

![Figure 2](image)

**Figure (2)** Effect of cultivar, compost and humic acid on leaf P content (%) of sweet cherry.

Manifests the interaction between (4 kg/tree) of compost with (100 ppm) of humic acid, produced the better treatment which registered (0.917) when compared with other treatments.

The same table illustrates that the interaction effect of cultivar Nefertity + (4 kg/tree) compost + (100 ppm) humic acid provided the best treatment which was (1.26 %) when compared with another treatments.
Table (2): Interactions effect of cultivar, compost and humic acid on leaf P content (%) of sweet cherry.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Humic acid (ppm)</th>
<th>Compost (Kg/tree)</th>
<th>Cultivar × Compost</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Berlit</td>
<td>0</td>
<td>0.17 e</td>
<td>0.21 c-e</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.27 c-e</td>
<td>0.33 b-e</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.60 b-d</td>
<td>0.50 b-e</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.63 bc</td>
<td>0.67 bc</td>
</tr>
<tr>
<td>Nefertity</td>
<td>0</td>
<td>0.22de</td>
<td>0.54 de</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.40 de</td>
<td>0.68 bc</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.53 b-e</td>
<td>0.61 bc</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.65 bc</td>
<td>0.60 b-d</td>
</tr>
</tbody>
</table>

Means of each factor and their interaction followed by the same letters are not significantly different from each others according to Duncans multiple ranges test at 5% level.

The interaction between cultivar Berlit + (100 ppm) was the best treatment when compared with other treatments. The interaction between (6 kg/tree) of compost + (200 ppm) of humic acid produced the best treatment.

3. Leaf potassium content (%):

Figure (3) shows that the leaf potassium in cultivar Berlit had significant effect compared to cultivar Nefertity. Obviously application of compost at a level (4 kg/tree) was the best treatments which noted (1.701 %), when compared with other treatment. The foliar spray of humic acid at a (100 ppm) was a better treatment among the humic concentration which registered (1.746 %), value (1.926 %), when compared with other treatments. In the same table the interaction between cultivar Nefertity + (6 kg/tree) of compost + (200 ppm) of humic acid provided the maximum value (2.011 %), when compared with other treatments. The interaction of cultivars Berlit plus (2 kg/tree) of compost has the best treatment to increase the leaf potassium, (1.608 %) when compared with other treatments.
Figure (3): Effect of cultivar, compost and humic acid on leaf K content (%) of sweet cherry.

Table (3): Interactions effect of cultivar, compost and humic acid on leaf K content (%) of sweet cherry.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Humic acid (ppm)</th>
<th>Compost (Kg/tree)</th>
<th>Cultivar × Compost</th>
<th>Humic acid × Compost</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0  2   4  6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berlit</td>
<td>0 1.21 jk</td>
<td>1.39 g-j 1.41 f-j</td>
<td>1.48 d-j 1.37 d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 1.84 a-c</td>
<td>1.87 a-c 1.93 ab</td>
<td>1.66 b-i 1.82 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 1.77 a-e</td>
<td>1.77 a-e 1.85 a-c</td>
<td>1.84 a-c 1.81 ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300 1.689 a-h</td>
<td>1.75 a-f 1.84 a-c</td>
<td>1.91 a-c 1.80 ab</td>
<td></td>
</tr>
<tr>
<td>Nefertity</td>
<td>0 1.04 k</td>
<td>1.32 i-k 1.42 f-j</td>
<td>1.36 h-i 1.29 d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 1.61 b-i</td>
<td>1.64 b-i 1.81 a-d</td>
<td>1.57 c-i 1.66 bc</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 1.63 b-i</td>
<td>1.74 a-f 1.70 a-g</td>
<td>2.01 a 1.77 ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300 1.68 a-h</td>
<td>1.70 a-g 1.63 b-i</td>
<td>1.47 e-j 1.62 c</td>
<td></td>
</tr>
</tbody>
</table>

Means of each factor and their interaction followed by the same letters are not significantly different from each others according to Duncans multiple ranges test at 5% level.
4. Leaf calcium content (%)

Figure (4) effect of cultivar, compost and humic acid on leaf calcium content (%) of sweet cherry.

Table (4) interactions effect of cultivar, compost and humic acid on leaf Ca content (%) of sweet cherry.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Humic acid (ppm)</th>
<th>Compost (Kg/tree)</th>
<th>Cultivar × Humic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Berlit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2.05 b-d</td>
<td>2.08 a-d</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.14 a-d</td>
<td>2.08 a-d</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2.20 a-d</td>
<td>2.15 a-d</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>2.30 a-c</td>
<td>2.31 a-c</td>
</tr>
<tr>
<td>Nefertity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.66 d</td>
<td>1.74 cd</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.78 cd</td>
<td>1.85 cd</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.84 cd</td>
<td>2.06 a-d</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>1.92 b-d</td>
<td>1.89 cd</td>
</tr>
</tbody>
</table>

Means of each their interaction followed by the same letters are not significantly different from each others according to Duncans multiple ranges test at 5% level.
Figure (4) shows leaf Ca content in cultivar Berlit had a significant effect when compared with cultivar Nefertity. The results clearly show the soil application of compost at a level (6 kg/tree) was significantly increased the leaf Ca content, (2.201 %), when compared with other treatments.

Foliar spray of humic acid at (300 ppm) was better treatment which registered (2.224 ppm), when compared with other treatments. Table (4), the interaction between cultivar Berlit plus (2 kg/tree) of compost was the best treatment when compared with other treatments. The results of combining between cultivar Berlit plus humic acid at a (300 %) indicated the highest significant effect when compared with other treatments. Also, the interaction between (6 kg/tree) compost plus plus (300 ppm) humic acid produced the best treatment (2.400 ppm), when compared with control. The interaction between cultivar Berlit + (6 kg/tree) compost + (300 ppm) humic acid which provided the best treatment (2.673 %) when compared with other treatments.

DISCUSSION

1. The effect of cultivar on leaf nutritional state, may be ascribed to the differences in genotype characteristics for root growth, nutrient absorption efficiency and photosynthesis process efficiency (Jorda, et al., 1999). Also, the response of different cultivars to the local environmental condition according to the genetic variation between the cultivars (Gaafar and Saker, 2006 ; Khalif, 2007).

2. The effect of compost may be due to the improvement of soil physical, biological properties and chemical properties resulting more release of nutrient elements available which absorbed by plant root and its effect on the physiological process, in addition to water use efficiency, also adequate nutrient quantities of nitrogen, phosphorus, and potassium, which increase both rate of leaf expansion as well as cell division which subsequently leads to larger individual leaves and higher photosynthesis activities (Abd El-Wahab, 2011). May be attributed to a higher nutritional uptake mainly by greater expansion of root system due to increased supply of photosynthetic productions in the leaves, attributed to presence of plant growth regulators, which are produced by increased activity of microbes such as fungi, bacteria, yeasts, actinomycetes and algae (Arancon et al., 2004).

3. The effect of humic acid may be acting on mechanisms involved in: cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, enzyme activities. (Ali et al., 2007). The hormone like activities of (HA) is well documented in various papers, in particular auxin-, cytokinin and gibberellins like effects (Pizzeghello et al., 2002). Also, the effect of humic acid may be due to the role of (HA) to increase of cation exchange capacity which affects the retention and availability of nutrients, or due to a hormonal effect, or a combination of both. (Chun hua et al., 1998).

Conclusions:

1. The Nefertity cultivar was superior on the Berlit cultivar in increasing vegetative growth, and most leaf nutrients.

2. Soil application of compost at a level 4 kg/tree was more than other levels and control in increasing most vegetative growth parameters and most leaf nutrients.

3. Foliar spraying of humic acid at concentration 300 ppm had more effects.

4. The interaction of Nefertity cultivar + 4 Kg of compost + 300 ppm of humic acid had more effect on increasing in most studied parameter.

2. Recommendations:

The flowing points of view can be recommended:

1. Testing other cultivars of sweet cherry.

2. Testing the different level and time of application of compost as well testing other organic fertilizer such as animal manure chicken manure and municipal.

3. Testing the effect of spraying with humic acid on the other sweet cherry cultivar.

4. Farmers are recomended to use the compost in their orchards like other organic fertilizer, such as animal manure without any fear of toxic materials, soil pollution and harmful effect of heavy metals.

References:


كارتيكرون د رضاء كرنا

N, Ca

كانت أكتريكون د رضاء كرنا

Cation كارتيكرون د ترابي

عن بدلونا و ناسم 3 كيلو لبومستي كارتيكرون د ترابي

CationCd, Fe

كانت أكتريكون د رضاء كرنا و يوكي، ترابي

P, N

كانت أكتريكون د ترابي

200 ppm

كانت أكتريكون د ترابي

P, K

كانت أكتريكون د ترابي

دغقل رضائنا نيميك نمسيدي

P, N

كانت أكتريكون د ترابي

4 كيلو

كانت أكتريكون د ترابي

300 ppm

كانت أكتريكون د ترابي

Prunus

Tأثير الصنف والكروست وحضاوض الهيوميك وتدخالها على مستوى الغذائيات في الكرز الحلو

Prunus avium L.

الخلاصة:

تم إجراء الدراسة على الباكيجات الكرز الحلو (Prunus avium L.) خلال الموسم 2013- 2014 في بستان أهلية مرزعة بصراع الكرز الحلو، الواقع في قرية برد قرب مدينة العمانية / محافظة دهوك / إقليم كردستان / العراق.

استخدم كروست بارعة مستويات من سماد (0, 2, 4, 6 كيلو/ شجرة) وباكيجات اسبي. اول الروع الوعلي

باكيجات اسبي بارعة تراكيز (0, 100, 200, 300 ppm) بحدود بعد التزهير الكامل و وكر نفس الراقه بعد

أوسين من الروع الأول.

تأثير الصنف: كان للصنف تاويemu معوي و تفوق على الصنف بولايت في مستوى الغذائيات في الورقة من

N, Ca, K ن

وكذلك الصنف بولايت لها تأثير معوي في مستوى الغذائيات في الورقة من

P

تأثير الكروست: استخدم كروست متروكة أقفة الكرز الحلو في مستوى 4 كيلو/ شجرة للنظام معوي على مستويات الغذائيات في الورقة من

N, Ca, K

وكمثال المستوي 6 كيلو/ شجرة كان له تأثير معوي في زيادة مستويات الغذائيات في الورقة

P

TARGET

400 ppm

TARGET

300 ppm

TARGET

N, Ca, K

TARGET

P, N, Ca

TARGET

100 ppm

تأثير التداخل الثلاثي: بين الصنف، الكروست مع الباكيجات اسبي: التداخل الثلاثي كان له تأثير في زيادة معظم

الغذائيات، لكن العامل الأكثر فاعلاً هو التداخل بين الصنف مع الكرز + 4 كيلو من الكروست + المستوي

300 ppm من الباكيجات.
SOME TECHNOLOGICAL PROPERTIES OF SAWN BOARD *Eucalyptus camaldulensis* Denh. GROWN IN ASKIKALAK

Mohammedamin Yasin Taha  
School of Forestry Dept. of Forestry, Faculty of Agriculture, University of Duhok, Kurdistan Region-Iraq.  
(Accepted for publication: May 5, 2015)

**ABSTRACT**

This study deals with wood density and static bending boards property of *Eucalyptus camaldulensis* Den. It was conducted using standardized, defect-free test specimens. Boards of air drying showed lowest value (749.117 kg/cm²) of static bending when compared with kiln (812.267kg/cm²) and solar (815.267kg/cm²) drying respectively, whereas, the quarter sawing boards recorded the lowest rates (586.633kg/cm²) when compared with flat sawn boards (1008.467kg/cm²), Also 2cm thickness level achieved the lowest rate (747.367 kg/cm²) when compared with other two levels 4cm (812.650 kg/cm²) and 6cm (837.633kg/cm²) of thickness. But, there were no any significant effects of wood density on the studied factors. *E. camaldulensis* in Kurdistan Region of Iraq has potential for traditional uses and by itself it can be harvested by applying suitable techniques at plantation areas, in saw mills and drying for utilization.

**KEYWORDS:** Wood Density, Static bending, *E. camaldulensis*, Sawn Board

**Introduction**

Kurdistan Region of Iraq is located in Northern Iraq. According to its fertile soil and appropriate environmental conditions have made this region known for its natural forests. Most prominently, the flowing of a number of big rivers through this region such as Tigris and some others lead to the prospect of establish artificial stands depending on river for irrigation.

About 30-40 species of the genus were introduced to Iraq during the last century Shahbaz, (2010).

Literature mentioned that the two main of them being *E.camaldulensis* and *E. microtithaca*, were considerably succeeded in middle and southern of country Roolitsch and Reader, (1969). *E. camaldulensis* Den. has been used for pulp, chipboard, fire wood, shelter belts and others. Appropriate temperature and humidity rates as well as precipitation in Kurdistan provided well situations for Eucalypt throughout the year. The shortage of manufacturing wood products in Iraq, so including in Kurdistan Region makes it difficult to have a clear overview of the prospects of eucalypt exploitation (Taha, 2013).

Eucalypts species are recognized simultaneously of the fastest wood producing trees. It has the capability to produce approximately100m³/capita. Some investigations in their studies (Myburg, et al., 2006,Acosta, et al.,2008 and Iglesias, et al., 2008) reported that there are about 18 Million hectares of Eucalypt in the world, and they expected the total areas of *Eucalyptus* will increase to reach up to 20 Million hectares in 2010 cited by (Taha,2013). It is distinguished that environmental circumstances might have their important belongings on the wood properties, and for the reason of the lack of researches on eucalypts wood in Iraq principally those associated to its drying, this study was designed to investigate the wood density and static bending (MOE) properties of dried sawn board of trees grown in Kurdistan Region.

**Materials and Methods**

Ten trees of *Eucalyptus camaldulensis* with DBH 35-45cm were selected, felled, and logged in a stand at Khabat district (Asikikalak) in Erbil Governorate, Kurdistan Region of Iraq where it lies at N 36° 15'; E 43° 38' and located at 252m, It is37km far from Erbil city. All logs were bucked (2m length) then they were sawed by sawing table with band saw (SIPA 100 Saw) preliminarily; the logs then were converted to planks. After that, the resulted planks were sawed into two types of board; quarter sawn board and flat sawn board at three different thickness 2,4, and 6cm. Logs were used for producing lumber for air drying, kiln drying, and solar drying methods respectively.

**Method of randomizing**

The following two characteristics were examined:

Wood density and static bending, before applying the test, make sure that the samples be
free from defects and splits. The tests concerned load the experiment specimens at Mid-length at prepared sawed boards.

With the aim of collect material to be used for present study determined by using ASTM D-143-94 and ASTM D-2395-93 procedures (Anonymous 1996) get 10 store dried (Air, Solar, and kiln) boards of (*Eucalyptus camaldulensis*) for each properties randomly. To evaluate wood properties of sawed boards, in situation (temperature 20-25°C, 35-40% relative humidity and wood moisture content 12%) defect free specimens (5×2×2cm) for wood density (kg/ cm²). And (30×2×2cm) for static bending (MOE) were tested by using the universal Strength Testing Machine.

The collected data so collected were analyzed statistically using experiment; it was included of three factors:
Factor A: Drying method; with 3 levels: (AD; Air Drying, KD; Kiln Drying and SD; Solar Drying)
Factor B: Thickness of board; with 3 levels: (Th1; 2cm, Th2; 4cm, and Th3; 6cm)
Factor C: Board kind; with 2 levels: (FS; Flat Sawn board and QS; Quarter Sawn board).

Numbers of treatment combinations were 18. Ten boards were chosen randomly to represents replication of each treatment combination. The experiment was statistically analyzed as factorial RCBD by using SAS program version 0.9 SAS, (2002). Statistical differences between treatment combination means were tested by Duncan Multiple Range test at 5% level Duncan, (1955).

**Results and Discussion**

The influence of interaction between drying methods, board thickness, and sawing methods on the studied parameters:

Effect of drying method, board thickness, and sawing method on wood density and static bending of dried board as shown in Table (1)

<table>
<thead>
<tr>
<th>Variable</th>
<th>d.f</th>
<th>Wood Density F Value</th>
<th>Wood Density Pr &gt; F</th>
<th>Static Bending F Value</th>
<th>Static Bending Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drying Methods (DM)</td>
<td>2</td>
<td>2.09</td>
<td>0.1276</td>
<td>4.45</td>
<td>0.0123</td>
</tr>
<tr>
<td>Thickness (Th)</td>
<td>2</td>
<td>2.21</td>
<td>0.1135</td>
<td>13.72</td>
<td>0.0001</td>
</tr>
<tr>
<td>Board Kind (BK)</td>
<td>1</td>
<td>3.08</td>
<td>0.0815</td>
<td>750.65</td>
<td>0.0001</td>
</tr>
<tr>
<td>DM×Th</td>
<td>4</td>
<td>1.06</td>
<td>0.3780</td>
<td>3.83</td>
<td>0.0054</td>
</tr>
<tr>
<td>DM× BK</td>
<td>2</td>
<td>0.73</td>
<td>0.4814</td>
<td>3.77</td>
<td>0.0252</td>
</tr>
<tr>
<td>Th× BK</td>
<td>2</td>
<td>0.96</td>
<td>0.3846</td>
<td>2.66</td>
<td>0.0731</td>
</tr>
<tr>
<td><strong>DM×Th×BK</strong></td>
<td>4</td>
<td>1.06</td>
<td>0.3778</td>
<td>3.77</td>
<td>0.0060</td>
</tr>
</tbody>
</table>
Table (1) refers that drying method (DM), thickness (TH), and board kind (BK) and their interaction could not affect significantly on wood density. Mean values of (Tab. 2) indicate that moderate values of wood density have been obtained in almost all treatments. Accordingly, differences should be so small that they would not be enough to give statistical significances. The results, also agreed with what has been found by Lima et al., (2008) who mentioned that density varies from a minimum of 0.319 g/cm³ to a maximum of 0.731 g/cm³. In general, wood structures formed in early stages of tree growing that have low density.

**Table (2):** Mean values of wood density as affected by drying method, board thickness, and board kind.

<table>
<thead>
<tr>
<th>Drying Methods (DM)</th>
<th>Board Thickness (TH) cm</th>
<th>Board Kind (BK)</th>
<th>MD $\rho$</th>
<th>TH Mean of (DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Air Drying (A)</strong></td>
<td>2</td>
<td>Flat</td>
<td>0.570</td>
<td>0.609</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Quarter</td>
<td>0.601</td>
<td>0.680</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>0.608</td>
<td>0.789</td>
</tr>
<tr>
<td><strong>Kiln Drying (K)</strong></td>
<td>2</td>
<td>Flat</td>
<td>0.570</td>
<td>0.609</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Quarter</td>
<td>0.601</td>
<td>0.680</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>0.608</td>
<td>0.789</td>
</tr>
<tr>
<td><strong>Solar Drying (S)</strong></td>
<td>2</td>
<td>Flat</td>
<td>0.570</td>
<td>0.609</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Quarter</td>
<td>0.601</td>
<td>0.680</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>0.608</td>
<td>0.789</td>
</tr>
<tr>
<td><strong>Mean of (BK)</strong></td>
<td></td>
<td></td>
<td>0.644</td>
<td>0.780</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(a)</td>
<td>(a)</td>
</tr>
<tr>
<td><strong>TH x BK</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0.578</td>
<td>0.616</td>
<td>0.601(a)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>0.655</td>
<td>0.939</td>
<td>0.797(a)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>0.689</td>
<td>0.786</td>
<td>0.738(a)</td>
<td></td>
</tr>
</tbody>
</table>

However, the diminishing of wood density alongside tangential direction (flat sawn board) can be associated to differentiations between chemical compositions in wood structures and existing of heartwood near to the pith compare to sapwood Akhtari, et al., (2012). The results were achieved from the study confirmed that the density of dried boards is located within the middy category (0.56 -0.75 g/cm³) according to the classification of the IAWA, (1989).

The studied factors were affected differently on static bending (MOE) (Tab.1). While board thickness showed moderate significant effects, drying method affected at lower level, and board kind (BK) high levels of confidence (p<0.05). Drying method showed high statistical influences on static bending. Boards dried by (AD) possessed the lowest values (Tab.3) because the density values were obtained in this study show the difficulty of different drying situations clearly.
Table (3): Mean values of MOE as affected by drying method, board thickness, and board kinds.

<table>
<thead>
<tr>
<th>Drying Methods (DM)</th>
<th>Board Thickness (TH) cm</th>
<th>Board Kind (BK)</th>
<th>MD×TH Mean of (DM)</th>
<th>(a)</th>
<th>(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Drying(A)</td>
<td>2</td>
<td>Flat</td>
<td>746.000</td>
<td>553.400</td>
<td>649.700</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Flat</td>
<td>1036.200</td>
<td>584.500</td>
<td>810.350</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Flat</td>
<td>1056.200</td>
<td>614.400</td>
<td>835.300</td>
</tr>
<tr>
<td>Kiln Drying(K)</td>
<td>2</td>
<td>Flat</td>
<td>1018.000</td>
<td>556.400</td>
<td>787.200</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Flat</td>
<td>1038.200</td>
<td>586.400</td>
<td>812.300</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Flat</td>
<td>1058.200</td>
<td>616.400</td>
<td>837.300</td>
</tr>
<tr>
<td>Solar Drying(S)</td>
<td>2</td>
<td>Flat</td>
<td>1021.000</td>
<td>559.400</td>
<td>790.200</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Flat</td>
<td>1041.200</td>
<td>589.400</td>
<td>815.300</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Flat</td>
<td>1061.200</td>
<td>619.400</td>
<td>840.300</td>
</tr>
<tr>
<td>Mean of (BK)</td>
<td>1008.467</td>
<td>Flat</td>
<td>586.633</td>
<td>797.550</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flat</td>
<td></td>
<td>(a)</td>
<td>(b)</td>
</tr>
<tr>
<td>TH×BK</td>
<td>T1</td>
<td>Flat</td>
<td>928.333</td>
<td>556.400</td>
<td>742.367</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>Flat</td>
<td>1038.533</td>
<td>586.767</td>
<td>812.650</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>Flat</td>
<td>1058.533</td>
<td>616.733</td>
<td>837.633</td>
</tr>
<tr>
<td>DM×BK</td>
<td>K</td>
<td>Flat</td>
<td>946.133</td>
<td>584.100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>Flat</td>
<td>1038.133</td>
<td>589.400</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flat</td>
<td></td>
<td>(a)</td>
<td>(b)</td>
</tr>
</tbody>
</table>

*Means of each factor and their interactions followed by the same letters are not significantly different from each other according to Duncan's multiple ranges test at 5% level.

The air dried boards (765.117 kg/cm²), when compared with kiln and solar drying respectively. While board thickness increased, static bending values increased too. Thin boards (2 cm thick.) bended only by (742.367 kg/cm²), while thickest ones (6cm thick.) deflected from straight line by (837.633 kg/cm²)as a mean may be caused by existence of different drying situation. The scholarships Bolza and Kloot (1963), Bryce (1967) and Louppe et al. (2008) were attributed by reason of low age and fast growth level.

Ogunsanwo, (2000), Oluwafemi and Adegbenga, (2007) through their studies on *Eucalyptus camaldulensis* were clarified that the values of density and mechanical properties achieved, They could be of use its wood in various aspects as building, construction, flooring, cabinetry, and furniture.

References:


-Bolza E, NH Kloot. (1963). The mechanical properties of 174 Australian timbers. Division of Forest Products Technological paper no.25. Commonwealth scientific and


هدمة سالوريّة تكتولوجىيّة بن دهين

بوجته:

Eucalyptus camaldulensis Denh.

(Static bending) (Wood density)

دارة خرابياً وستاندارد هاتيون. دهين برناها هنديّون هشکنون کیمیون دندانه (177.849 kg/cm²) دهین برناها هشکنون کیمیون دندانه (177.849 kg/cm²) 380.327 kg/cm² نامین در بهراود دهین (5) بهراود دهین (5) نامین در بهراود دهین (5) (kg/cm²) (kg/cm²) (kg/cm²)

هاتيون دهین شیوايی Quarter

بهراود دهین شیوايی راست (108.846 kg/cm²) بهراود دهین (5) 380.327 kg/cm² نامین در بهراود دهین (5) (kg/cm²) (kg/cm²) (kg/cm²)

پیشگاه بن کارتهکن متشانیا داری لمسر فاکتورن تاکتیکی نهانه می‌کند. ل هنرمندا یک فیلماه و نوعی شبیه یک فیلم وکالیتس Eucalyptus camaldulensis

جامگی، کارگه‌کنی داهیتکنوی و هنرمندان.

درستا بعضاً الصفات الکترونشیولی لالوگانالیزجیکالپورکس النامیا فی منطقه غیرات -اقیمن کوردستان العراق

الخلصاء

تم دراسة تاعییرکتاف و معامل الارطخا خصائص اللالوگالیکالپورکس. تم استعمال النماذج الباشیة وخلایة من العرب اللالوگانالیزجیکالپورکس(749.117 kg/cm²) سجلوا اقل قیمة لمعامل الارطخا مقاینه مع الخفیفة بالفرن (741.5267 kg/cm²) والشمی (741.5267 kg/cm²) على التوالي. مع ذلك، اللالوگانالیزجیکالپورکس بطریقة الشعاعی سجلوا اقل ممست (582.7093 kg/cm²) عند المقارنة مع الطریقة الباشیة (741.5267 kg/cm²)؛ بالإضافة الى اللالوگانالیزجیکالپورکس ذو سمك 2 سم حصلوا على اقل نسبة (741.5267 kg/cm²) عند المقارنة مع مستويات الباشیة (741.5267 kg/cm²) ودم 3 سم (741.5267 kg/cm²)؛ بينما، تم تشادیة الاتحالات المعویة لتعامل كتافنة الخشب على معامل الدراسة. تم تمثل اللالوگالیکالپورکس في اقیمن کوردستان العراق امکانیة استعمالها في مجالات التقليدية ویا لیاکیة، معامل الباشیة، و التحییف لاستغلالها.
THE EFFECT OF COLOR PLASTIC MULCHES ON GROWTH, YIELD AND QUALITY OF TWO HYBRIDS OF SUMMER SQUASH (CUCURBITA PEPO L.)

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Department Of Horticulture, Faculty of Agriculture, University of Duhok, Kurdistan Region – Iraq.
(Accepted for publication: May 8, 2015)

Abstract
This experiment was carried out at the vegetable research farm of Horticulture Department/ Faculty of Agriculture /Duhok University on two hybrids of summer squash during spring in the season of 2014, to study the effect of five color plastic mulches (without cover, transparent, black, red and blue) on two hybrids of summer squash (Amjed and Alexandra F1). The results showed that covers plastic mulches led to positive significant differences in leaf area (cm²), leaves chlorophyll content%, fruit number/plant, early yield and total yield as well as fruit weight (g), fruit length (cm) and fruit diameter (mm) as compared to without cover. There were no significant differences between the two hybrids in all detected traits. The interaction between covers plastic mulches and two hybrids was significantly enhanced all detected traits, since summer squash (Alexandra hybrid) covered with transparent plastic mulch were characterized by the highest values of leaf area (cm²), leaves chlorophyll content% and fruit diameter (mm), and summer squash (Amjed hybrid) covered with blue plastic mulch gave positive significant of fruit number/plant and total yields ton/ hectare.

Keywords: mulches, summer squash, hybrids

Introduction
Summer squash (Cucurbita pepo L.) is one of the most important vegetable crops in Iraq especially during spring. In addition to cultivation in fall season in some areas it consists of annual crops including cucurbitaceae and Summer squashes are planted for its fruits, which are the edible parts of the plant after cooking. It has a medium food value due to some nutritional elements (P, Ca and Fe), and some vitamins (Matlob et al., 1989). Yield of summer squash per unit area is remained too low which is about (12.82 and 13.09 t/ha.) in 1999 and 2000 respectively (Annual Statistic Book, 2000), as compared to the world production.

To increase vegetable production, many applications such as coverage are used. Coverage vegetables can promote early yield and reduce fruit defects. However, coverage can reduce evaporation from the soil surface, prevent weed growth, raise soil temperature, reduce costs, reduce insect number and increase yield (Ekinci and Dursun, 2006). Polyethylene mulches benefit to adjust the soils microclimate in order to prolong the growing season and increase plant growth (Tarara, 2000). Black plastic mulch has intense shortwave transmittances and high shortwave absorption, which causes quickly increased soil temperatures (Heibner et al., 2005) white plastic mulch is prefers during the summer season in warm regions because white plastic maintain soil moisture and providing cooler temperature. Kasperhauer (1992) mentioned that red plastic increased yield in some crops, believed that it is generate a positive phytochrome response, found that improved yield quality due to used colored plastic (Brown and Channel-Butcher, 2001) This study aimed to determine the effect of different coverage Plastic color on growth, some quality properties and yield in two hybrids of summer squash.

Materials and Methods
The experiment was conducted at the vegetable research farm, Faculty of Agriculture, University of Duhok, on summer squash during spring season of 2014. Seedlings were growing in first of April 2014 at a distance 40 cm between plants and 1.5 m between the rows.

The experiment comprised the effect of two hybrids namely (Amjed and Alexandra), five coverage (without coverage, transparent, Black, Red and Blue). Each treatment was replicated three times. A replicate contained ten plants per one and was implicated in a completely randomized block design (RCBD). The soil was well softened, and then it was divided into rows and in this study all plants received the regular agricultural practices that usually carried out in the vegetable crops. Coverage was done before planting the seedling. Data were analyzed by using SAS program (SAS, 2001).
Experimental measurements

Three plants were selected randomly from each experimental unit to measure:

1-Vegetative growth characteristic
   a-Leaf area (cm²)
   b-Leaf chlorophyll content%

2-Yield characteristic
   a-Early yield: The first three harvests from each treatment were weighted to considered as an early yield.
   b-Total yield: the total yield was measured by harvested all fruit from each treatment along the harvesting period were weighted to calculate the total yield Kg per plant and ton per hectare.
   c- Fruit number per plant: Number of fruits per plant along the harvesting period was counted from each experimental unit, starting from the commence of harvesting and lasted to the end of the growing season and calculated.

3-Fruit quality:
   five fruits from each treatments were randomly taken for determining average fruit character as follows:
   a- Fruit fresh weight (g )
   b- Fruit length (cm)
   c- Fruit diameter (mm)
   d- Fruit dry weight (gm).

Results and Discussion

Table (1) shows that transparent cover plastic mulch caused significant increase in leaf area (cm²) as compared to without coverage and insignificant increase in Chlorophyll content %. As for there was no significant differences between its two hybrids on leaf area (cm²) and Chlorophyll content %.

Concerning the effect of interaction between covers plastic mulches and hybrids observed that interaction between transparent cover and Alexandra hybrids was significant in its effect in leaf area (cm²) and chlorophyll content% by the highest values of (317.20) cm² and (54.90) respectively. The increase in growth was attributed to sufficient soil moisture at the root zone and minimized the evaporation loss due to covers. The extended retention of moisture and availability of moisture also lead to higher uptake of nutrient for proper growth and development of plant. Similar findings have also been obtained by Dean Ban et al. (2004), Ansary and Roy (2005) in watermelon, Angrej-Ali and Gaur (2007) in strawberry, Aruna et al. (2007) in tomato.

Table (1) Effect of covers plastic mulches on leaf area (cm²) and Chlorophyll content % of two hybrids of summer squash

<table>
<thead>
<tr>
<th>covers</th>
<th>leaf area (cm²)</th>
<th>Chlorophyll content %</th>
<th>hybrids</th>
<th>Covers effect</th>
<th>hybrids</th>
<th>Covers effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>AL</td>
<td>AM</td>
<td>AL</td>
<td>AM</td>
<td>AL</td>
</tr>
<tr>
<td>without coverage</td>
<td>209.40bc</td>
<td>184.30c</td>
<td>196.80b</td>
<td>50.93b</td>
<td>53.00ab</td>
<td>51.97a</td>
</tr>
<tr>
<td>White</td>
<td>302.60ab</td>
<td>317.20a</td>
<td>309.90a</td>
<td>53.23ab</td>
<td>54.90a</td>
<td>53.62a</td>
</tr>
<tr>
<td>Black</td>
<td>220.20a-c</td>
<td>252.80a-c</td>
<td>236.50b</td>
<td>54.00ab</td>
<td>52.67ab</td>
<td>53.80a</td>
</tr>
<tr>
<td>Red</td>
<td>234.70a-c</td>
<td>276.90a-c</td>
<td>255.80ab</td>
<td>52.63ab</td>
<td>53.33ab</td>
<td>52.98a</td>
</tr>
<tr>
<td>Blue</td>
<td>269.20a-c</td>
<td>237.30a-c</td>
<td>253.20ab</td>
<td>52.40ab</td>
<td>52.33ab</td>
<td>52.37a</td>
</tr>
<tr>
<td>hybrids effect</td>
<td>247.30a</td>
<td>253.70a</td>
<td>52.83a</td>
<td>53.07a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means within a column, row and their interaction following with the same latter are not significantly different according to Duncan multiple range test at the probability of 0.05 level
Data presented in Table (2 and 3) are clearly shown that covers caused significant increases in all yield characteristics as compared with without coverage. In case of cultivars there was no significant increase in all yield characteristics.

The interaction between covers and hybrids was significant in its effect. Since Amjed hybrid and blue cover were confined by highest value in fruit number/plant (29.00), total yield (4.50 kg/plant) and total yield (65.68 t/ha) as compared with the lowest values of these traits for without covers which gave (17.33, 2.28kg/ plant and 33.36 t/ha) respectively and the interaction between red polyethylene and Alexandra hybrid gave by the highest value in early yield kg/plant as compared with without cover. Plant under polyethylene produced larger fruit and have higher yield per plant because of better plant growth due to favorable hydro-thermal regime and complete weed free environmental. Dickerson et al. (2003) reported earlier yield under plastic mulch. The above results were in agreement with those of Dean Ben et al. (2004), Ansary and Roy (2005), Cenobio et al. (2007), and Arancibia and Motsenbocker (2008) in watermelon.

**Table (2)** effect of covers plastic mulches on fruit number and early yield(kg) of two hybrids of summer squash

<table>
<thead>
<tr>
<th>Covers</th>
<th>Fruit number/plant</th>
<th>Early yield(kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>AL</td>
</tr>
<tr>
<td>without coverage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transparent</td>
<td>24.89ab</td>
<td>25.11ab</td>
</tr>
<tr>
<td>Black</td>
<td>26.89ab</td>
<td>24.22b</td>
</tr>
<tr>
<td>Red</td>
<td>25.11ab</td>
<td>26.00ab</td>
</tr>
<tr>
<td>Blue</td>
<td>29.00a</td>
<td>28.78a</td>
</tr>
<tr>
<td>hybrids effect</td>
<td>24.64a</td>
<td>24.62a</td>
</tr>
</tbody>
</table>

Means within a column, row and their interaction following with the same latter are not significantly different according to Duncan multiple range test at the probability of 0.05 level.

**Table (3)** Effect of covers plastic mulches on Total yield kg/plant and Total yield t/ha of two hybrids of summer squash.

<table>
<thead>
<tr>
<th>Covers</th>
<th>Total yield Kg/plant</th>
<th>Total yield t/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>AL</td>
</tr>
<tr>
<td>without coverage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transparent</td>
<td>4.03a</td>
<td>4.39a</td>
</tr>
<tr>
<td>Black</td>
<td>4.08a</td>
<td>3.73ab</td>
</tr>
<tr>
<td>Red</td>
<td>4.16a</td>
<td>4.34a</td>
</tr>
<tr>
<td>Blue</td>
<td>4.50a</td>
<td>4.42a</td>
</tr>
<tr>
<td>hybrids effect</td>
<td>3.81a</td>
<td>3.92a</td>
</tr>
</tbody>
</table>

Means within a column, row and their interaction following with the same latter are not significantly different according to Duncan multiple range test at the probability of 0.05 level.
Data in Table (4 and 5) show that transparent cover plastic mulch had positive effect on fruit length (cm) and fruit diameter (mm) and no positive effect on fruit weight (g) and fruit dry weight (g). For the effect of hybrids there was no significant effect between two hybrids on fruit weight (g), fruit length (cm) and fruit dry weight (g).

Also the interaction between transparent covers plastic mulches and Amjed hybrid gave high significant effect on fruit length (16.27 cm) and fruit diameter (40.51 mm) and the interaction between black cover and Amjed hybrid give high significant effect on fruit weight (188.59 g). Among all mulching treatment, maximum fruit weight was recorded in black cover. It appears that black polyethylene mulch have induced favorable conditions conducive to a attainment of fruits of higher weight. The highest fruit length was due to congenial soil moisture results higher uptake of nutrition for better growth of fruit, the reduction in evaporation losses of soil moisture caused by mulches covered the soil surface in row of summer squash. The above results were in agreement with those of Ansary and Roy (2004), and Arancibia and Motsenbocker (2008) in watermelon, Aruna et al. (2007) in tomato.

Table (4) Effect of covers plastic mulches on fruit weight (g) and fruit length (cm) of two hybrids of summer squash.

<table>
<thead>
<tr>
<th>Covers</th>
<th>Hybrid</th>
<th>Fruit weight (g)</th>
<th>Fruit length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>AL</td>
<td>AM</td>
</tr>
<tr>
<td>without coverage</td>
<td>AM</td>
<td>130.72b</td>
<td>136.61a</td>
</tr>
<tr>
<td>Transparent</td>
<td>AL</td>
<td>142.51ab</td>
<td>13.17b</td>
</tr>
<tr>
<td>Black</td>
<td>AM</td>
<td>161.29ab</td>
<td>167.97a</td>
</tr>
<tr>
<td>Red</td>
<td>AL</td>
<td>153.11ab</td>
<td>170.85a</td>
</tr>
<tr>
<td>Blue</td>
<td>AM</td>
<td>165.63ab</td>
<td>165.89a</td>
</tr>
<tr>
<td>Red</td>
<td>AL</td>
<td>166.16ab</td>
<td>154.20a</td>
</tr>
<tr>
<td>hybrids effect</td>
<td>AM</td>
<td>160.20</td>
<td>158.01a</td>
</tr>
<tr>
<td>Red</td>
<td>AL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means within a column, row and their interaction following with the same latter are not significantly different according to Duncan multiple range test at the probability of 0.05 level.

Table (5) Effect of covers plastic mulches on fruit diameter (mm) and fruit dry weight (g) of two hybrids of summer squash.

<table>
<thead>
<tr>
<th>Covers</th>
<th>Hybrid</th>
<th>Fruit diameter (mm)</th>
<th>Fruit dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>AL</td>
<td>AM</td>
</tr>
<tr>
<td>without coverage</td>
<td>AM</td>
<td>34.55a-c</td>
<td>32.21c</td>
</tr>
<tr>
<td>Transparent</td>
<td>AL</td>
<td>29.86c</td>
<td>3.71a</td>
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<tr>
<td>Black</td>
<td>AM</td>
<td>40.51a</td>
<td>40.05a</td>
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<tr>
<td>Red</td>
<td>AL</td>
<td>39.58a</td>
<td>3.94a</td>
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<tr>
<td>Blue</td>
<td>AM</td>
<td>34.53a-c</td>
<td>36.20a-c</td>
</tr>
<tr>
<td>Red</td>
<td>AL</td>
<td>36.32a-c</td>
<td>4.11a</td>
</tr>
<tr>
<td>Blue</td>
<td>AM</td>
<td>36.20a-c</td>
<td>4.14a</td>
</tr>
<tr>
<td>hybrids effect</td>
<td>AM</td>
<td>37.62a</td>
<td>4.14a</td>
</tr>
<tr>
<td>Red</td>
<td>AL</td>
<td>34.40b</td>
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</tr>
<tr>
<td>Blue</td>
<td>AM</td>
<td>33.64bc</td>
<td>3.83a</td>
</tr>
<tr>
<td>hybrids effect</td>
<td>AM</td>
<td>3.83a</td>
<td>4.01a</td>
</tr>
</tbody>
</table>

Means within a column, row and their interaction following with the same latter are not significantly different according to Duncan multiple range test at the probability of 0.05 level.
References


تأثر النغمة البلاستيكية الملون على غرو وحلاص ومكونات الحاسل هجينين من القرع (Cucurbita pepo L.)

الخلاصة

اجريت هذه الدراسة في حقل الخضروات التابع لفاكولتى الزراعة/جامعة دهوك على هجينين من القرع خلال موسم
النمو 2014 لدراسة تأثير النغمة في خمسة ألوان من البلاستيك (بدون نغمة، شفاف، أسود، أحمر، أزرق) على هجينين
من القرع (أحمد والكمندرا) أظهرت النتائج بأن النغمة البلاستيكية ادى الى اختلافات معنوية موجبة في المساحة الورقية
(سم²) والمساحة الزاوية للكلوروفيل وعدد النشارات والحاسل المبكر والحاسل الكلي وكذلك وزن النورة (سم). وطول
النورة (سم) وقطر النورة (ملم) مقارنة مع النيابات المروعة بدون نغمة. ولم يظهر اختلافات معنوية بين هجينين في
جميع الصفات المذكورة. التداخل بين النغمة البلاستيكية وعينا نغمة معيناً جميع الصفات المذكورة، وتميزت
نيابات القرع هجين (الكمندرا) والغطاء البلاستيك الشفاف بعاثان اعلى الفائدة في المساحة الورقية (سم²) والنسبة
المزية للكلوروفيل وقطر النورة (ملم) واهجين (أحمد) والغطاء البلاستيك الأزرق أعطى زيادة معنوية موجبة في عدد
النمار/نيابات والحاسل الكلي طن/هكتار.

Cucurbita ناقفنا نايلونتين ورقها ورقها لسالوعيات كتاسكية وبرهمي وبيكباتيني نورغليدي (Cucurbita pepo L.)

نبعه

نف فاكولتى هدنه احنباين لزلفين جاندنلي/زانكيا دهوك ل سر دورو ورويى دورو ل سار دورو ورويى دورو كوندا
نافيرنا كريقية ناقفنا بيج تروين روكا ورقها فوقيه/روهك وبرهمي رويي. هيلموه لسر
دور دورو دورو كوندا (أحمد والكمندرا) دناجا دايركرو ناخافن ب نايلونين جيرونا يش جاها ب نزريه هيلموه
روبرهي بيلها ونبيقالة كلونوفريل ورقها فوقيه/روهك وبرهمي رويي. هيلموه لسر
دور دورو ونبيقالة كلونوفريل ورقها فوقيه/روهك وبرهمي رويي. هيلموه لسر
دور دورو ونبيقالة كلونوفريل ورقها فوقيه/روهك وبرهمي رويي. هيلموه لسر
دور دورو ونبيقالة كلونوفريل ورقها فوقيه/روهك وبرهمي رويي. هيلموه لسر
دور دورو دورو كوندا (أحمد والكمندرا) ونبيقالة كلونوفريل ورقها فوقيه/روهك وبرهمي رويي. هيلموه لسر
دور دورو دورو كوندا (أحمد والكمندرا) ون لديكن زلفين جاندنلي/نانكيا دهوك ل سر دورو ورويى دورو ل سار دورو ورويى دورو كوندا
كريقية ناقفنا بيج تروين روكا ورقها فوقيه/روهك وبرهمي رويي. هيلموه لسر
دور دورو ونبيقالة كلونوفريل ورقها فوقيه/روهك وبرهمي رويي. هيلموه لسر
SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME DIPEPTIDE DERIVATIVES AND THEIR HETEROCYCLIC COMPOUNDS

Huda Ahmed Basheer, Sabir Ayob Mohammed and Aween Akram Ibrahim
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(Accepted for publication: May 20, 2015)

Abstract:
The protected dipeptide esters (3a-e, 4a-l) is prepared by the reaction of compounds (1a-e, 2a-d) with dicyclohexylcarbodiamide (as coupling reagent) and amino acid esters. Thereafter hydrazides (5a-e, 6a-j) are obtained by the reaction of corresponding esters with hydrazine hydrate. Hydrazones (7a-e, 8a-e) are synthesized by the reaction of the above hydrazides with p-nitro benzoaldehyde, which was cyclized to 2,5-disubstituted 1,3,4-oxadiazole (9a-e, 10a-e) through lead oxide and to phthalazines (13a-b) through hydrochloric acid. Hydrazides were reacted with ammonium thiocyanate to afford thiosemicarbazide (14a-c) which were cyclized to 1,2,4-triazole-thione (15a-b) in sodium hydroxide medium. The structures of the synthesized compounds were confirmed by physical and spectral methods. The antibacterial activity of the prepared compounds (5d, 7a, 9e, 10d, 11a, 12e, 13a, 14b) against the gram +ve and –gram –ve Bacteria (Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Proteus mirabilis) were studied and discussed.

Introduction
Oxadiazole derivatives displayed wide spectrum of activities such as antibacterial (Salimon et al 2011; Jumat et al 2010), antimicrobial (Karthiskeyan et al 2008; Gaonkar and Rai 2006), anti-fungal (Kanthiah et al 2011; Srivastava et al 2010) analgesic (Amir and Kumar 2007), as anticonvulsant (Zargahi et al 2005; Rastogi et al 2006), antitumor (Bezerra et al 2005), anti-tubercular (Dhoel et al 2005), antihypoglycemic (Goankar et al 2006) and antiviral (Tan et al 2006). Compounds bearing 1,3,4- Oxadiazole nucleus are known to exhibit unique anti-edema and anti-inflamatory activity (Husain and Ajmal 2009; Franski 2005; Amir and Kumar 2007). Some of 2,5-disubstituted-1,3,4-oxadiazole derivatives used against 60 tumor cell lines derived from nine cancer cell type, anti-tumor activity against leukemia, colon and breast cancer (Wagle et al 2008; Aboraia et al 2006) While compounds having 1,2,4-triazole nucleus are very important in the field of medicinal chemistry, as fungicidal (Royer et al 2005), antibacterial (Singh and Singh 2009), antimicrobial (Hussain et al 2008), antimycotic (Zamani et al 2004), antidepressive (Clerici et al 2001), cardiotonic (Onkol et al 2004) and anticonvulsant (Parmar et al 1974) activities. Triazole ring derivatives are known to possess anti-inflammatory (Moise et al 2009), analgesic (Shenone et al 2001) anti-HIV-1 activity by examination of their inhibition of HIV-1-induced cytopathogenicity in MT-4 cells and by determination of their inhibitory effect on HIV-1 reverse transcriptase (Wu et al 2007). On the other hand, heterocycles containing the phthalazine moiety are of interest due to their pharmacological and biological activities (Jain and Vederas 2004). Some of the phthalazinone derivatives have found application in clinical medicine due to interesting vasodilator (Demirayak et al 2004), anticonvulsant (Zhang et al 2009), anti-diabetic (Boland et al 1993), anti-allergic (Hamamoto et al 1993), and antiasthmatic (Yamaguchi et al 1993). Phthalazinone nucleus has been proved to be a versatile system in medicinal chemistry such as, aldose reductase inhibitor activities (Kashima et al 1998). According to the above and due to the significance of peptides in antibiotic activity; we made a combination of peptide and heterocycles nucleus in a goal to increase the biological effects.
DCC = \[
\begin{align*}
\text{N} & \quad \text{C} \\
\text{N} & \quad \text{C} \\
\end{align*}
\]

\[R, R' = \text{residue of amino acid}\]
Experimental

Uncorrected melting points were determined by using bibby scientific limited stone, Staffordshire, ST 15 OSA, UK., IR spectra were recorded as KBr disc in the (400-4000 cm⁻¹) range by using (spectrum one B FT-IR spectrometer), ¹H NMR spectra in δ units (ppm) relative to an internal standard of tetramethyl silane on ¹H NMR (NMR: BRUKER400 MHz Ultrashield™) in DMSO-d₆, In Department of chemistry, faculty of Science, Dicle University, in Turkey. Amino acids esters and benzoyl amino acids were prepared according to literature procedure (Basheer 2000), p-toluene sulfonyl amino acids were prepared following literature reported procedure (Qreenstien and winitz 1961).

Synthesis of protected dipeptide ester (3a-e, 4a-l)

To a solution of 0.01 mole protected amino acid and 0.01mole of amino acid ester in 50 ml. of dichloromethane is added 0.01 mole N,N'-dicyclohexylcarbodiimide the mixture is allowed stirring over night at room temperature ,The precipitated dicyclohexylurea is removed by filtration and the filtrate washed with water , diluted hydrochloric acid , water , half saturated sodium bicarbonate solution and water, and finally dried over anhydrous sodium sulfate. Evaporation of the solution gives residual mixture of crystals and oil. This is treated with a small amount of diethylether and filtrate; although the material is quite soluble in diethylether and hence is lost in appreciable amount when this solvent is employed, the white precipitate is recrystallized from acetone – petroleum ether.

Synthesis of protected dipeptide hydrazide (5a-e, 6a-j)

A mixture of protected dipeptide ester (0.01mole) and hydrazine hydrate (0.2mole) in absolute ethanol (70 ml) was refluxed for (3) hours. The solvent was evaporated under reduced pressure and the residue was crystallized from methanol.

Synthesis of 4-nitrobenzaldehyde protected dipeptide hydrazide (7a-e,8a-e)

A mixture of p-Nitrobenzaldehyde (0.01mole) and (0.01mole) of protected dipeptide hydrazide (5a-e,6a-e) in (20 ml) ethanol was refluxed for 2 hrs. The solvent was concentrated and the precipitate was filtered and recrystallized from benzene.

Synthesis of 2-protected dipeptide residue, 5-(4’-nitrophenyl) -1, 3, 4-oxadia-zole (9a-e, 10a-e)

To a homogenous solution of hydrazone (7a-e,8a-e) (0.01mole) in 20 ml glacial acetic acid, lead oxide (pbO₂) (0.01mole) was added, the mixture was stirred at 25 C’ for 1 hr. The reaction mixture was diluted with ice- water and left to stand for 24 hrs. The precipitate was filtered off and recrystallized from benzene.

Synthesis of 5-protected dipeptide residue - 1,3,4-oxadiaazole -2-thione(11a-b, 12a-e)

(0.05mole) of the protected dipeptide hydrazide (5a-b,6a-e) was dissolved separately in (70 ml) 0.5% ethanolic potassium hydroxide. (0.1 mole, 6 ml) of carbon disulfide was added gradually and the resulted mixture was refluxed for 16 hours until the evolution of hydrogen sulfide was ceased (checked by filter paper moistener with lead acetate). The solvent was evaporated under reduced pressure and the residue was poured on crushed ice, diluted with ice-water, acidifies with diluted HCl, filtered and dried recrystallized from chloroform.

Synthesis of 1-(benzoyl dipeptide residue)-7-nitrophthalazine (13a-b)

(0.002mole) of the hydrazones (8a-b) in (10 ml) of amyl alcohol (saturated with HCl gas) was heated on steam bath for (1.5 hrs.) and then refluxed for (1hr.). The reaction mixture was cooled, washed with (10 ml) of (20%) sodium hydroxide and then with water until neutralized. Evaporation of the solvent and recrystallization from ethanol afforded the product (13a-b).

Synthesis Substituted thiosemicarbazide (14a-c)

A mixture of (0.003mole) benzoyl dipeptide hydrazide (6a-c), (0.009mole) of ammonium thiocyanate, (4 ml) hydrochloric acid in (25 ml) absolute ethanol, was refluxed for 22 hrs. The solvent was evaporated and the residue poured on crushed ice. The precipitated product was filtered, dried and recrystallized from ethanol.

Synthesis 5-substituted-1,2,4-triazole-3-thione (15a-b)

A mixture of substituted thiosemicarbazide (14a-b) (0.0012mole) and (7.5 ml) 1% aqueous sodium hydroxide solution was refluxed for 3 hrs. , the mixture was treated with charcoal and...
the charcoal then removed by hot filtration. The solution was acidified by 10% hydrochloric acid with cooling; the precipitate was filtered, and recrystallized from methanol.

**Antibacterial assay**

Discs of filter paper (6mm diameter) were sterilized at 140 Cº for 1hr. and impregnated with 1ml. of stock solution (10mg. /ml, 1mg. /ml, 0.1mg. /ml, and 0.01mg. /ml) of each compound and then dried-DMSO (dimethyl sulfoxide) was used as a solvent for compounds (5d, 7a, 9e, 10d, 11a, 12e, 13a, 14d). The same solvent was used for antibiotics. Blank paper of DMSO was used as control. The inoculated plates were incubated at 37 Cº for 24 hrs. And the inhibition zones (mm) were measured. In all experiments, the mean of each triplicate was measured (Garrod et al 1981).

**Results & Discussion**

Protected dipeptide esters were prepared from the reaction of p-toluene sulfonyl amino acid with amino acid ester by using N,N-dicyclohexylcarbodiimide (DCC) as coupling group. The synthesized compounds (3a-e,4a-l) were characterized by their IR and 1H NMR spectra, the IR spectra data provide evidence in support of structures (3a-e,4a-l) for these series of compounds in which characteristic bands at 3373 -3268cm⁻¹ for N-H stretching, 1651-1610 cm⁻¹ for C=O (phCONH) stretching, 1660-1626cm⁻¹ for C=O amide stretching, 1751-1721cm⁻¹ for C=O ester stretching, as illustrated in Table (1). The 1H NMR spectra (Table 4) of the compounds (3a,e 4c,j) indicated the presence of the ethyl group resonating triplet signals at the region of 1.3 -0.7ppm for -CH₃ group and quartet for -CH₂- group at the region 4-3.1 ppm, aromatic protons appeared in the expected range δ 7.9-7.0 ppm, finally the two amide protons occurred at the relatively downfield positions of 8.9- 8.1. Protected dipeptide hydrazides were prepared by the reaction of their corresponding ester with hydrazine hydrate in absolute ethanol. The structures of hydrazide compounds (5a-e, 6a-j) are confirmed on the basis of the following evidence. The IR showed the characteristic absorption bands at 3373 -3268cm⁻¹ for N-H stretching, 1651-1610 cm⁻¹ for C=O (phCONH) stretching, 1660-1626cm⁻¹ for C=O amide stretching, 1751-1721cm⁻¹ for C=O ester stretching, as illustrated in Table (1). The 1H NMR spectra (Table 4) of the compounds (3a,e 4c,j) indicated the presence of the ethyl group resonating triplet signals at the region of 1.3 -0.7ppm for -CH₃ group and quartet for -CH₂- group at the region δ-3.1 ppm, aromatic protons appeared in the expected range δ 7.9-7.0 ppm, finally the two amide protons occurred at the relatively downfield positions of 8.9- 8.1. Protected dipeptide hydrazides were prepared by the reaction of their corresponding ester with hydrazine hydrate in absolute ethanol. The structures of hydrazide compounds (5a-e, 6a-j) are confirmed on the basis of the following evidence. The IR showed the characteristic absorption bands as follow 3253-3325 cm⁻¹ (NH), 1629-1603cm⁻¹ C=O (phCO-) and 1656-1605 cm⁻¹ (C=O amide). In addition of absence of band for C=O of ester, as illustrated in Table (2). The 1H NMR spectrum of the compounds (6e,6j) showed a signal at 4.5ppm indicating the presence of NHNH₂ protons, absence the protons of the ethyl group and other signals were observed at appropriate places, as illustrated in Table (4).

Hydrazide compounds (7a-e, 8a-e) were prepared by the condensation reaction of protected dipeptide hydrazide with 4-nitrobenzaldehyde. Hydrazide compounds (7a-e,8a-e) were confirmed by the IR spectroscopy which showed the absorption bands of (C=N, C=C), amide, C=O (phCO-) and amine groups appeared stretching vibration at (1600-1530cm⁻¹), (1690-1629cm⁻¹) (1625-1600cm⁻¹), and (3467-3251cm⁻¹), respectively. While at stretching vibration (1538-1513cm⁻¹) Asymmetric stretching of aromatic NO₂, and (1345 - 1326 cm⁻¹) Symmetric stretching of aromatic NO₂, as shown in Table (2). The 1H NMR spectrum of the compounds (7d,e, 8c,e ) showed a signal at ( δ 7.3- 7.1ppm) (parikh 1974) indicating the presence of CH=N proton and other signals were observed at appropriate places, Table (4).

The hydrazone were cyclized to 2,5-disubstituted- 1,3,4- Oxadiazole (9a-e,10a-e) by their reaction with lead oxide. The IR characterization absorption bands of the oxadiazoles (9a-e,10a-e) were given in Table (3).The main absorption bands for imine and amide groups appeared at (1600-1561cm⁻¹) ,(1656 -1600 cm⁻¹) and (1686-1633cm⁻¹) for(C=N, C=C),C=O (phCONH)and C=O, while at (1525-1519 cm⁻¹) (1346-1340cm⁻¹) represent NO₂ asymmetric, symmetric stretching, respectively. The N-H groups of these compounds appeared at (3435- 3258cm⁻¹) as broad bands.

The oxadiazole -2-thione (11a-b,12a-e) synthesis was performed by the reaction of hydrazides and carbon disulfide in alkaline medium. The mechanism of the reaction is accomplished by nucleophilic attack of the enol hydrazide form at the carbon atom of carbon disulfide. The formed xanthate salts underwent intra nucleophilic attack followed by hydrogen sulfide elimination. The IR spectra of the compounds (11a-b,12a-e) showed absorption bands at (1266-1154cm⁻¹) corresponds to the thione stretching vibration, and others gave the following vibrational
absorption bands (1597-1529 cm\(^{-1}\)) , (1667-1632 cm\(^{-1}\)) and (3430-3246 cm\(^{-1}\)) which were assigned to (C=C, C=N), (C=O amide), and (N-H) respectively as illustrated in Table (3). The \(^1\)H NMR spectrum of compounds (11a,12b,e) revealed the resonance peaks that appeared at 7.1-8.2 ppm could be assigned to the contribution of aromatic protons, and the remaining signals has appeared in the expectation of places, as illustrated in Table (5).

Phthalazine compounds (13a-b) were prepared through ring closure of hydrazone by using hydrochloric acid in amyl alcohol. The main absorption bands of substituted Phthalazine compounds which includes stretching vibrations of C=N, C=C, C=O amide at (1605-1585 cm\(^{-1}\)), and (1630, 1629 cm\(^{-1}\)) respectively. While the absorption bands at (3435, 3327 cm\(^{-1}\)) were assigned to (N-H) stretching vibrations, as shown in Table (3).

Hydrazides were converted to thiosemicarbarbazide (14a-c) by its reaction with ammonium thiocyanate / hydrochloric acidic. The structure of compounds (14a-c) was confirmed by IR in which strong bands for C=S, C=O, C=O (phCO) and N-H stretching were observed at (1225-1085), (1631–1630), (1615-1610) and (3152–3114) cm\(^{-1}\), respectively Table (3).

Cyclocondensation of the thiosemicarbarbazide with aqueous sodium hydroxide afford 5-substituted-1,2,4-triazole-3-thione. The IR spectra of the synthesized compound (15a-b) showed the presence of N-H stretching bond at(3326-3127cm\(^{-1}\)) and detection of C=N stretching bond at (1582 -1577 cm\(^{-1}\)) for evidence of ring closure of triazole ring Table (3).

The antibacterial activity of the compounds (5d, 7a, 9e, 10d, 11a, 12e, 13a, 14b) were evaluated using various species of bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Proteus mirabilis*. The result showed that the compounds (7a-12e) were active in inhibiting the growth of nearly all organisms used as indicated from the diameter of inhibition zone Table (6). Blank discs DMSO did not show any activity. According to the data (Table 6), it was evident that the activity of tested compounds decreased considerably at lower concentration (0.01mg/ml).

However, compounds (7a, 9e, 11a and 12e) showed the higher antibacterial effect on *Bacillus subtilis*, *Proteus mirabilis* then *Staphylococcus aureus* and *Escherichia coli*, as indicated from the diameter of inhibition zone.
Table (1): Physical properties and spectral data of compounds (3a-e, 4a-l)

<table>
<thead>
<tr>
<th>Comp No.</th>
<th>R, R'</th>
<th>M. p. °C</th>
<th>Yield %</th>
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Table (2): Physical properties and spectral data of compounds (5a-e, 6a-j, 7a-e, 8a-e)

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**Table (3):** Physical properties and spectral data of compounds (9a-e, 10a-e, 11a-b, 12a-c, 13a-b, 14a-c, 15a-b)

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## Table (4): 1H NMR spectral data of compounds (3a,e, 4c,j, 6e,j, 7d,e, 8c,e)

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<th>Com. No.</th>
<th>R , R'</th>
<th>1H NMR (400 MHz, DMSO-d6, δ, ppm)</th>
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<td>3a</td>
<td>-H , -H</td>
<td>1.2-1.3 (t, 3H) CH₂CH₂; 2.5 (s, 3H) CH₃ph; 3.3, 3.5 (s, 4H) 2CH₂CO; 3.6-3.7 (q, 2H) -CH₂CH₂; 7.4-7.8 (m, 4H) ArH; 8.9 (s, 1H) NH; 8.1 (s, 1H) NH.</td>
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<td>-CH₂CH₂CH₃</td>
<td>0.7-0.9 (m, 6H) 2CH₃; 1.2-1.3 (t, 3H) CH₂CH₂; 2.5 (s, 3H) CH₃ph; 1.0-1.1 (m, 2H) CH₂CH₂CH₂; 3.5-3.6 (m, 2H) CH₂CO; 4.0-4.1 (quartet, 2H) COCH₂CH₂; 1.5-1.7 (m, 2H) 2CH; 7.3-7.8 (m, 4H) ArH; 8.2-8.3 (s, 2H) 2NH.</td>
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<tr>
<td>3e</td>
<td>-CH₃</td>
<td>0.7 (t, 3H) CH₂CH₂; 3.1 (q, 2H) COCH₂CH₃; 3.4 (d, 2H) phCH₂CH; 3.9 (s, 2H) CH₂CO; 4.2 (d, 1H) COCH₂CH₂; 7.0-7.9 (m, 9H) 2ArH; 8.4 (s, 1H) NH; 8.7 (s, 1H) NH.</td>
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<td>3e</td>
<td>-CH₂C₆H₄OH</td>
<td>1.0 (t, 3H) CH₂CH₂; 3.1 (q, 2H) COCH₂CH₃; 3.4 (d, 2H) phCH₂CH; 3.9 (s, 2H) CH₂CO; 4.2 (d, 1H) COCH₂CH₂; 7.0-7.9 (m, 9H) 2ArH; 8.4 (s, 1H) NH; 8.7 (s, 1H) NH; 8.9 (s, 1H) OH.</td>
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<tr>
<td>4c</td>
<td>-H</td>
<td>1.0 (t, 3H) CH₂CH₂; 3.1 (q, 2H) COCH₂CH₃; 3.4 (d, 2H) phCH₂CH; 3.9 (s, 2H) CH₂CO; 4.2 (d, 1H) COCH₂CH₂; 7.0-7.9 (m, 9H) 2ArH; 8.4 (s, 1H) NH; 8.7 (s, 1H) NH; 8.9 (s, 1H) OH.</td>
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<td>0.7 (d, 6H) (CH₃)₂CH; 1.0 (m, 1H) CH₃CH₃; 2.1 (d, 2H) phCH₂CH; 2.8 (m, 2H) 2CH₂CO; 4.5 (d, 2H) NH₂; 7.2-8.2 (m, 10H) 2ArH; 8.5 (d, 1H) NH.</td>
</tr>
<tr>
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<td>-CH₃</td>
<td>0.7 (m, 6H) 2CH₃; 1.2 (m, 2H) CH₂CH₂; 1.7 (m, 1H) CH₂CH₂CH₂; 1.9 (d, 2H) phCH₂CH; 2.9 (m, 9H) 2CH₂CO; 4.5 (d, 2H) NH₂; 7.1-8.0 (m, 10H) 2ArH; 8.2 (s, 1H) NH; 8.9 (s, 1H) NH; 9.1 (s, 1H) NH.</td>
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<tr>
<td>6j</td>
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<td>7d</td>
<td>CH₂CH(CH₃)₂</td>
<td>0.8 (m, 6H) 2CH₃; 1.2 (m, 1H) CH₃CH₃; 1.4 (m, 2H) CH₂CH₂; 2.5 (s, 3H) CH₃ph; 4.2 (t, 1H) COCH₂CH₂; 5.1 (q, 2H) CH₂CO; 7.2 (s, 1H) CH=N; 7.1 (d, 4H) ArH; 7.4 (s, 1H) NH; 7.5 (8, 2H) ArH; 8.8 (s, 1H) NH; 8.9 (s, 1H) NH.</td>
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<td>-CHCH₂CH₃</td>
<td>0.9 (m, 6H) 2CH₂; 1.2 (m, 1H) CH; 1.5 (m, 2H) CH₂CH₂; 2.3 (3, 3H) CH₃ph; 3.6 (m, 1H) CH₂CO; 3.9 (d, 2H) CH₂CH₂; 7.3 (s, 1H) CH=N; 7.4 (s, 1H) NH; 7.7, 8.1 (d, 4H) ArH; 7.8 (s, 1H) NH; 7.9 (8, 1H) ArH; 8.4 (s, 1H) NH; 9.0 (s, 1H) NH.</td>
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<tr>
<td>8c</td>
<td>-CH₃</td>
<td>1.1 (d, 3H) CH₃CH; 2.4 (d, 2H) CH₂CH₂; 2.8 (m, 1H) COCH₂CH₂; 3.0 (t, 1H) COCH₂CH₂; 7.1 (s, 1H) CH=N; 7.3-8.3 (m, 14H) 3ArH; 4.2, 4.5 (b, 2H) 2NH; 9.1 (s, 1H) NH.</td>
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Table (5): 1H NMR spectral data of compounds (9a,c-d, 10b-d, 11a, 12b,e)

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<th>Com. No.</th>
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<th>1H NMR (400 MHz, DMSO-d6, δ, ppm)</th>
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<td>9a</td>
<td>H, H</td>
<td>2.4 (s, 3H) CH₃Ph; 3.9 (d, 2H) NHCH₂; 4.3 (s, 2H) CH₂CO; 7.4-7.7 (dd, 4H) ArH; 7.9-8.2 (dd, 4H) ArH; 8.0 (s, 2H) 2NH.</td>
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<td>CH₃, CH₂CH(CH₃)₂</td>
<td>0.9 (d, 6H) (CH₃)₂CH; 1.2 (m, H) CH(CH₃)₃; 1.5 (d, 3H) CH₃CH; 1.7 (m, 2H) CH₂(CH₃); 2.5 (s, 3H) CH₃Ph; 2.8 (t, H) NHCH₂CH₂; 7.1-7.9 (m, 8H) 2ArH; 8.9 (s, 1H) NH; 8.5 (s, 1H) NH.</td>
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<td>-CH₂CH(CH₃)₂, H</td>
<td>0.8 (d, 6H) (CH₃)₂CH; 1.3 (d, 2H) CH₂CH(CH₃)₂; 1.7 (m, 1H) CH(CH₃)₂; 2.4 (s, 3H) CH₃Ph; 3.7 (t, 2H) CH₂NH; 4.1 (m, 1H) COCH₂CH₂; 7.3, 7.9 (dd, 4H) ArH; 7.6-8.3 (dd, 4H) ArH; 8.5 (s, 2H) 2NH.</td>
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<td>-CH(CH₃)₂, CH₂C₆H₅</td>
<td>0.9 (d, 6H) (CH₃)₂CH; 1.8 (d, 2H) phCH₂CH₂; 1.4 (m, 1H) CH(CH₃)₂; 2.1 (t, 1H) COCH₂CH₂Ph; 2.8 (d, H) COCH₂CH₂; 4.5 (s, 1H) NH; 5.6 (s, 1H) NH; 7.1-8.2 (m, 14H) 3ArH.</td>
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<td>CH₃, CH₂C₆H₅</td>
<td>1.1 (d, 3H) CH₃CH; 1.6 (d, 2H) phCH₂CH₂; 2.0 (m, 1H) COCH₂CH₂; 2.3 (t, 1H) CH₂CH₂Ph; 4.5 (s, 1H) NH; 5.7 (s, 1H) NH; 7.2-8.2 (m, 14H) 3ArH.</td>
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<td>11a</td>
<td>CH(CH₃)₂, H</td>
<td>0.9 (d, 6H) (CH₃)₂CH; 1.3 (m, 1H) CH(CH₃)₂; 2.5 (s, 3H) CH₂Ph; 2.7 (d, 2H) CH₂NH; 4.1 (d, 1H) COCH₂CH₂; 5.6 (b, 1H) NH; 7.2-7.5 (dd, 4H) ArH; 7.4 (s, 1H) NH; 8.6 (s, 1H) NH.</td>
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Table (6): the antibacterial activity of compounds (5d, 7a, 9e, 10d, 11a, 12e, 13a, 14d); and diameter of inhibition zone (cm).

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Reference

تحتبر بعض المركبات الحلقية من المشتقات ثنائية الببتيد ودراسة فاعليتها البيولوجية

الخلاصة:

تعتبر الدراسات، التي تتعلق بتفاعل المركبات الدقيقة (1-13) مع الميكروبات، نتائجها إيجابية، حيث تم تحضير اسبرات الببتيدات الثنائية المحمية (1a-e, 2a-d) من تفاعل المركبات الأمينية. ووجد أن المركب ذو تركيز اثنائي مكون من الهيدراتيدات يتكاثر في الميكروبات. ونتيجة بتفاعل المركبات الدقيقة، تم تحضير اسبرات الببتيدات الثنائية المحمية (5a-e, 6a-j) وذلك بتفاعل المركبات مع الميكروبات المحمية. كما تم تحضير هيدراتيدات مع البارانايبوزنالدمي، ومن ثم حقله الميكروبات المحمية (7a-e, 8a-e) (9a-e, 10a-e) في تفاعل حامض الهيدروكلوريك، كما تم تحضير اسبرات الببتيدات الثنائية المحمية (11a-b) وذلك بتفاعل المركبات الدقيقة مع كارباونات السلفايد. ونتيجة بتفاعل الميكروبات الدقيقة مع كارباونات السلفايد، تم تحضير اسبرات الببتيدات الثنائية المحمية (14a-c) الذي تحقده إلى 1,2,3-ثابول -2-15а. 1b) في وسط حامض الهيدروكلوريك. التفاعل الفعالة (Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Proteus mirabilis)


الخلاصة:

تم تحضير اسبرات الببتيدات الثنائية المحمية (1a-e, 2a-d) مع اسبرات الأمينية (3a-e, 4a-l) من تفاعل المركبات الأمينية. ووجد أن المركب ذو تركيز اثنائي مكون من الهيدراتيدات يتكاثر في الميكروبات. ونتيجة بتفاعل المركبات الدقيقة مع الميكروبات المحمية، كلما تم تحضير اسبرات الببتيدات الثنائية المحمية (5a-e, 6a-j) وذلك بتفاعل المركبات مع الميكروبات المحمية. كما تم تحضير هيدراتيدات مع البارانايبوزنالدمي، ومن ثم حقله الميكروبات المحمية (7a-e, 8a-e) (9a-e, 10a-e) في تفاعل حامض الهيدروكلوريك، كما تم تحضير اسبرات الببتيدات الثنائية المحمية (11a-b) وذلك بتفاعل المركبات الدقيقة مع كارباونات السلفايد، ونتيجة بتفاعل المركبات الدقيقة مع كارباونات السلفايد، تم تحضير اسبرات الببتيدات الثنائية المحمية (14a-c) الذي تحقده إلى 1,2,3-ثابول -2-15а. 1b) في وسط حامض الهيدروكلوريك، ونتيجة بتفاعل المركبات الدقيقة، تم تحضير اسبرات الببتيدات الثنائية المحمية (11a-b) الذي تحقده إلى 1,2,3-ثابول -2-15а. 1b) في وسط حامض الهيدروكلوريك، ونتيجة بتفاعل المركبات الدقيقة مع كارباونات السلفايد، ونتيجة بتفاعل المركبات الدقيقة مع كارباونات السلفايد، تم تحضير اسبرات الببتيدات الثنائية المحمية (14a-c) الذي تحقده إلى 1,2,3-ثابول -2-15а. 1b) في وسط حامض الهيدروكلوريك، ونتيجة بتفاعل المركبات الدقيقة مع كارباونات السلفايد، ونتيجة بتفاعل المركبات الدقيقة مع كارباونات السلفايد، تم تحضير اسبرات الببتيدات الثنائية المحمية (14a-c) الذي تحقده إلى 1,2,3-ثابول -2-15а. 1b) في وسط حامض الهيدروكلوريك، ونتيجة بتفاعل المركبات الدقيقة مع كارباونات السلفايد، ونتيجة بتفاعل المركبات الدقيقة مع كارباونات السلفايد، تم تحضير اسبرات الببتيدات الثنائية المحمية (14a-c) الذي تحقده إلى 1,2,3-ثابول -2-15а. 1b) في وسط حامض الهيدروكلوريك.
ناماده کرونا همراه با تأثیرات ناقل‌پذیری از دهان‌شیاری در دومین هفته و خانم‌ها کاریگری نظامی;

پوسته:

ناماده کرونا تست‌رایت بیشتری در رویه بی‌پاراسیت (1-3) و کاریکترهای ناقل‌پذیری در دومین هفته و دهان‌شیاری در دومین هفته و خانم‌ها کاریگری نظامی.

در حال حال یافتگان هیپیدرازیدات (5a-e,6a-j) همان ناماده کرونا ب کاریکترهای ناقل‌پذیری و دهان‌شیاری در دومین هفته و خانم‌ها کاریگری نظامی و مبتلایان.

ناماده بازیابی هیپیدرازیدات کو هانه دوست‌کرن بو 3- دووبانی فرم‌بکری 4,3- نوکسادیازول (9a-e,10a-e، 15a-b) ب همیشه نوکسیدی فرم‌بکری و همر و هما فسیاژین (13a-b) ب همیشه هایدرکلوئین، دیسک ناماده کرونا 1,3,4- نوکسادیازول 2- سایون 15a-b) ب کاریکترهای هیپیدرازیدات بالاخره دگرگونی در دومین هفته و دهان‌شیاری دوم تنوع کو‌هست ب مدعی کاریکترهای هیپیدرازیدات بالاخره دگرگونی در دوم تنوع کو‌هست ب مدعی کاریکترهای هیپیدرازیدات بالاخره دگرگونی در دوم تنوع کو‌هست Bacteroides, Bacillus, Escherichia Coli and Proteus Mirabilis)

(5d,7a,9e,10d,11a,12e,13a,14b)
ON THE ENERGY OF SOME COMPOSITE GRAPHS

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ABSTRACT:
Eigenvalues of a graph are the eigenvalues of its adjacency matrix. The energy of a graph is the sum of the absolute values of its eigenvalues, was studied by (Gutman 1978). This paper divided in to three parts, in part one spectra and nullity of graphs are defined (Brouwer and Haemers, 2012) and (Harary, 1969). In the second part graph products are their spectra is studied (Shibata and Kikuchi 2000) and (Balakrishnan and Ranganathan , 2012). In the last part, we prove the energy of some graph products including Cartesian, tensor, strong, skew and inverse skew which are applied of some graphs.

Keywords: Graph product, Spectra, Energy.

1. INTRODUCTION

Let G be a graph of p vertices with adjacency matrix A, then A is a real symmetric matrix and so the eigenvalues of A are real and hence can be ordered. The eigenvalues of A, \( \lambda_1 \geq \lambda_2 \geq \cdots \geq \lambda_p \), are called the eigenvalues of G and form the spectrum of G. The energy E(G) of a graph G is defined as the sum of absolute values of its eigenvalues. That is E(G)=\( \sum_{i=1}^{p} |\lambda_i| \). The study of properties of E(G) was initiated by (Gutman, 1978). All graphs considered in this paper are finite, simple and undirected.

In this part, we look at the properties of graphs from their eigenvalues. The set of eigenvalues of a graph G with its multiplicities is known as the spectrum of G and denoted by \( S_p(G) \).

Definition 1.1: The adjacency matrix A(G) or \( A=[a_{ij}] \) of a labeled graph G with vertex set \( V(G)\)=\{v_1, v_2, ..., v_p\} is a \( p \times p \) matrix in which \( a_{ij}=1 \) if \( v_i \) and \( v_j \) are adjacent, and 0 if they are not.

Adjacency matrices define graphs up to isomorphism. Moreover, the adjacency matrix of a graph G is a symmetric 0, 1 matrix having zero entries along the main diagonal, and in which the sum of the entries in any row or column is equal to the degree of the corresponding vertex. Because of this correspondence between graphs and matrices, any graph theoretic concept is reflected in the adjacency matrix.

Definition 1.2: The characteristic polynomial of the adjacency matrix A(G) of a graph G with p vertices is called the characteristic polynomial of G, denoted by \( \phi(G; x) \) with the convention that the coefficient of the highest order term is positive:
\[
\phi(G; x) = \det(xI_p - A(G)) = (-1)^p \det(A(G) - xI_p).
\]
Therefore, the characteristic polynomial of a graph G of order p is a polynomial of degree p:
\[
\phi(G; x) = a_0x^p + a_1x^{p-1} + \cdots + a_{p-1}x + a_p.
\]
It has two practical forms, explicitly as a polynomial in the variable x, or as product of linear factors. Thus,
\[
\phi(G; x) = \sum_{i=0}^{p} a_i x^{p-i} = \prod_{i=1}^{p} (x - \lambda_i).
\]

Definition 1.3: The eigenvalues of a graph G of order p are defined to be the eigenvalues of the adjacency matrix associated with the graph G. That is, if G has adjacency matrix A(G), then the eigenvalues of G are those \( p \) (not necessarily distinct) numbers \( \lambda \) which satisfy the determinant equation \( \det(A(G) - \lambda I_p) = 0 \), viz each \( \lambda \) is a root of the polynomial equation \( |A(G) - \lambda I_p| = 0 \).

Equivalently, a number \( \lambda \) is an eigenvalue of G if there exists a non-zero \( p \times 1 \) vector \( X \) (called an eigenvector of \( \lambda \)) such that \( A(G)X = \lambda X \).

Since A(G) is a real symmetric (0, 1) matrix, its eigenvalues \( \{\lambda_1, \lambda_2, ..., \lambda_p\} \) must be real by the next theorem and can be ordered as \( \lambda_1 \geq \lambda_2 \geq \cdots \geq \lambda_p \). See (Balakrishnan, 2004)
Theorem 1.4: The eigenvalues of a real symmetric matrix are real.

Theorem 1.5: The sum of the eigenvalues of any simple graph is zero.

Definition 1.6: Let G be a graph of order p and H is a subgraph of G of order m, m ≤ p, then H is called a partial cover of G if every component of H is isomorphic with K₂ or a cycle graph. If m = p, then H is called a spanning cover of G.

Theorem 1.7: (Sachs’ Theorem) The coefficients aᵢ’s of φ(G; x) are given by

\[ aᵢ = \sum_{H} (-1)^{k(H)} 2^{c(H)}, \]

where the summation extends over all partial covers H on i vertices of G, and where k(H) and c(H) denote respectively, the number of components and of cycles in H.

Definition 1.8: The spectrum \(S_p(G)\) of a graph G is defined as the eigenvalues of its adjacency matrix, that is, another matrix of two rows, the first row consists of the eigenvalues of the graph G and the second row consists of the multiplicities of the corresponding eigenvalues. That is if the distinct eigenvalues of G are \(λ₁, λ₂, \ldots, λ_k\) and their multiplicities are \(m₁, m₂, \ldots, m_k\), respectively, then we write

\[ S_p(G) = \begin{pmatrix} λ₁ & λ₂ & \cdots & λ_k \\ m₁ & m₂ & \cdots & m_k \end{pmatrix} \]

Or just as \(λ₁^{m₁}, λ₂^{m₂}, \ldots, λ_k^{m_k}\).

If G is a disconnected graph with components \(G₁, G₂, \ldots, Gₖ\), then the spectrum of G is the “union” of the eigenvalues of the components of G in some manner because of the fact that \(φ(G; x) = \prod_{i=1}^{k} φ(Gᵢ; x)\).

Spectra of graphs can be obtained using the fact that the coefficients of the characteristic polynomial are integers. It follows that the sum of k-th powers of eigenvalues are integers too. Since the coefficient of the highest power term \(x^p\) of the characteristic polynomial \(φ(G; x)\) is equal to 1, hence any eigenvalue of G which is rational must be an integer, and for any square matrix with real entries, the sum of its eigenvalues is equal to its trace.

To specify the spectrum of a graph G with order p, the coefficients \(aᵢ’s\) of the characteristic polynomial \(φ(G; x) = ∑ aᵢ x^{p-i}\) are of important use. Thus, we seek the coefficients \(aᵢ’s\) first. Certainly \(a₀ = 1\), and using Theorem 1.7, we can easily verify that \(a₁ = 0, -a₂ = q\) and -a₃ is twice the number of triangles in G. And to find the remaining coefficients apply Theorem 1.7.

Definition 1.9: A graph G is said to be a singular graph provided that its adjacency matrix A(G) is a singular matrix. The algebraic multiplicity of the number zero in the spectrum of the graph G is called its nullity (degree of singularity), and is denoted by \(η(G)\).

Lemma 1.10:

i) The eigenvalues of the cycle graph \(C_p\) are of the form \(2\cos \frac{2πi}{p}, i = 0, \ldots, p - 1\), and
\[ η(C_p) = \begin{cases} 2, & \text{if } p \equiv 0 \text{ (mod } 4), \\ 0, & \text{otherwise.} \end{cases} \]

ii) The eigenvalues of the path graph \(P_p\) are of the form \(2\cos \frac{πi}{p+1}, i = 1, \ldots, p\), and
\[ η(P_p) = \begin{cases} 1, & \text{if } p \text{ is odd,} \\ 0, & \text{if } p \text{ is even.} \end{cases} \]

iii) The spectrum of the complete graph \(K_p\), \(S_p(K_p) = \begin{pmatrix} p-1 & -1 \\ 1 & p-1 \end{pmatrix}\), and \(η(K_p) = \begin{cases} 1, & \text{if } p = 1, \\ 0, & \text{if } p > 1. \end{cases} \)

iv) The spectrum of the complete bipartite graph \(K_{p₁,p₂}\), \(S_p(K_{p₁,p₂}) = \begin{pmatrix} \sqrt{p₁p₂} & 0 & -\sqrt{p₁p₂} \\ 1 & p₁+p₂-2 & 1 \end{pmatrix}\), and \(η(K_{p₁,p₂}) = p₁+p₂-2\), for all \(p₁, p₂\).

2. Graph Products
In this part, we study some graph products and determine the spectra of some of them.

Let \( G_1 = (V_1, E_1) \) and \( G_2 = (V_2, E_2) \) be vertex disjoint non-trivial graphs.

**Definition 2.1:** The **Cartesian product** \( G_1 \times G_2 \) of the two graphs \( G_1 \) and \( G_2 \) is the graph with vertex set \( V(G_1 \times G_2) = V_1 \times V_2 \) and two vertices \( (u_1, v_1) \) and \( (u_2, v_2) \) are adjacent in \( G_1 \times G_2 \) if, and only if, \( [u_1 = u_2 \text{ and } v_1v_2 \in E(G_2)] \) or \([u_1u_2 \in E(G_1) \text{ and } v_1 = v_2] \).

It is clear that: \( p(G_1 \times G_2) = p(G_1)p(G_2) \) and \( q(G_1 \times G_2) = p(G_1)q(G_2) + q(G_1)p(G_2) \).

**Definition 2.2:** The **tensor product** \( G_1 \otimes G_2 \) of the two graphs \( G_1 \) and \( G_2 \) is the graph with vertex set \( V(G_1 \otimes G_2) = V_1 \times V_2 \) and two vertices \( (u_1, v_1) \) and \( (u_2, v_2) \) are adjacent in \( G_1 \otimes G_2 \) if, and only if, \( [u_1u_2 \in E(G_1) \text{ and } v_1v_2 \in E(G_2)] \).

It is clear that: \( p(G_1 \otimes G_2) = p(G_1)p(G_2) \) and \( q(G_1 \otimes G_2) = 2q(G_1)q(G_2) \).

**Definition 2.3:** The **strong product** \( G_1 \oslash G_2 \) of the two graphs \( G_1 \) and \( G_2 \) is the graph with vertex set \( V(G_1 \oslash G_2) = V_1 \times V_2 \) and two vertices \( (u_1, v_1) \) and \( (u_2, v_2) \) are adjacent in \( G_1 \oslash G_2 \) if, and only if, \( [u_1 \equiv u_2 \text{ and } v_1v_2 \in E(G_2)] \) or \([u_1u_2 \in E(G_1) \text{ and } v_1 \equiv v_2] \) or \([u_1u_2 \in E(G_1) \text{ and } v_1v_2 \in E(G_2)] \).

It is clear that: \( p(G_1 \oslash G_2) = p(G_1)p(G_2) \) and \( q(G_1 \oslash G_2) = p(G_1)q(G_2) + q(G_1)p(G_2) + 2q(G_1)q(G_2) \).

**Definition 2.4:** The **skew product** \( G_1 \triangleleft G_2 \) of the two graphs \( G_1 \) and \( G_2 \) is the graph with vertex set \( V(G_1 \triangleleft G_2) = V_1 \times V_2 \) and where \( (u_1, v_1) \) and \( (u_2, v_2) \) are adjacent in \( G_1 \triangleleft G_2 \) if, and only if, \( [u_1 \equiv u_2 \text{ and } v_1v_2 \in E(G_2)] \) or \([u_1u_2 \in E(G_1) \text{ and } v_1v_2 \in E(G_2)] \).

It is clear that: \( p(G_1 \triangleleft G_2) = p(G_1)p(G_2) \) and \( q(G_1 \triangleleft G_2) = p(G_1)q(G_2) + 2q(G_1)q(G_2) \).

**Definition 2.5:** Let \( G_1 = (V_1, E_1) \) and \( G_2 = (V_2, E_2) \) be vertex disjoint non-trivial graphs. The **inverse skew product** \( G_1 \triangleright G_2 \) of the two graphs \( G_1 \) and \( G_2 \) is the graph with vertex set \( V(G_1 \triangleright G_2) = V_1 \times V_2 \) and where \( (u_1, v_1) \) and \( (u_2, v_2) \) are adjacent in \( G_1 \triangleright G_2 \) if, and only if, \( [u_1u_2 \in E(G_1) \text{ and } v_1 \equiv v_2] \) or \([u_1u_2 \in E(G_1) \text{ and } v_1v_2 \in E(G_2)] \).

It is clear that: \( p(G_1 \triangleright G_2) = p(G_1)p(G_2) \) and \( q(G_1 \triangleright G_2) = q(G_1)p(G_2) + 2q(G_1)q(G_2) \).

**Lemma 2.6:** (Shibata and Kikuchi, 2000) Let \( G_1 \) and \( G_2 \) be two graphs with orders \( p_1 \) and \( p_2 \), respectively. Then

i) \( A(G_1 \triangleleft G_2) = (I_{p_1} \ast A_2) + (A_1 \ast I_{p_2}) \).

ii) \( A(G_1 \triangleright G_2) = (A_1 \ast I_{p_2}) + (A_1 \ast I_{p_2}) \).

**Corollary 2.7:** Let \( S_\rho(G_1) = \{ \lambda_1, ..., \lambda_{p_1} \} \) and \( S_\rho(G_2) = \{ \mu_1, ..., \mu_{p_2} \} \), and let \( A_1 \) and \( A_2 \) be the adjacency matrices of \( G_1 \) and \( G_2 \), respectively. Then

i) \( A(G_1 \times G_2) = (I_{p_1} \ast A_2) + (A_1 \ast I_{p_2}) \) and \( S_\rho(G_1 \times G_2) = \{ \lambda_i + \mu_j : 1 \leq i \leq p_1; 1 \leq j \leq p_2 \} \).

ii) \( A(G_1 \otimes G_2) = (A_1 \ast A_2) \) and \( S_\rho(G_1 \otimes G_2) = \{ \lambda_i \mu_j : 1 \leq i \leq p_1; 1 \leq j \leq p_2 \} \).

iii) \( A(G_1 \oslash G_2) = (I_{p_1} \ast A_2) + (A_1 \ast I_{p_2}) \) and \( S_\rho(G_1 \oslash G_2) = \{ \lambda_i + \mu_j : 1 \leq i \leq p_1; 1 \leq j \leq p_2 \} \).

**3. Energy of a Product Graphs**

In this part, we discuss another application of eigenvalues of graphs. The energy \( E(G) \) of a graph \( G \) is defined as follows:

**Definition 3.1:** Let \( G \) be a graph on \( p \) vertices, and let its ordinary spectrum (i.e., the spectrum of its adjacency matrix) consist of the numbers \( \lambda_1, \lambda_2, ..., \lambda_p \). Then, the energy \( E(G) \) of a graph \( G \) is defined as follows:

\[ E(G) = \sum_{i=1}^{p} |\lambda_i| . \]

In the next proposition, the energy of some special graphs is studied by (Balakrishnan, 2004) and (Li, Shi, and Gutman, 2012).

**Proposition 3.2:** The energy of some special graphs is defined as follows:

1) \( E(K_p) = 2p - 2 \).
2) \( E(K_{p_1p_2}) = 2 \left( \sin \left( \frac{\pi}{2(p_1+1)} \right) \right) \), if \( p = 0 \) (mod 2),

3) \( E(P_p) = \left( \frac{2 \cos \left( \frac{\pi}{2(p+1)} \right)}{\sin \left( \frac{\pi}{2p} \right)} \right) - 2 \), if \( p = 1 \) (mod 2).

4) \( E(C_p) = \left( \frac{4 \cos \frac{\pi}{p}}{\sin \theta} \right) - 2 \), if \( p = 0 \) (mod 4),

\[ \text{if } p = 2 \text{ (mod 4),} \]

\( \left( \frac{2}{\sin \frac{\pi}{p}} \right) \), if \( p = 1 \) (mod 2).

In the following, we determine the spectra of skew and inverse skew products.

Let \( A_1 \) and \( A_2 \) be the \( p_1 \times p_1 \) and \( p_2 \times p_2 \) adjacency matrices of \( G_1 \) and \( G_2 \) have eigenvalues \( \lambda_i, 1 \leq i \leq p_1 \) and \( \mu_j, 1 \leq j \leq p_2 \), respectively.

**Theorem 3.3:** The \( p_1p_2 \) eigenvalues of the skew product \( G_1 \circ G_2 \) are \( \mu_j + \lambda_i \mu_j, \forall i, j; 1 \leq i \leq p_1; 1 \leq j \leq p_2 \).

Moreover, if \( X_1, ..., X_{p_1} \) are the eigenvectors of \( A_1 \) corresponding to \( \lambda_1, ..., \lambda_{p_1} \), and \( Y_1, ..., Y_{p_2} \) are the eigenvectors of \( A_2 \) corresponding to \( \mu_1, ..., \mu_{p_2} \), then \( X_i \circ Y_j \) are the eigenvectors of \( G_1 \circ G_2 \) corresponding to \( \mu_j + \lambda_i \mu_j, 1 \leq i \leq p_1; 1 \leq j \leq p_2 \).

**Proof:** By Lemma 2.6, we have:

\( A(G_1 \circ G_2) = [(I_{p_1} \circ A_2) + (A_1 \circ A_2)] \)

Assume that \( X(Y) \) is an eigenvector of \( G_1(G_2) \) corresponding to the eigenvalue \( \lambda(\mu) \). Then

\[ A(G_1 \circ G_2) (X \circ Y) = [(I_{p_1} \circ A_2) + (A_1 \circ A_2)] (X \circ Y) = (X \circ A_2 Y) + (A_1 X \circ A_2 Y) = (X \circ \mu Y) + (\lambda X \circ \mu Y) = (\lambda + \mu)(X \circ Y). \]

**Theorem 3.4:** The \( p_1p_2 \) eigenvalues of the inverse skew product \( G_1 \triangledown G_2 \) are \( \lambda_i + \mu_i, \forall i, j; 1 \leq i \leq p_1; 1 \leq j \leq p_2 \).

Moreover, if \( X_1, ..., X_{p_1} \) are the eigenvectors of \( A_1 \) corresponding to \( \lambda_1, ..., \lambda_{p_1} \), and \( Y_1, ..., Y_{p_2} \) are the eigenvectors of \( A_2 \) corresponding to \( \mu_1, ..., \mu_{p_2} \), then \( X_i \triangledown Y_j \) are the eigenvectors of \( G_1 \triangledown G_2 \) corresponding to \( \lambda_i + \mu_i, 1 \leq i \leq p_1; 1 \leq j \leq p_2 \).

**Proof:** By Lemma 2.6, we have:

\( A(G_1 \triangledown G_2) = [(A_1 \circ I_{p_2}) + (A_1 \circ A_2)] \)

Also, assume that \( X(Y) \) is an eigenvector of \( G_1(G_2) \) corresponding to the eigenvalue \( \lambda(\mu) \). Then

\[ A(G_1 \triangledown G_2) (X \circ Y) = [(A_1 \circ I_{p_2}) + (A_1 \circ A_2)] (X \circ Y) = (A_1 X \circ Y) + (A_1 X \circ A_2 Y) = (\lambda X \circ Y) + (\lambda X \circ \mu Y) = (\lambda + \mu)(X \circ Y). \]

Thus, as a result for the spectra of the above two products we have:

**Corollary 3.5:** Let \( S_p(G_1) = \{ \lambda_1, ..., \lambda_{p_1} \} \) and \( S_p(G_2) = \{ \mu_1, ..., \mu_{p_2} \} \). Then

(i) \( S_p(G_1 \circ G_2) = \{ \mu_j + \lambda_i \mu_j : 1 \leq i \leq p_1; 1 \leq j \leq p_2 \}. \)

(ii) \( S_p(G_1 \triangledown G_2) = \{ \lambda_i + \mu_i : 1 \leq i \leq p_1; 1 \leq j \leq p_2 \}. \)

**Proposition 3.6:** The energy of the Cartesian product of \( K_{p_1} \) and \( K_{p_2} \) where \( p_1, p_2 > 1 \), is given by

\[ E(K_{p_1} \times K_{p_2}) = 4(p_1p_2-p_1-p_2+1). \]

**Proof:** By Corollary 2.7, we have:
Thus, by Definition 3.1, we get:
\[
E(Kp_1 \times Kp_2) = p_1 + p_2 - 2 + 2p_1p_2 - p_1 - 2p_2 + 2 = 4(p_1 - p_2 + 1).
\]
Therefore, \( Kp_1 \times Kp_2 \) is singular, if and only if \( p_1 = p_2 = 1 \), this gives the case \( K_1 \times K_1 = K_1 \).

\[\]

**Proposition 3.7:** The energy of the Cartesian product of \( K_p \) and \( K_{p_1, p_2} \) is
\[
E(K_p \times K_{p_1, p_2}) = 2(p - 1)(p_1 + p_2 + \sqrt{p_p} - 1).
\]

**Proof:** By Lemma 1.10 and Corollary 2.7 it follows that the spectrum of \( K_p \times K_{p_1, p_2} \) is
\[
(p - 1) + \left( \begin{array}{cc}
p_1p_2 & 0 \\
p_1 + p_2 - 2 & 1
\end{array} \right)
\]
\[
\begin{pmatrix}
p + \sqrt{p_1p_2} - 1 & p - 1 & p - \sqrt{p_1p_2} - 1 & -1 & -(\sqrt{p_1p_2} + 1) \\
p_1 + p_2 - 2 & 1 & p - 1 & (p - 1)(p_1 + p_2 - 2) & p - 1
\end{pmatrix}
\]
Thus, by Definition 3.1, we have:
\[
E(K_p \times K_{p_1, p_2}) = (p + \sqrt{p_1p_2} - 1) + (pp_1 + pp_2 - 2p - p_1 - p_2 + 2) + (p - \sqrt{p_1p_2} - 1) + \\
\frac{(p_1p_2 - \sqrt{p_1p_2} - p_1 - p_2 + 1)}{1}
\]
\[
= 2p(p_1 + p_2 + \sqrt{p_1p_2} - 1) - (p_1 + p_2 + \sqrt{p_1p_2} - 1) = 2(p - 1)(p_1 + p_2 + \sqrt{p_1p_2} - 1).
\]
Moreover, if \( p \neq \sqrt{p_1p_2} + 1 \), so
\[i)\] If \( p_1 = p_2 = 1 \), then \( K_p \times K_{1, 1} \) is \((p - 1)\) singular.
\[ii)\] If either \( p_1 \) or \( p_2 \) is not \( 1 \) while \( p = \sqrt{p_1p_2} + 1 \), then \( K_p \times K_{p_1, p_2} \) is \((p - 1)\) singular.

**Lemma 3.8:** The energy of the Cartesian product of \( K_{p_1} \) and \( K_{p_2, p_2} \) is
\[
E(K_{p_1} \times K_{p_2, p_2}) = (p_1 - 1)(6p_2 - 2).
\]

**Proof:** Put \( p_1 = p_2 \) in Proposition 3.7, we get the result.

**Proposition 3.9:** The energy of the Cartesian product of \( K_{p_1, p_2} \) and \( K_{p_1, p_2} \) is
\[
E(K_{p_1, p_2} \times K_{p_1, p_2}) = 4p(p_1 + p_2 - 1).
\]

**Proof:** By Corollary 2.7, we have
\[
\begin{pmatrix}
p_1p_2 & 0 \\
p_1 + p_2 - 2 & 1
\end{pmatrix} + \left( \begin{array}{cc}
p_1p_2 & 0 \\
p_1 + p_2 - 2 & 1
\end{array} \right)
\]
\[
\begin{pmatrix}
p + \sqrt{p_1p_2} - 1 & p - 1 & p - \sqrt{p_1p_2} - 1 & -1 & -(\sqrt{p_1p_2} + 1) \\
p_1 + p_2 - 2 & 1 & p - 1 & (p - 1)(p_1 + p_2 - 2) & p - 1
\end{pmatrix}
\]
Thus, by Definition 3.1, we have:
\[
E(K_{p_1, p_2} \times K_{p_1, p_2}) = 2\sqrt{p_1p_2} + 2p_1\sqrt{p_1p_2} + 2p_2\sqrt{p_1p_2} - 4\sqrt{p_1p_2} + 2p_1\sqrt{p_1p_2} + 2p_2\sqrt{p_1p_2} - 4\sqrt{p_1p_2} + 2\sqrt{p_1p_2}
\]
\[
E(K_{p_1, p_2} \times K_{p_1, p_2}) = 4p(\sqrt{p_1p_2} + 2p_1\sqrt{p_1p_2} - 4\sqrt{p_1p_2} = 4\sqrt{p_1p_2}(p_1 + p_2 - 1)).
\]
Moreover, the nullity of \( K_{p_1, p_2} \times K_{p_1, p_2} \) is \( 2p_1 + 2p_2 - 2 \).
Lemma 3.10: The energy of the Cartesian product of $K_{p_1,p_1}$ and $K_{p_2,p_2}$ is
\[ E(K_{p_1,p_1} \times K_{p_2,p_2}) = 2(4p_1p_2 - p_1 - p_2). \]

Proof: By Corollary 2.7, we have:
\[
\begin{pmatrix}
 p_1 & 0 & -p_1 \\
 1 & 2p_1 - 2 & 1 \\
 p_2 & 0 & -p_2 \\
 1 & 2p_2 - 2 & 1
\end{pmatrix}
+ \begin{pmatrix}
 p_1 & p_1 & p_1 - p_2 & p_2 \\
 1 & 2p_1 - 2 & (2p_1 - 2)(2p_2 - 2) & 2p_1 - 2 \\
 0 & -p_2 & p_2 - p_1 & -p_1 - (p_1 + p_2) \\
 1 & 2p_2 - 2 & 1 & 2p_2 - 2
\end{pmatrix}
= \begin{pmatrix}
 p_1 & 0 & -p_1 & -p_2 & -p_1 - (p_1 + p_2) \\
 1 & 2p_1 - 2 & (2p_1 - 2)(2p_2 - 2) & 2p_1 - 2 & 1 \\
 0 & -p_2 & p_2 - p_1 & -p_1 & - (p_1 + p_2) \\
 1 & 2p_2 - 2 & 1 & 2p_2 - 2 & 1
\end{pmatrix}
\]

Thus, by Definition 3.1, we get:
\[ E(K_{p_1,p_1} \times K_{p_2,p_2}) = 8p_1p_2 - 2p_1 - 2p_2 = 2(4p_1p_2 - p_1 - p_2). \]

Moreover, the nullity of $K_{p_1,p_1} \times K_{p_2,p_2}$ is $(2p_1-2)(2p_2-2)$, provided that neither $p_1$ nor $p_2$ is 1. If $p_1=p_2=1$, then the nullity of $K_{1,1} \times K_{1,1}$ is 2.

Proposition 3.11: Let $G_1$ and $G_2$ be two graphs on $p_1$ and $p_2$ vertices, respectively. Then \( E(G_1 \times G_2) \leq p_2 E(G_1) + p_1 E(G_2). \)

Proof: Let $\lambda_1, \lambda_2, \ldots, \lambda_{p_1}$ and $\mu_1, \mu_2, \ldots, \mu_{p_2}$ be the eigenvalues of $G_1$ and $G_2$; respectively. Then
\[
E(G_1 \times G_2) = \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\lambda_i + \mu_j| \leq \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\lambda_i| + |\mu_j| = \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\lambda_i| + \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\mu_j| \leq p_2 E(G_1) + p_1 E(G_2). \]

Proposition 3.12: The energy of the tensor product $K_{p_1} \otimes K_{p_2}$ is given by:
\[ E(K_{p_1} \otimes K_{p_2}) = 4(p_1-1)(p_2-1). \]

Proof: By Lemma 1.10 and Corollary 2.7, we have:
\[
\begin{pmatrix}
 p_1 - 1 & -1 \\
 1 & p_1 - 1
\end{pmatrix}
\times
\begin{pmatrix}
 p_2 - 1 & -1 \\
 1 & p_2 - 1
\end{pmatrix}
= \begin{pmatrix}
 p_1 & p_2 - 1 & -p_1 + 1 & -p_2 + 1 \\
 1 & p_2 - 1 & p_1 - 1 & (p_1 - 1)(p_2 - 1)
\end{pmatrix}
\]

Thus, by Definition 3.1, we get:
\[ E(K_{p_1} \otimes K_{p_2}) = p_1p_2 - 2p_1 + 2p_2 - 2(p_1 + p_2 - 2)^2 + p_1 - p_2 + p_1 - p_2 + 2 \]
\[ \leq p_2 E(G_1) + p_1 E(G_2). \]

Proposition 3.13: The energy of the tensor product $K_{p_1,p_2} \otimes K_{p_1,p_2}$ is given by:
\[ E(K_{p_1,p_2} \otimes K_{p_1,p_2}) = 4p_1p_2. \]

Proof: By Corollary 2.7, we have:
\[
\begin{pmatrix}
 p_1 & 0 & -p_1 & -p_1 \\
 1 & p_1 + p_2 - 2 & 1 & 0 \\
 0 & -p_2 & p_1 + p_2 - 2 & 1 \\
 1 & 0 & 0 & p_1p_2
\end{pmatrix}
= \begin{pmatrix}
 \sqrt{p_1p_2} & 0 & \sqrt{p_1p_2} & \sqrt{p_1p_2} \\
 1 & p_1 + p_2 - 2 & (p_1 + p_2 - 2)^2 & 0 \\
 0 & p_1 + p_2 - 2 & (p_1 + p_2 - 2)^2 & 2 \\
 1 & 0 & 0 & p_1p_2
\end{pmatrix}
\]

Then, by Definition 3.1, we get:
\[ E(K_{p_1,p_2} \otimes K_{p_1,p_2}) = 4p_1p_2. \]

And the nullity of $K_{p_1,p_2} \otimes K_{p_1,p_2}$ is $4(p_1 + p_2 - 2) + (p_1 + p_2 - 2)^2$. ■

Proposition 4.14: (Balakrishnan 2004) Let $G_1$ and $G_2$ be two graphs on $p_1$ and $p_2$ vertices, respectively. Then $E(G_1 \otimes G_2) = E(G_1) E(G_2)$.

Proof: By Definition 3.1, we have:
\[ E(G_1 \otimes G_2) = \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\lambda_i \mu_j| = \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\lambda_i| |\mu_j| = \sum_{i=1}^{p_1} |\lambda_i| \sum_{j=1}^{p_2} |\mu_j| = E(G_1) E(G_2). \]
Proposition 3.15: The energy of the strong product of two graphs is the sum of energy of their Cartesian and tensor products.

Proposition 3.16: The energy of the skew product of two graphs $G_1$ and $G_2$ is related as:

$$E(G_1 \ast G_2) \leq p_1E(G_2) + E(G_1)E(G_2).$$

Proof: Let the spectra of $G_1$ and $G_2$ be $\{\lambda_1, ..., \lambda_{p_1}\}$ and $\{\mu_1, ..., \mu_{p_2}\}$; respectively.

Then

$$E(G_1 \ast G_2) = \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\lambda_i + \mu_j|^2 \leq \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\lambda_i|^2 + |\mu_j|^2 \leq \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\lambda_i| + \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\mu_j| \leq p_1E(G_2) + E(G_1)E(G_2).$$

Example 3.17: Let $G_1=K_2$ and $G_2=P_3$, then the skew product of $K_2$ and $P_3$ is given by:

$$S_p(K_2) = \begin{pmatrix} 1 & -1 \\ 1 & 1 \end{pmatrix} \text{ and } S_p(P_3) = \begin{pmatrix} \sqrt{2} & 0 & -\sqrt{2} \\ 0 & 1 & 1 \end{pmatrix}$$

For $j=1$ and $i=1, 2$, we have: $\lambda_1 + \lambda_1 \mu_1 = 2\sqrt{2}$, $\lambda_1 + \lambda_2 \mu_1 = 0$. For $j=2$ and $i=1, 2$, we have: $\mu_2 + \lambda_1 \mu_2 = 0$, $\mu_2 + \lambda_2 \mu_2 = 0$. For $j=3$ and $i=1, 2$, we have: $\mu_3 + \lambda_1 \mu_3 = -2\sqrt{2}$, $\mu_3 + \lambda_2 \mu_3 = 0$.

Then, $E(K_2 \ast P_3) = 2\sqrt{2}$.

And by Proposition 3.16, we have $E(K_2 \ast P_3) \leq 2 \ast 2\sqrt{2} + 2 \ast 2\sqrt{2} = 8\sqrt{2}$. Moreover, equality does not hold for any pair of non-empty simple graphs.

Proposition 3.18: The energy of the inverse skew product is given by

$$E(G_1 \mathbin{\#} G_2) \leq p_2E(G_1) + E(G_1)E(G_2).$$

Proof: Let the spectra of $G_1$ and $G_2$ be $\{\lambda_1, ..., \lambda_{p_1}\}$ and $\{\mu_1, ..., \mu_{p_2}\}$, respectively.

Then

$$E(G_1 \mathbin{\#} G_2) = \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\lambda_i + \lambda_i \mu_j|^2 \leq \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\lambda_i|^2 + |\lambda_i|^2 \leq \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\lambda_i| + \sum_{i=1}^{p_1} \sum_{j=1}^{p_2} |\mu_j| \leq p_2E(G_1) + E(G_1)E(G_2).$$

Example 3.19: Let $G_1=K_2$ and $G_2=P_3$, then the inverse skew product of $K_2$ and $P_3$ is given by:

$$S_p(K_2) = \begin{pmatrix} 1 & -1 \\ 1 & 1 \end{pmatrix} \text{ and } S_p(P_3) = \begin{pmatrix} \sqrt{2} & 0 & -\sqrt{2} \\ 0 & 1 & 1 \end{pmatrix}$$

For $i=1$ and $j=1, 2, 3$, we have: $\lambda_1 + \lambda_1 \mu_1 = 1 + \sqrt{2}$, $\lambda_1 + \lambda_3 \mu_2 = 1, \lambda_1 + \lambda_4 \mu_3 = 1 - \sqrt{2}$. For $i=2$ and $j=1, 2, 3$, we have: $\lambda_2 + \lambda_2 \mu_1 = -1 - \sqrt{2}, \lambda_2 + \lambda_2 \mu_2 = -1, \lambda_2 + \lambda_2 \mu_3 = -1 + \sqrt{2}$.

Then, $E(K_2 \mathbin{\#} P_3) = 2 + 4\sqrt{2}$.

And by Proposition 3.18, we have $E(K_2 \mathbin{\#} P_3) \leq 3 \ast 2 + 2 \ast 2\sqrt{2} = 6 + 4\sqrt{2}$.

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حوصل الطاقة لبعض البيانات المركبة

الخلاصة


ل دور هيزة هندسه و راغفين تاوهه

كورت

بائه خويتي بي ويد جراف نمو بيهاء خويتي هو ودى، هيزة جراف نمو كرمالا بيهاء رووته با بيهين خويتي، وا هاته خوندند ز لاي (Gutman 1978) هاته دايهك لب ششكي، د پشکا. (Harary 1969) نيکپنا سپر و پا نابو اک جرافان هاته دیارکن (Brouwer and Haemers 2012) د پشکا دوویدا لیکپانا هندهک جرافا و سپر وان هاته خواندن (Shibata and Kikuchi 2000) ور (skew) د پشکا دوماهپیدا سپرا لیکپانا خوار (inverse skew) لیکپانا خوار با زفري (and Ranganathan 2012 Balakrishnan و) ور (inverse skew) مه سفالاندن و هیزة هندسه جرافین تاوهه مه دیارکن ل سمر هندهک لیکپانا جرافا.
CONVERGENCE OF THE BARZILAI-BORWEIN METHOD FOR SOLVING SLIGHTLY UNSYMMETRIC LINEAR SYSTEMS

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Abstract:
Due to its simplicity and numerical efficiency, the Barzilai and Borwein (BB) gradient method has received numerous attentions in different scientific fields. In this paper, the sufficient condition for convergence of the BB method when the coefficient matrix of linear algebraic equations is slightly unsymmetric with positive definite symmetric part is presented.

Keywords: Unsymmetric linear algebraic equations, Barzilai and Borwein gradient method, symmetric and skew-symmetric matrices, eigenvalues, condition number.

1. Introduction

A significant development that has completely changed our perspectives on the effectiveness of gradient methods is due to Barzilai and Borwein (Barzilai and Borwein, 1988). They proposed two choices of step size the gradient method. The computational cost and typical behaviour of the algorithm for both choices of step size are quite similar. Their method aimed to accelerate the convergence of the steepest descent (SD) method.

The main idea of Barzilai and Borwein’s approach is to use the information in the previous iteration to decide the step size in the current iteration. The Barzilai-Borwein (BB) method requires few storage locations and inexpensive computations. Therefore, several authors have paid attention to the BB method. Their method is strongly related to Quasi-Newton (QN) algorithms (Dennis and Schnabel, 1983) and (Fletcher, 2000).

It is known that the (BB) method (Barzilai and Borwein, 1988) converge when this method is applied to solve linear systems of the form

\[ Ax = b, \]

where \( A \) is symmetric and positive definite. For some finite difference discretizations of elliptic problems, one gets positive definite matrices that are almost symmetric. Practically, the BB method works for these matrices. However, the convergence of this method is not guaranteed theoretically.

For quadratics that the BB method has been shown to converge (Raydan, 1993) and its convergence is R-linear (Dai and Liao, 2002). For the case of \( n = 2 \), the method is R-superlinearly convergent (Barzilai and Borwein, 1988). For more details on the Barzilai and Borwein method see (Raydan, 1991) and (Dai and R. Fletcher, 2005). The BB algorithm for quadratic case is summarized in Algorithm 1.

Algorithm 1 Barzilai and Borwein (BB)
1. Given \( x_0 \in \mathbb{R}^n \), choose arbitrary \( y_0 > 0 \), for instance, \( y_k = \frac{(g_0, g_0)}{(g_0, A g_0)} \), (or \( y_k = \frac{(A g_0, g_0)}{(A g_0, A g_0)} \)
2. For \( k = 0, 1, 2, \ldots \) (until convergence) do
3. \( g_0 = Ax_0 - b \)
4. \( x_{k+1} = x_k - y_k g_k \)
5. \( g_{k+1} = g_k - y_k A g_k \)
6. \( y_k = \frac{(g_{k+1}, g_{k+1})}{(g_k, A g_k)} \), (or \( y_k = \frac{(A g_{k-1}, g_{k-1})}{(A g_{k-1}, A g_{k-1})} \)
7. End for

This paper presents the proof of convergence of the BB method when the Euclidian norm (\( f^2 \)) of the unsymmetric part of a positive definite matrix is less than some value related to the smallest and the largest eigenvalues of the symmetric part of the given matrix. This means that the restriction of \( A \) be symmetric is removed, and required only that its symmetric part \((A + A^T)/2\) be positive definite.
Let us begin with some notations:

1- The Euclidean inner product of any two vectors \( u, v \in \mathbb{R}^n \) is defined by \( (u, v) = u^T v \), and the induced Euclidean norm (or 2-norm) of \( u \) is \( \| u \| = \sqrt{(u, u)} \).

2- Let \( A \) be an \( n \times n \) matrix and the associated matrix norm is given by
\[
A := \sup_{\|u\|=1} \|Au\|.
\]
It is known that \( A \) can be represented as \( A = A_0 + A_1 \), where \( A_0 \) and \( A_1 \) the symmetric and skew-symmetric of a matrix \( A \), i.e. \( A_0 = (A + A^T)/2 \), \( A_1 = (A - A^T)/2 \).

4- Let \( A \) be positive definite, then the symmetric part \( A_0 \) is also positive definite. Hence \( A \) and \( A_0 \) are invertible and the eigenvalues of \( A_0 \) are all positive real numbers. If \( \lambda_{\text{min}}, \ldots, \lambda_{\text{max}} \) are the eigenvalues of \( A_0 \) such that
\[
0 < \lambda_{\text{min}} \leq \ldots \leq \lambda_{\text{max}}
\]
then \( \lambda_{\text{min}}^{-1}, \ldots, \lambda_{\text{max}}^{-1} \) are the eigenvalues of \( A_0^{-1} \).

5- The condition number of \( A_0 \) is defined to be \( \rho := \frac{\|A_0\|}{\|A_0^{-1}\|} \).
Since \( A_0 \) is symmetric,
\[
\rho = \frac{\lambda_{\text{max}}}{\lambda_{\text{min}}} \geq 1.
\]

6- The Rayleigh-quotient of any vector \( u \in \mathbb{R}^n \) with respect to \( A \) is defined by
\[
r(u) = \frac{(u, Au)}{(u, u)}.
\]
The lemmas that will be used frequently in the proof of the main theorem are follows:

**Lemma 1.** For any vector \( u \),
\[
\lambda_{\text{min}} \| u \|^2 \leq (u, A_0 u) \leq \lambda_{\text{max}} \| u \|^2.
\]

**Proof.** For a symmetric matrix positive definite matrix \( A_0 \), the Rayleigh quotients are bounded by the smallest and the largest eigenvalues of the matrix. Thus, the following relation holds for any \( u \)
\[
\lambda_{\text{min}} \leq \frac{(u, A_0 u)}{\|u\|^2} \leq \lambda_{\text{max}}.
\]
Hence the proof is complete.

**Lemma 2.** For any vector \( u \),
\[
\lambda_{\text{max}}^{-1} \| u \|^2 \leq (u, A_0^{-1} u) \leq \lambda_{\text{min}}^{-1} \| u \|^2.
\]

**Proof.** Similar to proof Lemma 1.

**Lemma 3.** For any vector \( u \),
\[
(u, Au) = (u, A_0 u) \quad \text{and} \quad (u, A_1 u) = 0
\]

**Proof.** We have
\[
(u, Au) = (u, A_0 u) + (u, A_1 u).
\]
Since \( u^TA_1 u \) is a real number,
\[
u^TA_1 u = (u^T A_1 u)^T = u^T A_1^T u = -u^T A_1 u.
\]
Therefore \( (u, A_1 u) = 0 \) and \( (u, Au) = (u, A_0 u) \).

**Lemma 4** (Cauchy–Schwarz inequality for a positive definite matrix).
If \( A_0 \) is a positive definite matrix, then for any \( u \) and \( v \)
\[
| (u, A_0 v) | \leq \sqrt{(u, A_0 u)} \sqrt{(v, A_0 v)}.
\]
2. Convergence of the BB Method

In this section, the proof of convergence of the BB method applied to the slightly unsymmetric linear system $Ax = b$ with the positive definite symmetric part is given.

**Theorem 1.** If
\[\|A_1\| \leq \lambda_{\text{min}}\sqrt{\rho^{-1}} (-1 + \sqrt{1 + \rho^{-1}}),\]

then the BB method, defined by
\[g_{k+1} = g_k - \gamma_k A g_k\]
where
\[\gamma_k = \frac{(g_k - 1, g_k - 1)}{(g_k, A g_k)}\]
( or \[\gamma_k = \frac{(A g_k - 1, g_k - 1)}{(A g_k, A g_k)}\]),
converges.

**Proof.** Using (2.1), (2.2), Lemma (3), Lemma (1) and since $\gamma_k$ is the Rayleigh quotient of the symmetric positive definite matrix $A_0$ (i.e. $0 < \lambda_{\text{min}} \leq \gamma_k \leq \lambda_{\text{max}}$ for all $k$), we have
\[g_k, A_0 g_k = (g_k - 1, A_0 g_k - 1) - 2\gamma_k (A g_k - 1, A_0 g_k - 1) + \gamma_k^2 (A g_k - 1, A_0 g_k - 1)
+ \gamma_k^2 (A g_k - 1, A_0 A g_k - 1)
= (g_k - 1, A_0 g_k - 1) - 2\gamma_k (A g_k - 1, A_0 g_k - 1) + \gamma_k^2 (A g_k - 1, A_0 A g_k - 1)
+ \gamma_k^2 (A g_k - 1, A_0 A g_k - 1)
= (g_k, A_0 g_k) - \gamma_k (g_k, g_k) - 2\gamma_k (A g_k - 1, A_0 g_k - 1)
+ \gamma_k^2 (A g_k - 1, A_0 g_k - 1)\]
(2.3)
Assuming $\delta = \|A_1\|$ and $c_k := (g_k - 1, A_0 g_k - 1)$ for any $k$, using Lemma 2 and since $\lambda_{\text{max}} \geq \gamma_k \geq \lambda_{\text{min}}$, we obtain
\[\gamma_k (g_k - 1, g_k - 1) \geq \lambda_{\text{max}} \lambda_{\text{min}} c_k - 1 = \rho^{-1} c_k - 1.\]
(2.5)
By using Lemma 2 twice, we have
\[\|A_1 g_k - 1, A_0 g_k - 1)\| \leq \lambda_{\text{max}} \|A_1 g_k - 1\| \|A_0 g_k - 1\| \leq \lambda_{\text{max}} \rho^2 \|g_k - 1\|\]
(2.6)
Using Lemma 4, we get
\[\|A_1 g_k - 1, A_0 g_k - 1\| \leq \sqrt{(A_1 g_k - 1, A_0 g_k - 1)c_k - 1} = \sqrt{\rho \delta c_k - 1}.\]
(2.7)
From (2.3) to (2.7) and utilizing Lemma 3,
\[c_k \leq c_k - 1 - \rho^{-1} c_k - 1 + 2\lambda_{\text{max}} \rho^{-1} \delta c_k - 1 + \lambda_{\text{max}} \rho \delta c_k - 1\]
\[c_k - 1 - \rho^{-1} + 2\lambda_{\text{max}} \rho^{-1} \delta + \lambda_{\text{max}} \rho \delta^2 c_k - 1\]
For the purpose of convergence, we need
\[1 - \rho^{-1} + 2\lambda_{\text{max}} \rho^{-1} \delta + \lambda_{\text{max}} \rho \delta^2 < 1,
2\lambda_{\text{max}} \rho^{-1} \delta + \lambda_{\text{max}} \rho \delta^2 - \rho^{-1} < 0,
\]
multiplying by $\lambda_{\text{max}} \rho^{-1}$, we have
\[\delta^2 + 2\lambda_{\text{max}} \rho \delta^2 - \lambda_{\text{max}} \rho^{-1} \delta < 0.
\]
This satisfies when
\[\delta < -\lambda_{\text{min}} \sqrt{\rho^{-1} - \lambda_{\text{max}} \rho^{-1} \delta^2 + \lambda_{\text{max}} \rho \delta^2} = \lambda_{\text{min}} \sqrt{\rho^{-1} \left(-1 + \sqrt{1 + \rho^{-1}}\right)}.
\]
This completes the proof. □

3. Numerical Experiments

In this section, two numerical experiments are presented to show the rate of convergence of the Barzilai-Borwein (BB) algorithm for solving the linear system of equations $Ax = b$ where $A$ is slightly unsymmetric positive definite matrix. They demonstrate that if the sufficient condition
Satisfies, then the BB algorithm convergence. Simulations were run in MatLab 27.

Example 1. For the first experiment, a matrix $A$ with size $d \times d$ can be taken as

$$
\begin{bmatrix}
1.0000 & -0.0009 & -0.0001 & -0.0013 & -0.0007 & 0.0020 & 0.0021 \\
-0.0007 & 1.6667 & -0.0000 & -0.0022 & -0.0013 & 0.0012 & 0.0008 \\
0.0013 & 0.0015 & 2.3333 & -0.0007 & -0.0029 & 0.0018 & 0.0003 \\
-0.0004 & -0.0014 & -0.0023 & 3.0000 & -0.0007 & -0.0019 & 0.0027 \\
0.0000 & 0.0024 & -0.0014 & 0.0026 & 3.6667 & -0.0024 & 0.0026 \\
-0.0017 & 0.0011 & -0.0020 & 0.0007 & -0.0033 & 4.3333 & 0.0011 \\
-0.0021 & 0.0021 & 0.0028 & -0.0027 & 0.0023 & 0.0020 & 5.0000 
\end{bmatrix}
$$

where $n = 7$, $A = [a_{ij}], i = 1,2,\ldots,n; j = 1,2,\ldots,n$ such that $a_{ij}$ are equally spaced real numbers between 1 and 5 when $i = j$, and $a_{ij}$ is a random number between -1 and 1 when $i \neq j$. The symmetric part of $A$ ($A_0$) is symmetric positive definite matrix and its condition number $\rho = 5$ where $\lambda_{\min} = 1$ and $\lambda_{\max} = 5$. $\|A_1\| = 0.0041$. The value of $\lambda_{\min}\sqrt{\rho^{-1}} (1 + \sqrt{1 + \rho^{-1}}) = 0.0427$.

Figure 1 shows the rate of convergence $r_k = \frac{(g_{k+1} - g_k)}{(g_0 - g_k)}$ of the BB algorithm as a function of number of iteration $k=300$.

![Figure 1](image-url)

**Figure 1:** Rate $r_k$ of convergence as a function of $k$ for Example 1.

Example 2. For the second experiment, a matrix $A$ with size $d \times d$ can be taken as

$$
\begin{bmatrix}
11.0000 & 0.7621 & 0.7621 & 0.4057 & 0.0579 \\
0.0000 & 11.0000 & 0.7919 & 0.9355 & 0.3529 \\
0.0000 & 0.0000 & 11.0000 & 0.9169 & 0.8132 \\
0.0000 & 0.0000 & 0.0000 & 11.0000 & 0.0099 \\
0.0000 & 0.0000 & 0.0000 & 0.0000 & 11.0000 
\end{bmatrix}
$$

where $d = 5$, $A = [a_{ij}], i = 1,2,\ldots,n; j = 1,2,\ldots,n$ such that $a_{ij} = 11$ when $i = j$, and $a_{ij} = \text{rand}(d)$ when $i \neq j$. $A_0$ is symmetric positive definite matrix and its condition number $\rho \approx 1.1801$ where $\lambda_{\min} = 10.3449$ and $\lambda_{\max} = 12.2082$. $\|A_1\| = 1.0117$ and the value of $\lambda_{\min}\sqrt{\rho^{-1}} (1 + \sqrt{1 + \rho^{-1}}) \approx 3.4204$.

Figure 2 shows the rate of convergence $r_k = \frac{(g_{k+1} - g_k)}{(g_0 - g_k)}$ of the BB algorithm as a function of number of iteration $k = 100$. 

![Figure 2](image-url)
Figure 2: Rate $r_k$ of convergence as a function of $k$ for Example 2.

References


ON GENERALIZED REGULAR LOCAL RING

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Abstract:
A ring $R$ is called a generalized Von Neumann regular local ring (GVNL-ring) if for any $a \in R$, either $a$ or $(1-a)$ is $\pi$-regular element. In this paper, we give some characterization and properties of generalized regular local rings. And we studied the relation between generalized regular local rings, Von Neumann regular rings, Von Neumann regular local rings (VNL-rings) and exchange rings.

Key words: local, $\pi$-regular , exchange rings .

1- Introduction:
Throughout this paper, $R$ will be an associative ring with identity. For $a \in R$, $r(a)$, $\ell(a)$ denote the right (left) annihilator of $a$. We write $Y(R)$, $J(R)$ for the right singular ideal and the Jacobson radical of $R$, respectively. An ideal $I$ of a ring $R$ is said to be essential if and only if $I$ has a non-zero intersection with every non-zero ideal of an $R$. A ring $R$ is reduced if $R$ contains no non-zero nilpotent element. A ring $R$ is called Von Neumann regular (or just regular) if and only if for each $a$ in $R$, there exists $b$ in $R$ such that $a = aba$ [7]. In [3] Contessa first introduced and characterized a VNL-ring, and gave many properties, a ring $R$ is called Von Neumann regular local rings (VNL-rings), if for any $a \in R$, either $a$ or $(1-a)$ is Von Neumann regular element. A ring $R$ is called local ring, if it has exactly one maximal ideal [1]. A ring $R$ is called $\pi$-regular ring if and only if for each $a$ in $R$, there exists $b$ in $R$ and a positive integer $n$ such that $a^n = a^n b a^n$ [4]. Clearly that every $\pi$-regular ring is GVNL-ring. A ring $R$ is said to be strongly commuting regular if for each $x, y$ in $R$ there exists $a \in R$ such that $(x y) = (y x) a (y x)^2$ [6].

A ring $R$ is called Exchange ring if for any $a \in R$, there exists an idempotent element $e \in R$ such that $e a R$ and $(1-e) \in (1-a)R$ [5].

2- GVNL-Rings
This section is devoted to give the definition of generalized Von Neumann regular local ring(GVNL-rings) with some of its characterization and basic properties .

Definition 2.1: [2]
A ring $R$ is called a generalized Von Neumann regular local ring(GVNL-ring) if a or $(1-a)$ is $\pi$-regular for every $a \in R$ .

Examples:
1-) Let $(Z_2, +, .)$ be a ring and let $G=\langle g : g^2 = 1 \rangle$ is cyclic group, then $Z_2 G=\langle 0, 1, g, 1 + g \rangle$ is not regular, but $\pi$-regular ring.
2-) Let $R$ be the set of all matrix in $Z_2$ which defined as:

$$R=\left\{ \begin{bmatrix} a & b \\ 0 & d \end{bmatrix} : a, b, d \in Z_2 \right\}$$

It is easy to find the elements of $R$.

$$R=\left\{ A_0, A_1, A_2, A_3, A_4, A_5, A_6, A_7 \right\}$$

$$A_0 = \begin{bmatrix} 0 & 0 \\ 0 & 0 \end{bmatrix}, A_1 = \begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix}, A_2 = \begin{bmatrix} 0 & 1 \\ 0 & 0 \end{bmatrix}, A_3 = \begin{bmatrix} 0 & 0 \\ 0 & 1 \end{bmatrix}$$

$$A_4 = \begin{bmatrix} 0 & 1 \\ 0 & 1 \end{bmatrix}, A_5 = \begin{bmatrix} 1 & 1 \\ 0 & 1 \end{bmatrix}, A_6 = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}, A_7 = \begin{bmatrix} 1 & 1 \\ 1 & 1 \end{bmatrix}$$

Thus $R$ is not regular but $R$ is $\pi$-regular.

3-) Let $R=Z_4 \oplus Z_4$ then it is easy to check that ,

$$(3, 2) \text{ and } (1, 1) - (3, 2) \text{ are not regular. So } R \text{ is not a VNL-ring, but } R \text{ is } \pi\text{-regular. Thus } R \text{ is a GVNL-ring.}$$

Proposition: 2.2:
A ring $R$ is GVNL-ring iff $a^n R$ is generated by an idempotent element for every $a \in R$ and some positive integer $n$. 

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**Proposition 2.3:** Let \( R \) be a commutative ring and let \( P \) be a primary ideal of \( R \). Then \( P \) is a maximal ideal if \( R/P \) is a division ring. Therefore \( P \) is a maximal ideal in \( R \).

**Proof:**
Let \( R \), then \( a + P \in R/P \). Since \( R/P \) is a division ring, then either \((a+p)\) or \((1-a)+P\) is a regular element in \( R/P \). Let \( a \in R \) and \( \alpha \in Z^+ \) such that \( \alpha \cdot (1-a)^n = (a+p)^n \). Therefore \( P \) is a maximal ideal in \( R \).

Now, let \( z \in R \), then \( a \) is an idempotent in \( R \) such that \( a^nR = a \cdot (1-a)^n \). Hence \( a^nR = (a+p)^n \) and \( (a+p)^n \) is regular in \( R/P \). Then let \( (a+p)^n \) be \( (a+p)^n \) is regular in \( R/P \). Therefore \( R/P \) is a division ring. Therefore \( P \) is a maximal ideal in \( R \).

#
**Theorem 2.4:** Let I be a regular ideal of a ring R. Then R is GVNL-ring if and only if $R/I$ is GVNL-ring.

**Proof:**
Let R be GVNL-ring. Then either a or (1-a) is $\pi$- regular element in R, for all a\in R.
Now, if a is $\pi$-regular, then there exists b\in R and n\in Z such that $a^n = a^n b a^n$
Hence $(a+I)^n = a^n + I = a^n b a^n + I$
\[= (a^n + I)(b+I)(a^n + I)\]
\[= (a+I)^n (b+I)(a+I)^n\]
Thus $(a+I)$ is $\pi$-regular element in $R/I$
Now, if $(1-a)$ is $\pi$-regular in R, then there exists d\in R and n\in Z such that
\[(1-a)^n = (1-a)^n d (1-a)^n\]
Hence $((1-a)+I)^n = ((1-a)+I) (d+I) ((1-a)+I)^n$
\[= (1-a)^n (d+I) ((1-a)^n + I)\]
\[= (1-a)^n d (1-a)^n + I\]
\[= (1-a)^n + I\]
Therefore $((1-a)+I)$ is $\pi$-regular in $R/I$
Conversely, let $R/I$ be GVNL and a\in R. Then either $(a+I)$ or $((1-a)+I)$ is $\pi$-regular element in $R/I$.
Then there exists $(b+I)\in R/I$ and a positive integer n such that
\[a^n = (a^n + b) a^n + I\]
Hence $a^n = a^n b a^n + I$, so $a^n = a^n b a^n \in I$
Since I is regular, there exists c\in I such that
\[a^n = a^n b a^n c a^n - a^n a^n c a^n + a^n a^n b a^n + a^n - a^n b a^n = a^n (b + c - b a^n b a^n + b a^n c a^n) a^n\]
\[= a^n w a^n, \quad \text{where } w = (b + c - b a^n c a^n + b a^n c a^n) a^n\]
Thus a is $\pi$-regular in R.
Now, if $(1-a)+I$ is $\pi$-regular, then there exists $q+I$ and n\in Z such that,
\[(1-a)^n q \equiv (1-a)^n q (1-a)^n + I\]
So $(1-a)^n q (1-a)^n + I = (1-a)^n q (1-a)^n + I$ since I is regular then
\[(1-a)^n q (1-a)^n = (1-a)^n q (1-a)^n - (1-a)^n q (1-a)^n\]
\[= (1-a)^n q (1-a)^n w (1-a)^n q (1-a)^n + (1-a)^n q (1-a)^n w (1-a)^n q (1-a)^n\]
\[= (1-a)^n q (1-a)^n w (1-a)^n q (1-a)^n + (1-a)^n q (1-a)^n w (1-a)^n q (1-a)^n\]
\[= (1-a)^n q (1-a)^n w (1-a)^n q (1-a)^n + (1-a)^n q (1-a)^n w (1-a)^n q (1-a)^n\]
\[= (1-a)^n q (1-a)^n w (1-a)^n q (1-a)^n + (1-a)^n q (1-a)^n w (1-a)^n q (1-a)^n\]
\[= (1-a)^n Z (1-a)^n\]
Thus $(1-a)$ is $\pi$- regular in R. Therefore R is a GVNL ring.

**Proposition 2.5:**
Let R be a GVNL-ring. Then $J(R)$ is nil ideal.

**Proof:**
Let 0\ne a\in J(R) Since R is GVNL-ring, then either a or (1-a) is $\pi$- regular element of R, if a is $\pi$-regular, then there exists an element b\in R and n\in Z such that $a^n = a^n b a^n$, then $a^n - a^n b a^n = 0$. Hence $(1-a^n) a^n = 0$ Since $a\in J(R)$, therefore $a^n \in J(R)$, and $a^n b \in J(R)$. Thus $1-a^n b$ is invertible, so there exists u\in R such that u = 1, it follows that u $(a^n b a^n) = a^n$, thus a is nilpotent element. Now, if $(1-a)$ is $\pi$-regular, since $a \in J(R)$, then $(1-a)$ is invertible, and then $(1-a)^n$ invert able, thus $(1-a)$ is nilpotent element and Therefore $J(R)$ is a nilideal.

**Corollary 2.6:**
Let R be a reduced GVNL ring Then $J(R) = 0$

**Proof:**
Suppose that $J(R) \neq 0$, then there exists $a \in J(R)$ and by (prop 2.5) a is a nilpotent element in R. But R is reduced, then $a = 0$. Therefore $J(R) = 0$. #
Corollary 2.7:
Let R be a reduced GVNL-ring, then R is VNL-ring.

Proof:
R is exchange ring [2, Theorem 2.2].

Now, R is reduced exchange ring for any a ∈ R.

If a is a regular ring in R, then \( a^n = a^n x \) for some positive integer n and x ∈ R. Clearly e = a^n x where e is an idempotent element in R, so ((1-e) a^n) = (1-e) a^n e = 0, and hence (1-a) e = 0. Therefore a = e, a = (a^{n+1} x) a, is regular.

Now, if (1-a) is regular, then (1-a)^n = (1-a)^n e (1-a)^n = 0.

Hence (1-a) (1-a) = 0.

Thus (1-a) = (1-a)^n e (1-a) = (1-a) (1-a) = (1-a) w (1-a).

That is (1-a) is regular. Therefore R is a VNL-ring.

Proposition 2.8:
Let R be a GVNL-ring with \( r(a) \) \( R \) for any a ∈ R, and let \( Y(R) \) be the nilideal of R.

Proof:
Let a ≠ \{ 0, 1 \} be an element in Y(R). Then Y(R) is an essential right ideal of R. Now, since r(a) \( R \) \( a^n \), then r(a) is also an essential right ideal of R.

Since R is a GVNL-ring, then either a or (1-a) is a regular element in R. If a is regular, then there exists b ∈ R such that a = b a b.

Now, consider r(a) \( R \) \( a^n \), and let x ∈ r(a) ∩ ba^n R, then a^n x = 0 and x = ba^n r for some r ∈ R. So, a^n b a^n r = 0, which implies a^n r = 0, yielding x = 0.

Therefore r \( a^n \) \( ba^n R = 0 \), since r(a) is a non-zero essential right ideal of R, then ba^n = 0, and hence a^n = 0.

Now, if (1-a) is regular, then there exists c ∈ R and e \( Y(R) \) such that (1-a)^n = (1-a)^n = c(1-a)^n.

Now, since r(a) \( Y(R) \) \( r(a^n) \) \( (1-a)^n \), then r((1-a)^n) is also an essential ideal of R. Consider r((1-a)^n) \( Y(R) \) \( c(1-a)^n \), and let y ∈ r((1-a)^n) \( Y(R) \) \( c(1-a)^n \), then (1-a)^n y = 0, and y = c(1-a)^n r for some r ∈ R. So, (1-a)^n c(1-a)^n r = 0, which implies (1-a)^n r = 0, yielding y = 0. Therefore r((1-a)^n) \( Y(R) \) \( (1-a)^n R = 0 \).

Since r((1-a)^n) is a non-zero essential right ideal of R, then c(1-a)^n = 0.

And hence (1-a)^n = 0, and then 1 = a, a contradiction.

Thus a^n = 0 and therefore Y(R) is nilideal.

Theorem 2.9:
Let R be a ring with \( r(a) \) \( a^n \) and \( r(a(1-a)^n) \) \( R \), then R is GVNL-ring.

Proof:
Assume that R/r(a) be a GVNL-ring, then either a + r(a) ∈ R/a or
(1-a)+r(a) is regular element. Now, if a + r(a) is a regular element for a ∈ R then there exists b + r(a) ∈ R/a (a + r(a))^n = (a + r(a))(a + r(a))^n = (a^n + r(a))(a + r(a)) = a^n b a^n + r(a)

Then a^n = a^n b a^n ∈ Y(R), that is
a a^n b a^n ) = 0, hence a^{n+1} = 0, then (1-b a^n ) ∈ r(a^{n+1}) \( R = 0 \), which implies that a^n = 0.

Thus a is regular element in R.

Now, if (1-a)+r(a) ∈ R/ r(a) is regular for a ∈ R, then there exists c+r(a) ∈ R/ r(a) and a ∈ Y(R) such that (1-a)^n + r(a) = ((1-a) + r(a))^n.
Proposition 2.11:  
\[a^n = (1-a)^n + r(a^n) = ((1-a)^n + r(a^n))c + r(a^n)((1-a)^n + r(a^n)) = (1-a)^n + r(a^n) \]

Proof:  
\[a^n = (1-a)^n + r(a^n), \text{ then } (1-a)^n - (1-a)^n c (1-a)^n = r(a^n), \text{ that is} \]
\[a((1-a)^n)(1-a)^n = 0, \text{ so } a(1-a)^n = 0 \text{ then} \]
\[(1-a)^n \in r(a(1-a)^n) \subseteq r(1-a)^n, \text{ hence } (1-a)^n(1-a)^n = 0 \]
Thus \((1-a)^n - (1-a)^n c (1-a)^n = 0 \). Hence \((1-a)^n\) is \(\pi\)-regular element in \(R\)  
Therefore \(R\) is GVNL-ring. #

Theorem 2.10:  
If \(R\) is a reduced ring and every maximal ideal of \(R\) is a right annihilator.  
Then \(R\) is GVNL-ring.

Proof:  
Let \(a \in R\), we shall prove that \(a^n R + r(a^n) = R\), if not there exists a right maximal ideal \(M\) containing \(a^n R + r(a^n)\). If \(M = r(b)\) for some \(0 \neq b \in R\), we have  
\[b \in (a^n R + r(a^n)) \subseteq \ell(a^n) = r(a^n), \text{ which implies that } b \in M = r(b), \text{ then } b^2 = 0, \]
and \(b = 0\) a contradiction. Therefore \(a^n R + r(a^n) = R\).  
In particular \(a^n c + d = 1\), \(c \in R\), \(d \in r(a^n)\), then \(a^n c a^n = a^n\) which proves \(a\) is \(\pi\)-regular element. Now, if \((1-a) \in R\), we shall prove that.  
\[(1-a)^n R + r((1-a)^n) = R\], if not there exists a maximal right ideal \(M\) containing \((1-a)^n R + r((1-a)^n)\), if \(M = r(c)\) for some \(0 \neq c \in R\), we have  
\[c \in (1-a)^n R + r((1-a)^n) \subseteq \ell((1-a)^n) = r((1-a)^n). \text{ Which implies } c \in M = r(c), \text{ then } c^2 = 0. \]
And hence \(c = 0\) a contradiction. Therefore \((1-a)^n R + r((1-a)^n) = R\)  
In particular \((1-a)^n x + y = 1\) Where \(x \in R\) and \(y \in r((1-a)^n)\) Then,  
\[(1-a)^n x + (1-a)^n y = 0 + (1-a)^n. \]
Thus \((1-a)^n\) is \(\pi\)-regular element in \(R\). Therefore \(R\) is GVNL-ring. #

Now, we have the following result to obtain the relation between GVNL-ring and exchange ring  

Proposition 2.11:  
If \(R\) is GVNL-ring, then \(eRe\) is also GVNL-ring for every idempotent element \(e \in R\).

Proof:  
For \(a \in eRe\), \(a (1-a)\) is \(\pi\)-regular in \(R\). If \(a\) is \(\pi\)-regular, then there exists \(b \in R\) and \(b \neq 0\) such that \(a^n = a^n b a^n\), so, \(a^n = (a^n e) b (e a^n) = a^n (e b e) a^n\). Thus \(a\) is \(\pi\)-regular in \(eRe\).  
If \((1-a)^n\) is \(\pi\)-regular, then there exists \(c \in R\) and \(n \in \mathbb{Z}^+\) such that \((1-a)^n = (1-a)^n c(1-a)^n\).  
Now, \((e-a)^n = (e-a)^n = (e(1-a))^n = e(1-a)^n e\).  
Hence \(e-a\) is \(\pi\)-regular in \(eRe\).  
Therefore, \(eRe\) is a GVNL-ring. #

Now, we give the following proposition which is due to YING Zhi-ling in [8]

Proposition 2.12:  
If \(R\) a GVNL-ring, then for every idempotent element \(e \in R\), either \(eRe\) or \((1-e) R(1-e)\) is a \(\pi\)-regular ring.

Proof:  
Let \(R\) be a GVNL-ring and \(e \in R\) be an idempotent element in \(R\). Then  
\[R \cong \begin{pmatrix} eRe & eR(1-e) \\ (1-e)R(e) & (1-e)R(1-e) \end{pmatrix} \]
If \(x \in eRe\) and \(y \in (1-e)R(1-e)\) are two non-\(\pi\)-regular elements. Then both  
\[a = \begin{pmatrix} 1-x & 0 \\ 0 & 1-y \end{pmatrix} \]
and \(1-a = \begin{pmatrix} 0 & 0 \\ 1-x & 0 \end{pmatrix} \)
are also non-\(\pi\)-regular, a contradiction. #
From proposition 2.12, clearly every GVNL-ring is an exchange ring.
The following corollary is given in [8]

**Corollary 2.13:**
For an abelian ring $R$, $R$ is GVNL-ring if $R$ is an exchange ring

**Proof:**
For $a \in R$, let $a_1, a_2 \in R$ and $a_3, a_4 \in R$. Then $a_1 R + a_2 R = R$, then by [5, proposition 1-11], there exists an orthogonal idempotent $e_1$ and $e_2$ such that $e_1 e_2 R = e_1 R + e_2 R$. Now, $e_1 a_1 + e_2 a_2$ is regular. Thus we can suppose that $e_1 a_1 = e_2 a_2$ and $e_2 a_2 = e_1 a_1$, implying that $e_1 a_1 = (e_1 a_1)^2 (e_1 b_1)$ and $e_2 a_2 = (e_2 a_2)^2 (e_2 b_2)$. By (proposition 2.12) either $e_1 R = e_1 R e_1$ and $e_2 R = e_2 R e_2$ or $(1-e_1) R (1-e_1) R (1-e_2) R (1-e_2)$ are $\pi$-regular. If $(1-e_1) R (1-e_1)$ is $\pi$-regular for $j=1, 2$, then there exists $m > 0$ and $C_j \in R$ for $j=1, 2$ such that $(1-e_1) a_j = (1-e_1) a_j (1-e_1) a_j = (1-e_1) a_j (1-e_1) a_j = (1-e_1) a_j (1-e_1) a_j = (1-e_1) a_j (1-e_1) a_j$. Hence, $(1-e_1) a_j = (1-e_1) a_j (1-e_1) a_j = (1-e_1) a_j (1-e_1) a_j = (1-e_1) a_j (1-e_1) a_j = (1-e_1) a_j (1-e_1) a_j = (1-e_1) a_j (1-e_1) a_j = (1-e_1) a_j (1-e_1) a_j = (1-e_1) a_j (1-e_1) a_j = (1-e_1) a_j (1-e_1) a_j$. Therefore $R$ is GVNL-ring. #

Now, to give the relation between GVNL-ring and strongly commuting regular rings.

**Theorem 2.14:** [6]
Suppose that $R$ is a strongly commuting regular ring. Then $R$ is $\pi$-regular.

**Proof:**
By strongly commuting regularity of $R$, for each $x \in R$, there exists an element $c$ in $R$ such that $x^2 = x$. Now suppose $x = x$. Then we have $x = x$. Which implies that $R$ is $\pi$-regular. #

We observe the following corollary.

**Corollary 2.15:**
If $R$ is a strongly commuting regular. Then $R$ is GVNL-ring.

3- The GVNL – Ring without zero Divisor element .

In this section, we give some results about GVNL-ring without zero –divisors and some relation with other rings like division ring, local ring, $\pi$-regular ring, simple ring, VNL-ring.

**Proposition 3.1:**
Let $R$ be a GVNL-ring without zero divisors. Then every element $a \not\in \{0, 1\}$ in $R$ is invertable.

**Proof:**
Let $R$ be a GVNL-ring and $0 \neq a \in R$. Then either $a = 0$ or $(1-a)$ is $\pi$-regular element in $R$. If $a$ is $\pi$-regular element in $R$, then there exists $b \in R$ and $n \in \mathbb{Z}^+$ such that $a^n = a^n b a^n$ and then we have $a^n - a^n b a^n = 0$. That is, $a^n - a^n b a^n = (1-b a^n) a = (a^n b (1-b a^n)) = 0$. Since $R$ is without zero divisors then, $a^n b (1-b a^n) = 0$, thus $a^n b (1-b a^n) = 0$ and hence $a^n b (1-b a^n) = 0$. Thus $1 = b a^n$, which implies that $1 = b a^n$. Therefore $a$ has left inverse.

Now, since $1 = b a^n$, then $a = a (b a^n) a$ and thus $(1-ab a^n) a = 0$. Hence $(1-ab a^n) a = 0$. Which implies that $1 = a (b a^n)$, Thus $a$ has right inverse and therefore $a$ is invertable element in $R$.

Now, if $(1-a)$ is $\pi$-regular element in $R$, then there exists $c \in R$ and $n \in \mathbb{Z}^+$ such that $(1-a)^n - (1-a)^n c (1-a)^n = 0$, that is $(1-a)^n [1-c(1-a)^n] = (1-a)^n (1-a)^n [1-c(1-a)^n] = 0$. 150
Since \( R \) is without zero divisor and \( a \notin \{0, 1\} \).

Thus \((1-a)(1-a)^n; (1-c(1-a)^n) = 0\), and hence … \((1-a)(1-c(1-a)^n) = 0\).

Then \(1-c(1-a)^n = 0\), which implies that \(1 = c(1-a)^n\).

That is \(1 = (c(1-a)^{n+1})(1-a)\). Hence \((1-a)\) has left inverse.

Now, since \(1 = (c(1-a)^{n+1})(1-a)\), then,
\[(1-a) = (1-a)(c(1-a)^n)(1-a)\], and then \((1-a)(c(1-a)^n)(1-a) = 0\) thus,
\[(1-a)(c(1-a)^n) \in \mathcal{I}(1-a) = 0\] which implies that \(1 = (c(1-a)^n)(1-a)^n\).

And hence \((1-a)\) has right inverse. Therefore \((1-a)\) is invertible element in \(R\).

**Corollary 3.2:**
Let \( R \) be a GVNL-ring without zero-divisors. Then \( R \) is a division ring.

**Corollary 3.3:**
Let \( R \) be a GVNL-ring without zero divisors. Then \( R \) is;
1- Local ring.
2- VN-regular ring and reduced ring.
3- \(\pi\)-regular ring.

Finally we give the following result.

**Proposition 3.4:**
If \( I \) is a proper ideal of GVNL-ring \( R \). Then every element of \( I \) is zero divisors in \( R \). Especially every GVNL-ring has non-zero divisors is simple.

**Proof:**
Let \( 0 \neq a \in I \), since \( R \) is GVNL-ring, then either \( a \) or \((1-a)\) is \(\pi\)-regular element in \( R \). If \( a \) is \(\pi\)-regular element, then there exists an element \( b \in R \) and a positive integer \( n \) such that \(a^n = a^n b a^n\), if \( a \) is not zero divisors then \(a^n (1-b a^n) = 0\) gives \((1-b a^n) = 0\), and hence \(1 \notin I\), a contradiction.

Hence, \( a \) is zero divisors. Now, if \((1-a)\) is \(\pi\)-regular element, then there exists \( c \in R \) and a positive integer \( n \) such that \((1-a)^n = (1-a)^n c (1-a)^n\). If \((1-a)\) is not zero divisor, then \((1-a)^n (1-c(1-a)^n) = 0\) gives, \(1-c(1-a)^n) = 0\) thus \(1 = c (1-a)^n \in I\) a contradiction.

If \( R \) has nonzero divisors, there is not a proper ideal \( I \) of \( R \). Hence \( R \) is simple.

**REFERENCES**
پویش:

دی‌بینه‌ی عملکرد ( (a ∈ R ) کو ضرب‌هایی ریکشسی و گشتمکره، بو همرنیک ز ( (π یا ( 1-a ( هنیه کو ضرب‌هایی ریکشسی و ضرب‌هایی گشتمکره ای‌فوکلتی‌ی دا مه هندک تایبندی و ساختمانی عملکرد نافذ‌وی بین ریکشسی و نافذ‌وی گشتمکره دایره، ورودی مه پی‌هودنی دانشرا خملکین نافذ‌وی بین گشتمکره و خملکین نافذ‌وی خالی‌بین، عملکرد کین ریکشسی ل پذی تیگ‌هشتنا فون نیومان، خملکین فون نیومان بین ریکشسیتین نافذ‌وی (عملکرد ز ( ۷۷۰۸۲۵ (VNL- و خملکین هم‌داز.

المتخصّل:

یقال للحلقة R بأنّها حلقة محلّیة متونّة معمّمة، إذا كان لكلّ (1-a) (a ∈ R ) هو عنصر متونّ من النظام – π. في هذا البحث أعطينا بعض ميزات و خواص الحلقة محلّیة المتونّة المعمّمة وذلك درستنا العلاقة بين الحلقات المحلّیة المتونّة المعمّمة و الحلقات المحلّیة المتونّة حسب مفهوم فون نیومان، حلقات فون نیومان المتونّة المحلّیة (VNL-حلقات من النظام وحلقات المقابلة. 
SURFACE ROUGHNESS EFFECT ON DISCHARGE COEFFICIENT OF COMBINED CYLINDRICAL WEIR GATE STRUCTURE

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ABSTRACT:

The aim of this research is to investigate the effect of surface roughness on the performance of weir and gate. An experimental study in a laboratory flume is carried out to study flow over and under cylindrical weir gate in combined structure as flow measurement device. Four models having different diameters were tested in a laboratory flume. In each model, the surface was roughed four times. The results of the test show logical negative effect of the increase of surface roughness on the performance. The performance of the combined structure improved with decrease ratio of roughness to the upstream head (Ks/H) and with the increase of the total head to the diameter of the weir (H/d). Empirical relations were obtained to estimate the variation of discharge coefficient (Ca) in terms of some dimensionless parameters. Within the limitations of the present experimental work an equation to predict the discharge is proposed with R² of 0.936. Finely the contribution of the gate increases relative to the weir when the surface roughness increases.

KEYWORDS: Combined discharge measuring structure, cylindrical weir, gate, surface roughness, discharge coefficient.

1. INTRODUCTION

The most common and important water measurement structures that used for controlling, adjusting the flow in irrigation channel and diverting the flow from a main channel to a secondary channel are weirs and gates. One of the weirs demerits is they need to be cleaned of sediment and trash periodically. Sluice gates are used extensively for flow control and water measurement for long time. One disadvantage of the gates is they retained the floating materials. In order to maximize their advantages, weirs and gates can be combined together in one device, so that water could pass over the weir and below the gate simultaneously. One of the combined weir-gate structures is cylindrical weir-gate structure. Regarding the form of the combined cylindrical weir-gate structures, it has some advantages including easy design by using commercial pipes, sediments and floating materials flow, high flow discharge coefficient than other replaceable structures and its being economic and combination of those structures in one device can minimize the disadvantages of separate use of each device. The performance of the combination has been studied by many investigations, Negm et al. (2002) presented the effects of hydraulic and geometrical parameters, viscosity and surface tension in the combined flow over rectangular weirs and rectangular gates with sharp crested.

They found that the trend of variation in sloping bed cases was similar to that horizontal bed. Hayawi et al. (2008) studied the characteristics of free flow through the combined triangular weir and rectangular gate. They found that the values of theoretical discharge was inversely proportional to the geometrical dimensionless parameters and directly to distance between the bottom of the weir and the upper edge of the gate. Hayawi et al. (2009) investigated the coefficient of discharge (Ca) for a combined rectangular weir with semi-circular gate. They found that the average value of (Ca) was equal to 0.695. Also, obtained that the value of (Ca) increase with the increases of the distance between the weir and gate opening with average value of (Ca) 0.74. Jalil and Sarhan (2013) studied the flow over a sharp crested weir and under gate in combined oblique structure as flow measurement structure. They found that the value of $C_{ld}$ range from 0.403 to 0.623 with different effect parameters. Masoudian et al. (2013) experimentally studied the effects of canal size on discharge coefficients of cylindrical weir-gate by used two flumes. They found that the value of $C_{ld}$ in large canals were (0.75 to 1.05) more than in small canals ($C_{ld}$ = 0.55 to 0.9) which can be as a result of surpassing effects of walls, canal size, surface tension and viscosity on discharge coefficient in
small canals since the ratio of boundary layer thickness to the canal width in small canals was considerably more than the ratio in large canals and whereas, the amount of flow velocity in boundary layer was low, led to reduce discharge coefficient. Severi et al. (2013) performed the effect of vertical movement of cylindrical weir-gate on free flow hydraulic. Experimentally resulted that the gate opening changes has inverse effect related to discharge coefficient, so that the maximum and minimum discharge coefficient were visible in cylindrical weir and cylindrical gate, respectively. Furthermore, in a constant diameter and constant discharge, discharge coefficient changes had an increasing process by the decreases of the gate opening height. Besides, in a constant discharge and gate opening height, discharge coefficient decreases with increase in the structure diameter. Khassaf et al. (2013) investigated the coefficient of discharge and characteristic of free flow for a combined weir (have two rectangular notches with trapezoidal notch between them) and semicircular sluice gate. The results show that the values of (Cd) range from (0.358 to 0.426) with an average value of (0.392). On the other side the effect of surface roughness are studied by numerous research on different hydraulic structure, such as, Othman et al. (2011) and Ghobadian et al (2013) studied the effect of size and surface roughness of cylindrical weir. Mohammed et al. (2011) studied the effect of bed roughness of free overfall in a rectangular channel with different bed slope. Jalil et al. (2014) investigate the effects of surface roughness sizes on the discharge coefficient for broad crested weirs.

Previous studied sources the discharge coefficient of combined weir-gate structure usually expressed for a smooth case. Therefore, the main objective of this investigation is to study the effects of different diameters and surface roughness on the hydraulic characteristics of the combined cylindrical weir and gate structure.

2. THEORETICAL BACKGROUND

The conservation principles of the energy and continuity can lead to the theoretical base of the free flow over weir and under the gate as two parts of the hydraulic flow measurements structure. Theoretically if no energy lost, a well-known equations for evaluating the discharge over the weir and under the gate can be presented as in Equations (1 and 2) respectively, the overall discharge of the structure is the addition of the two equations which presented in Equation 3. Figure 1 shows the definition sketch for the flow with the geometric parameters.

\[ Q_{th} = \frac{2}{3} B \sqrt{2gh_1^2} \]  
\[ Q_{g1h} = aB \sqrt{2gH} \]  
\[ Q_{toh} = Ba \sqrt{2gH} + \frac{2}{3} B \sqrt{2gh_1^2} \]  

![Figure 1. Combined weir gate structure](image)

Where: \( Q_{th} \) = discharge passing over the weir and under gate (L³/T),
Q_w = discharge passing over the weir (L^3/T),
Q_g = discharge passing under gate (L^3/T),
H = upstream head (L),
d = diameter of the pipe (L),
h = head depth of water over the weir (L),
a = gate opening (L),
B = weir and gate length (L), and
g = acceleration due to gravity (L/T^2).

The actual value of discharge is affected by all the factors imposed in the flow phenomena. This coefficient simulates the effect of all factors entering the physical process of flow. Based on Equation 4 and used the dimensionless parameters introduced by earlier studies, the following wide function relationship can be written as in Equation 5.

\[ C_d = \frac{K_s}{\alpha} \left( \frac{K_s}{\alpha} \cdot \frac{h}{H} \cdot \frac{h}{h'} \cdot \frac{h}{h''} \cdot \frac{h}{h'''} \cdot \frac{a'}{a} \cdot \frac{d}{d'} \cdot \frac{H}{H'} \cdot \frac{R_w}{R_w'} \cdot \frac{W_e}{W_e'} \right) \]  
(5)

Where: \( K_s = \) roughness size (L),
\( R_w = \) Reynolds number, and
\( W_e = \) Weber number.

The values of Reynolds number and Weber number are not affected due to turbulent flow and neglecting surface tension. Equation 5 can be written as

\[ C_d = f_d \left( \frac{K_s}{\alpha} \cdot \frac{K_s}{\alpha} \cdot \frac{h}{H} \cdot \frac{h}{h'} \cdot \frac{h}{h''} \cdot \frac{h}{h'''} \cdot \frac{a'}{a} \cdot \frac{d}{d'} \cdot \frac{H}{H'} \cdot \frac{R_w}{R_w'} \cdot \frac{W_e}{W_e'} \right) \]  
(6)

3. EXPERIMENTAL WORK

The models of study were made from plastic pipes have four different diameters (d = 4, 6.3, 9, and 11 cm). Each model was roughed four, three models were roughed by uniform aggregates of size 2.36, 3.35, and 4.75 mm, and the fourth model left as it is a smooth plastic, see Figure 2. The experimental investigation was carried out in a horizontal flume of working length 2.4 m, having a rectangular cross section of 0.25m height and 0.075m width. The depth of flow was measured, using point gauge with venier scale reading to 0.1 mm, at center line of flume. The flow rate was measured by a volumetric tank. Details of the experimental program are shown in Table 1.

Figure 2. Cylindrical weir-gate structure model
Table 1. Details of the cylindrical weir-gate structure models tested during the experimental program

<table>
<thead>
<tr>
<th>Models</th>
<th>Diameter (m)</th>
<th>State of Surface Ks (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smooth</td>
<td>2.36</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Smooth</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.75</td>
</tr>
<tr>
<td></td>
<td>Smooth</td>
<td>2.36</td>
</tr>
<tr>
<td>2</td>
<td>0.063</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td>Smooth</td>
<td>4.75</td>
</tr>
<tr>
<td></td>
<td>Smooth</td>
<td>2.36</td>
</tr>
<tr>
<td>3</td>
<td>0.09</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td>Smooth</td>
<td>4.74</td>
</tr>
<tr>
<td></td>
<td>Smooth</td>
<td>2.36</td>
</tr>
<tr>
<td>4</td>
<td>0.11</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.75</td>
</tr>
</tbody>
</table>

4. RESULTS AND DISCUSSION

The data collected from the tests of the four structural diameter sand two roughness are presented in Figure 3, which shows the comparison between the smooth surface of the cylindrical combined structure and the rough surface of Ks=4.35 mm. It is clear that the total discharge flowing over weir and under the gate is increase with the total head. The effect of surface roughness is also clear, it can be notice that for a certain total head and pipe diameter the total discharge is more for the smooth, so the head increase with increase of surface roughness (Ks) for a certain value of discharge. This increase in head caused by the head loss due to increase of friction force.

![Figure 3](image_url)

Figure 3. Variation of the discharge with total head for different pipe diameter and roughness

The experimental data is presented in Figure 4, which shows the effect of structure diameter on the discharge for all surface roughness heights (Ks). It can be pointed that there is a slight decrease in discharge value with the increase in surface roughness for large pipe diameter, while the effect of surface roughness seen to be more effective for the small pipe diameter.
The calculated value of discharge coefficient ($C_d$) in Equation 4 was studied with the dimensionless parameters in Equation 5. It is clear that the value of ($C_d$) decreases with the increase of roughness ($K_s$) for a fixed value of ($h/H$) and increases with the increase of the diameter of the cylindrical combined structure as shown in Figure 5. It can be also noticed that for a fixed value of surface roughness the rate of changes in value of ($C_d$) depends on the value of the diameter.

In addition, the value of the coefficient of discharge increases with the increase of ($H/d$), the rate of change in value is also depends on the diameter, the negative effect of the surface roughness on the performance of the structure by reducing the value of ($C_d$) is also can noted in Figure 6.
The collected experimental data of sixteen models and the geometric parameters are used to calculate the dimensionless parameters in Equation 6. The calculated discharge coefficient \( C_d \) from Equation 4 varies in the range from 0.4616 to 0.7876, with Standard Error 0.0067091 and Standard Deviation 0.0867006, as shown in Table 2 with other parameters which they have highest significant correlation with the independent variable \( C_d \). The highest negative Pearson Correlation Factor 0.913 between the \( (C_d) \) and \( (Ks/a) \) at the 0.01 level (2-tailed).

**Table 2.** Descriptive analysis of the calculated coefficient of discharge

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Range</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Error</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>cd</td>
<td>167</td>
<td>.3260</td>
<td>.4616</td>
<td>.7876</td>
<td>.60877</td>
<td>.0067091</td>
<td>.0008</td>
</tr>
<tr>
<td>Ks/a</td>
<td>167</td>
<td>.9837</td>
<td>.0001</td>
<td>.9830</td>
<td>.22048</td>
<td>.0111425</td>
<td>.021</td>
</tr>
<tr>
<td>Ks/B</td>
<td>167</td>
<td>.0825</td>
<td>.0000</td>
<td>.0825</td>
<td>.03446</td>
<td>.0017984</td>
<td>.001</td>
</tr>
<tr>
<td>Ks/H</td>
<td>167</td>
<td>.0658</td>
<td>.0000</td>
<td>.0658</td>
<td>.02243</td>
<td>.0013393</td>
<td>.000</td>
</tr>
<tr>
<td>Ks/d</td>
<td>167</td>
<td>.1187</td>
<td>.0000</td>
<td>.1187</td>
<td>.04090</td>
<td>.0026003</td>
<td>.001</td>
</tr>
<tr>
<td>Valid N (listwise)</td>
<td>167</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The facilities built in the features of SPSS 17 (statistical software package) are employed to get statistical analysis and mathematical relations between the variables. The regression has been used to find correlation models between the dependent \( C_d \) variable and the independent parameters in Equation 6. The models studied include 18 mathematical relations of three type’s models, linear, square and power. The best and simplest forms of equations were the linear models; they have the highest coefficient of determination reaches to 0.940. Nonlinear Regression shows that the square and power models haven’t very high value of \( R^2 \) as the linear models, the highest power equation has \( R^2 \) is equal to 0.731. To show some of the models, 9 models have been chosen Table 3

**Table 3.** The regression models analysis

<table>
<thead>
<tr>
<th>No.</th>
<th>Equation</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[ C_d = 0.134 - 1.075 \frac{Ks}{a} - 0.269 \frac{h}{a} + 2.144 \frac{h}{B} + 0.407 \frac{H}{d} - 1.210 \frac{h}{H} + 0.031 \frac{d}{h} - 2.676 \frac{Ks}{d} + 5.172 \frac{Ks}{H} ]</td>
<td>0.940</td>
</tr>
<tr>
<td>2</td>
<td>[ C_d = 0.228 - 0.957 \frac{Ks}{a} - 0.983 \frac{h}{H} + 0.414 \frac{H}{d} - 2.407 \frac{Ks}{H} + 0.026 \frac{d}{h} + 0.094 \frac{h}{B} + 4.079 \frac{Ks}{B} - 0.263 \frac{Ks}{h} ]</td>
<td>0.935</td>
</tr>
<tr>
<td>3</td>
<td>[ C_d = 0.216 - 0.297 \frac{Ks}{a} - 1.072 \frac{h}{H} + 0.428 \frac{H}{d} - 2.522 \frac{Ks}{H} + 0.021 \frac{d}{h} + 0.150 \frac{h}{B} ]</td>
<td>0.928</td>
</tr>
<tr>
<td>4</td>
<td>[ C_d = 0.396 - 0.149 \frac{Ks}{a} - 1.069 \frac{h}{H} + 0.385 \frac{H}{d} - 3.661 \frac{Ks}{H} ]</td>
<td>0.912</td>
</tr>
<tr>
<td>5</td>
<td>[ C_d = 0.074 \left( \frac{Ks}{a} \right)^{0.044} \left( \frac{Ks}{B} \right)^{-0.06} \left( \frac{h}{B} \right)^{-0.039} \left( \frac{H}{a} \right)^{0.40} \left( \frac{Ks}{H} \right)^{-0.349} ]</td>
<td>0.731</td>
</tr>
<tr>
<td>6</td>
<td>[ C_d = 0.076 \left( \frac{Ks}{a} \right)^{0.115} \left( \frac{Ks}{B} \right)^{-0.109} \left( \frac{Ks}{d} \right)^{0.162} \left( \frac{H}{a} \right)^{0.444} ]</td>
<td>0.710</td>
</tr>
<tr>
<td>7</td>
<td>[ C_d = 0.520 \left( \frac{Ks}{a} \right)^{-0.042} \left( \frac{Ks}{d} \right)^{0.087} \left( \frac{Ks}{H} \right)^{-0.104} \left( \frac{H}{a} \right)^{0.153} \left( \frac{d}{a} \right)^{-0.040} ]</td>
<td>0.686</td>
</tr>
<tr>
<td>8</td>
<td>[ C_d = 0.524 \left( \frac{Ks}{a} \right)^{0.24} \left( \frac{H}{a} \right) \left( \frac{Ks}{d} \right) \left( \frac{d}{a} \right)^{-0.029} ]</td>
<td>0.671</td>
</tr>
<tr>
<td>9</td>
<td>[ C_d = -3.560 \left( \frac{Ks}{a} \right)^{2} + 123.06 \left( \frac{Ks}{B} \right)^{2} - 0.03 \left( \frac{d}{a} \right)^{2} + 0.229 \left( \frac{h}{a} \right) - 61.693 \left( \frac{Ks}{H} \right)^{2} - 1.346 \left( \frac{h}{H} \right)^{2} + 0.05 \left( \frac{d}{H} \right)^{2} + 0.001 \left( \frac{d}{a} \right)^{2} ]</td>
<td>0.418</td>
</tr>
</tbody>
</table>
The first four equations in the table have $R^2$ more than 0.9 and have the simplest form. These equations have been found by stepwise linear regression of the dependent variable $(C_d)$ with all twelve independent dimensionless variables in Equation 5. The first linear equation in the table can be suggested to calculate the discharge coefficient for rough combined hydraulic structures, which based on stepwise method of including independent variables in model to determine statistical significance predictors of the higher correlation in the matrix at confidence level of 95%. After including seven independent variables the Adjusted $R$ square is equal to 0.937 and standard Error of estimate equal to 0.0364, this relation is presented in Equation 7.

$$Cd = 0.134 - 1.075 \frac{K_s}{a} - 0.269 \frac{h}{a} + 2.144 \frac{h}{B} + 0.499 \frac{H}{d} - 1.210 \frac{h}{H} + 0.031 \frac{d}{H} - 2.676 \frac{K_s}{d} + 5.173 \frac{K_s}{H} \quad (7)$$

The statistical analysis output details for the proposed Equation 7 are shown in Table (4 to 6).

Table 4. Stepwise regression analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>$R$</th>
<th>$R^2$</th>
<th>Adjusted $R^2$</th>
<th>Std. Error of Estimate</th>
<th>Change Statistics</th>
<th>Durbin-Watson</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>.970</td>
<td>.940</td>
<td>.937</td>
<td>.023</td>
<td>.002</td>
<td>6.098</td>
</tr>
</tbody>
</table>

j. Predictors: (Constant), $k_s/a$, $h/a$, $h/B$, $H/d$, $h/H$, $d/H$, $k_s/d$, $k_s/H$

k. Dependent Variable: $cd$

Table 5. Stepwise regression analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Squares</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.417</td>
<td>8</td>
<td>.177</td>
<td>311.835</td>
<td>.000</td>
</tr>
<tr>
<td>Residual</td>
<td>.090</td>
<td>158</td>
<td>.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.507</td>
<td>166</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

j. Predictors: (Constant), $k_s/a$, $h/a$, $h/B$, $H/d$, $h/H$, $d/H$, $k_s/d$, $k_s/H$

k. Dependent Variable: $cd$

Table 6. Stepwise regression coefficients

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>95.0% Confidence Interval for B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
</tr>
<tr>
<td>10</td>
<td>(Constant)</td>
<td>.134</td>
<td>.057</td>
</tr>
<tr>
<td></td>
<td>$k_s/a$</td>
<td>-1.075</td>
<td>.159</td>
</tr>
<tr>
<td></td>
<td>$h/a$</td>
<td>-.269</td>
<td>.047</td>
</tr>
<tr>
<td></td>
<td>$h/B$</td>
<td>2.144</td>
<td>.358</td>
</tr>
<tr>
<td></td>
<td>$H/d$</td>
<td>.499</td>
<td>.032</td>
</tr>
<tr>
<td></td>
<td>$h/H$</td>
<td>-.1210</td>
<td>.086</td>
</tr>
<tr>
<td></td>
<td>$d/H$</td>
<td>.031</td>
<td>.005</td>
</tr>
<tr>
<td></td>
<td>$k_s/d$</td>
<td>-2.676</td>
<td>.793</td>
</tr>
<tr>
<td></td>
<td>$k_s/H$</td>
<td>5.173</td>
<td>2.095</td>
</tr>
</tbody>
</table>

a. Dependent Variable: $cd$

The suggested coefficient of discharge in the formulations above based on the assumption that the same discharge coefficient for the weir and the gate, but the flow in this combined structure is composed of two parts, each of them performs on its phenomena of flow. Within the limitation of this experimental work, the contribution percent of weir and gate in the total discharge can be found by linear regression analysis. The model of the relation is presented in Equation 8.
\[ Q_{act} = F \left( C_g \cdot Q_{th. \ gate} + C_w \cdot Q_{Th. \ weir} \right) \]  \hspace{1cm} (8)

Where: \( C_g \) = percent of theoretical flow under gate, \( C_w \) = percent of theoretical flow over weir and 
\( F \) = interaction factor which is a function of structure geometry. The model summary and ANOVA of 
regression analysis for the experimental measurements are shown in Table 7, while the coefficients in 
Table 8.

**Table 7.** The regression models analysis for Equation 8

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.976a</td>
<td>.953</td>
<td>.953</td>
<td>.22416</td>
</tr>
<tr>
<td>2</td>
<td>.989c</td>
<td>.978</td>
<td>.977</td>
<td>.15492</td>
</tr>
</tbody>
</table>

a. Predictors: H/d  
b. For regression through the origin (the no-intercept model), R  
c. Predictors: H/d, ks/H  
d. Dependent Variable: F  
e. Linear Regression through the Origin

**ANOVA**

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Regression</td>
<td>169.616</td>
<td>169.616</td>
<td>3375.575</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>8.341</td>
<td>86.999</td>
<td>3624.884</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>177.957^2</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Regression</td>
<td>173.997</td>
<td>2</td>
<td>86.999</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>3.960</td>
<td>165</td>
<td>.024</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>177.957^2</td>
<td>167</td>
<td></td>
</tr>
</tbody>
</table>

a. Dependent Variable: F  
b. Linear Regression through the Origin  
c. Predictors: H/d  
d. This total sum of squares is not corrected for the constant because the constant is zero  
e. Predictors: H/d, ks/H
Table 8. Coefficients for models in Equation 8

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>95.0% Confidence Interval for B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
</tr>
<tr>
<td>1</td>
<td>H/d</td>
<td>0.608</td>
<td>0.010</td>
</tr>
<tr>
<td>2</td>
<td>H/d</td>
<td>0.754</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>ks/H</td>
<td>-10.004</td>
<td>0.740</td>
</tr>
</tbody>
</table>

a. Dependent Variable: F
b. Linear Regression through the Origin

The above analysis shows that contribution percent of the theoretical flow is different. The relation which give an idea on the contribution percent is presented in Equation 9 and 10. It can be also notice that contribution of the gate increase with the increase of roughness as shown in Figure 7.

\[ Q_{act} = F(0.677 Q_{th. gate} + 0.498 Q_{th. weir}) \]  \( (9) \)

\[ F = 0.754 \frac{H}{d} - 10.004 \frac{Ks}{H} \]  \( (10) \)

Figure 7. Relation between weir and gate discharge for different roughness’s
5. CONCLUSION

The effect of surface roughness on the performance of flow over rounded weir and under gate in combined structure was studied experimentally. From the statistical analysis of experimental data the following conclusions may be fixed with in the experimental limitations:

1. The performance of the combined structure affected negatively with increases of surface roughness, $C_d$ decreases with increase of $K_s/H$

2. The value of $C_d$ increases with the increase of $H/d$ for constant ($K_s$) and decreases with the increase of $h/H$

3. The value of $C_d$ range from 0.4616 to 0.7876.

4. Within the limitations of the present experimental work a discharge prediction Equation 7 is developed with mean percent error of 0.036%.

5. The contribution of the gate increases with increase of roughness, percent contribution is 49.8% for the weir while for the gate 67.7% from its theoretical discharges.

6. REFERENCES


الخلاصة:

الهدف من هذا البحث هو دراسة تأثير خشونة السطح على أداء المنتشات المتكونة من الهدار والبوبون. إجريت دراسة تجريبية في قناة المختبرية على جراني فوق هدار دائر، وتحتها فتحة ببوية دائرة الشكل في منشأ مشترك يعمل كجهاز لقياس التصريف. تم اختيار أربعة نماذج مختلفة أقطار في قناة مختبرية، لكل نموذج تم تحصي السطحة أربع مرات. بين النتائج: الاختبار تأثير سلبي منطقي لزيادة خشونة السطح على الأداء. أثبت أن أداء المنتش يتحسن بإخفاق قيمة النسبة (Ks/H) مع زيادة قيمة النسبة (R2=0.936) كم، مما يشير إلى تأثير تزايد مساهمة بوابة في التصريف. تم اقتراح تقدير تأثير في قيمة معامل التصريف (H/d) لتحديد قيمة معامل التصريف ذات معامل التحديدي (R2=0.936).
دليول

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