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Jordan Journal of Biological Sciences

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Effects of Ramadan Fasting on Some Haematological and Biochemical Parameters

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Abstract

Ramadan is the month during which Muslims refrain from food, liquids and smoking during daylight hours and eat a large meal after sundown. This custom provides a unique opportunity to study the biochemical changes over Ramadan time. The study was performed on 57 healthy females and was carried out in the month of Ramadan (October- November 2004). Blood samples were collected four times: one week before the beginning of Ramadan, at the end of the first week, at the end of the second week, and at the end the last week of Ramadan. Haematological indices including haemoglobin, hematocrit, red blood cell count, and platelets count were determined twice (one week before Ramadan and mid of Ramadan) on whole blood samples. Serum was evaluated for creatinine, urea, albumin, uric acid, and lipids (triglycerides), total cholesterol, high density lipoprotein (HDL-C) and low density lipoprotein- cholesterol (LDL-C) was calculated.

Haematologically, platelets count was significantly decreased (p = 0.002) during Ramadan while other parameters remained relatively stable. Biochemical analysis showed a significant reduction in serum triacylglycerols (TAGs) after the mid of Ramadan (p = 0.007). A slight but not significant increase (p=0.073) in HDL–C was observed. The changes in the other parameters were not significant. In Jordanian healthy females, Ramadan fasting resulted in a statistical effect on platelets count and serum triglycerides.

الملخص

أجريت هذه الدراسة على 57 من الإناث اللواتي لا بعانين من مشاكل صحية خلال شهر رمضان 2004 وقد تم جمع عينات الدم من المشاركات على أربع فترات الأولى فبل بداية شهر رمضان والثانية في الأسبوع الأول من الشهر و والثيالثة في منتصف الشهر والرابعة في نهايته. وقد تم تقدير الهيموجلوبين والهيماتو كريت وعدد كريات الدم الحمراء والصفائح الدموية قبل بداية شهر رمضان وفي منتصفة بالاضافة إلى تقدير كمية الكرياتينين والبوريا والالبيومين وحمض البوليك والجليسريدات الثلاثية والكولسترول الكلي والكولسترول عالي الكثافة ومنخفض الكثافة. وإشارات النتائج إلى انخفاض ملحوظ في عدد الصفائح الدموية خلال شهر رمضان وانخفاض في والجليسريدات الثلاثية بعد منتصف الشهر مع عدم حدوث تغيرات معنوية على المؤشرات الأخرى.

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Keywords: Ramadan, fasting, platelets, lipids.

1. Introduction

Ramadan is the holiest month in the Islamic calendar (The Holy Quran). Fasting in this month is one of the five pillars of Islam. Fasting is obligatory for all adults and healthy Muslims during the day hours for the whole month every year. Ramadan month occurs 11 days earlier every year due to the difference between the solar and lunar years, and may occur in any of the four seasons, making the length of fasting hours variable from 11-18 hours in tropical countries (Sakr, 1975). Ramadan is the month during which Muslims refrain from food, liquids and tobacco smoking during daylight hours and eat a main meal after sundown. Free eating is allowed from sunset to

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dawn. Ramadan teach Muslims self –restraint and remind them of the feelings of the impoverished. Ramadan is observed by over 400 million of Muslims who spread across the globe; and live under various geographical, climatic, social, cultural and economic conditions. This provides a unique opportunity to study the haematological and biochemical changes over Ramadan time.

Effect of Ramadan on biochemical parameters is still a matter of debate. Energy intake decreases during Ramadan (Sweileh *et al.* 1992). Several studies have reported on the effect of Ramadan fasting on the values of certain haematological factors (El-Hazmi *et al.* 1987; Azizi and Rasouli 1986; Al Tufail *et al.* 1992; Sarraf *et al.* 2000; Ramadan, 2002). Changes in serum urea and creatinine were small (El-Hazmi *et al.* 1987; Sliman and Khatib 1988). Serum uric acid showed a slight increase (El-Hazmi *et al.* 1987; Ramadan *et al.* 1994). Concerning serum protein levels, an increase was demonstrated for total proteins (El-Hazmi *et al.* 1987; Ramadan *et al.* 1994; Aybak *et al.* 1996) and albumin ((El-Hazmi *et al.* 1987)

Ramadan Islamic fasting is an excellent model of how dietary modifications may affect serum total cholesterol (TC), triacylglycerols (TAGs), low density lipoprotein – cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). Effect of Ramadan fasting on serum lipid profile is not so clear; some studies record improvements in serum profile, while others show deterioration within healthy subjects (Hussain *et al.* 1987; Maislos *et al.* 1998; Adlouni *et al.* 1998; Bilto, 1998; Rahman *et al.* 2004; Ziaee *et al.* 2006; Asgary *et al.* 2000). The aim of the present study is to evaluate the effect of Ramadan fasting on some haematological and biochemical parameters on healthy young females.

2. Material and Method

This study was performed during Ramadan of October – November 2004 (Hijri year 1425). The subjects were students of The Hashemite University in Jordan. All subjects were interviewed; a questionnaire was used to collect data regarding age, marital status, and medical history. Non-healthy volunteers were excluded. Volunteers gave informed consent for participation in the study.

Venous blood was taken one week before Ramadan (T1), first week of Ramadan (T2), and second week of Ramadan (T3), and last week of Ramadan (T4) after an average fast of eight hours. Anthropometric measurements were performed at the same time of blood sampling. Blood was collected in plain and EDTA tubes. Serum was obtained by low speed centrifugation at 1000g for 15 minutes, and samples were immediately separated into aliquot and stored at -20C until analysis. All serum samples were analyzed in a single batch to avoid day-today laboratory variation. Haematological and biochemical measurements took place in the Research Laboratory for the department of medical laboratories at The Hashemite University. Fresh EDTA blood was used to determine haematological parameters using Cell - Tac a (Nihon-Kohden, Japan)

Serum total cholesterol (TC) and high density lipoprotein- cholesterol (HDL-C) were measured by an enzymatic colorimetric method using cholesterol oxidase, perioxidase, and the chromogen 4-aminophenazone/phenol Allain et al. 1974. Serum triacylglycerols (TAGs) levels were determined by an enzymatic colorimetric method using lipoprotein lipase glycerokinase, glycerphosphate oxidase, and the chromogen 4-aminophenazone/N-ethyl-N (3-sulphopropyl)-nramisidine (Fossati, 1982). Low-density lipoprotein – cholesterol (LDL–C) was calculated using Friedwald *et al.* equation (Friedwald *et al.* 1972).

Urea, Serum albumin and uric acid were quantitatively estimated in serum by enzymatic colorimetric test. Creatinine was determined using JAFFE method by commercially provided kits provided by Biocon diagnostic (Germany)

All data were expressed as mean \pm standard deviation (SD). Paired t-test was used to compare pre and during Ramadan fasting variables. ANOVA was used to analyze repeated measures. Differences were considered significant when *p* values were less than 0.05. All analysis was performed using the statistical package (SPSS) version 10.0 (Chicago, IL, USA).

3. Results

Fifty-seven healthy volunteer females were included in this study. The mean age of the subjects was 21.6 years (ranging from 18 to 29, SD 4.14). No significant changes were observed in haemoglobin, hematocrit, and red blood cell count values before and during Ramadan fasting. Platelets was decreased significantly during Ramadan compared to before Ramadan (P= 0.002) as shown in Table 1.

Table 1. Hematological indices of the subjects.

| Haematological indices | Before Ramadan | During Ramadan | P value |
|-------------------------------------|-------------------|-------------------|---------|
| Red blood cell count (x 106/mm3) | 4.30 ± 0.69 | 4.43 ± 0.37 | 0.16 |
| Hemoglobin gm/dl | 12.3 ± 2.0 | 12.3 ± 1.3 | 0.935 |
| Hematocrit % | 35.8 ± 4.1 | 36.0 ± 3.3 | 0.697 |
| Platelets (1000) | 165.1 ± 66.0 | 126.9 ± 80.3 | 0.002 |

Data are mean \pm SD

Table 2 showed no significant changes in serum creatinine, urea, albumin and uric acid values during Ramadan fasting compared to that before Ramadan.

Results of the effect of Ramadan fasting on plasma lipids are shown in Table 3. A significant reduction of serum triglycerides was observed after the mid of Ramadan (p = 0.007). A slight but not significant increase (p=0.073) in HDL – C was observed. No significant changes were observed on total cholesterol and LDL – C.

4. Discussion

During the fasting month of Ramadan, Muslims are obliged to fast during daytime hours and restrict food and drink intake to the period after sunset. Long lasting modifications in the circadian distribution of the eating and sleeping schedule result in various changes in metabolism. This will provide a unique opportunity to study the effect

| | T1 | T2 | Т3 | T4 |
|--------------------|-----------------|-----------------|-----------------|---------------|
| Creatinine (mg/dl) | 0.73 ± 0.21 | 0.78 ± 0.29 | 0.70 ± 0.16 | 0.75 ± 0.29 |
| Urea (mg/dl) | 23.2 ± 7.9 | 23.7 ± 6.2 | 21.4 ± 6.2 | 20.4 ± 5.9 |
| Albumin (mg/dl) | 4.7 ± 0.58 | 4.7 ± 0.47 | 4.7 ± 0.49 | 4.7 ± 0.81 |
| Uric acid (mg/dl) | 5.8 ± 3.8 | 5.5 ± 2.7 | 5.2 ± 3.0 | 5.7 ± 4.2 |

Table 2. Serum creatinine, urea, albumin and uric acid.

Data are mean \pm SD

Table 3. Plasma lipids and lipoprotein levels.

| | T1 | T2 | Т3 | T4 |
|---------------------------|------------------|------------------|---------------------|-------------------|
| Total Cholesterol (mg/dl) | 157.9 ± 33.2 | 154.8 ± 26.8 | 155.4 ± 37.9 | 154.1 ± 28.2 |
| Triacylglycerols (mg/dl) | 88.3 ± 62.5 | 70.7 ± 24.1 | 62.9 ± 24.6^{1} | $65.4^2 \pm 20.8$ |
| HDL Cholesterol (mg/dl) | 59.3 ± 9.5 | 57.8 ± 11.4 | 58.8 ± 13.1 | $62.3^3 \pm 14.6$ |
| LDL Cholesterol (mg/dl) | 81.7 ± 26.4 | 84.5 ± 24.9 | 88.8 ± 33.3 | 83.4 ± 29.9 |

Data are mean \pm SD

 $^{1} p = 0.007, ^{2} p = 0.052, ^{3} p = 0.073$

meal frequency reduction on haematological and biological indices.

Studies reported in literature on the effect of Ramadan fasting on various haematological indices have been conflicting and inconsistent. In this study, red blood cells count, haemoglobin and hematocrit remained unchanged, which was consistent with (Azizi and Rasouli 1986; Sarraf et al. 2000; Azizi, 2002). Although, other studies showed a slight degree of hemoconcentration (El-Hazmi et al. 1987). Conversely, Dewanti et al. (2006) showed a significant decrease in haemoglobin and hematocrit. These controversial results may be due to geographical, climatic, economical, and nutritional variations. This study showed a significant reduction in the platelets count, which was consistent with Ramadan et al., (1994) this may due to deficit or redistribution of specific micronutrients (iron and vitamins) that may account for reduction in platelets count (Ramadan et al. 1999).

Many previous studies have been published on the effect of Ramadan fasting on serum creatinine and urea in healthy individuals and reported small changes that were statistically not significant. The results of this study were consistent with the previous studies (El-Hazmi *et al.* 1987; Sliman and Khatib 1988; Mafauzy *et al.* 1990; Aksunger *et al.* 2005).

Studies on serum uric acid among healthy individuals showed normal to temporary slight increase that doesn't deviate from normal range ,which is probably due to decrease in glomerular filtration rate and uric acid clearance(Azizi, 2002). The results of this study showed no significant increase in the level of uric acid despite a significant weight loss of the subjects (Al Hourani and Atoum 2007), which could be explained either by body fat loss rather than catabolism of body cell mass or by the nature of Ramadan fasting which is short lasting and intermittent.

To best of our knowledge, none of the previous studies reported an alteration in serum albumin among healthy individuals (Azizi and Rasouli 1986; Maislos *et al.* 1998) No significant changes in serum albumin detected in the results of this study, which was consistent with the previous reports.

Many reports have been published on the effect of Ramadan fasting on blood lipids among healthy individuals, with inconsistent and even conflicting findings. The discrepancy might be attributed to the amount and type of food intake, physical activity, ethnic, and genetic background of studied populations.

In line with the reports of Asgary *et al.* (2000) and Mahboob *et al.* (1999), we found a significant decrease in serum triacylglycerols after mid of Ramadan. The reduction in serum triacylglycerols can be explained either by changes in fat intake or inherent metabolic changes during Ramadan. In previous report (Al Hourani and Atoum, 2007)[•] we found that fat intake during Ramadan was similar to pre Ramadan in healthy young Jordanian females; therefore, our explanation is in favour with the fact that inherent metabolic changes during Ramadan may lower serum triglycerides.

Concerning levels of serum total cholesterol, LDLcholesterol, and HDL- cholesterol, the changes were not statistically significant. However, most previous studies on HDL cholesterol showed a significant increase in plasma HDL cholesterol (Maislos *et al.* 1998; Rahman *et al.* 2004; Maislos *et al.* 1993; Fakhrzadeh *et al.* 2003; Adlouni *et al.* 1997). Plasma concentration of HDL is a protective factor against the development of atherosclerosis and cardiovascular diseases and usually quite stable. Since we have observed a gradual increase in HDL cholesterol during Ramadan which didn't reach a significant level over the period of one month fasting, our results are in accord with the previous reports that showed an elevation of plasma HDL cholesterol levels. Although, mechanism(s) by which fasting increases level of HDL cholesterol are not clear, loss of weight in the studied population may increase HDL-Cholesterol.

In conclusion, Ramadan fasting is a healthy nonpharmacological method for improving lipid profile. In view of the fact that many factors influence the effect of Ramadan fasting on haematological and biochemical parameters; we recommend a large – scale coordinated studies, with standardization of research methods regarding the season, gender, food habits, and ethnic background of the subjects, to explore the issue more comprehensively.

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Formulation and Fuzzy Modeling of Viscosity of Orange Beverages Fortified with Carboxymethylcellulose-Whey Protein Isolate Emulsions

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Abstract

The aim of this study was to employ whey protein isolate (WPI) and carboxymethylcelulose (CMC) as stabilizers in beverage production and to evaluate viscosity of the products. Three beverages were formulated using 6% WPI combined with three different concentrations of CMC (0.1, 0.5, and 1%). The combination of 6% WPI/1.0% CMC was selected and added to three different ratios of pure orange juice (50:50 "T1", 40: 60 "T2", and 15: 85 "T3). The apparent viscosity decreased as both shear rate and temperature increased. Additionally, the apparent viscosity for the same treatment at certain shear rate/same temperature increased after storage. In addition, an adaptive neurofuzzy inference system (ANFIS) was used to model and identify the viscosity of the resulted beverages. Experimental validation runs were conducted to compare the measured values and the predicted ones. ANFIS models achieved an average prediction error of viscosity of only 9%. It is believed that this approach can be applied to predict many other parameters and properties in beverage industry.

هدفت الدراسة لاستخدام بروتين مصل الحليب (المنتج الثانوي لصناعة الاجبان) (WPI) والكاربوكسي ميثل سليليوز (CMC) كمادة مثبتة لانتاج العصيروكذلك هدفت الى دراسة لزوجة المنتج. تم تحضير ثلاث عصائر باستخدام 6% (WPI) مع ثلاث تراكيز مختلفة (CMC) (0.0% ،5.0% و 1%). تم اختيار التركيبة 6% WPI -1% CMC) وتم اضافتها الى ثلاثة نسب من عصير البرتقال النقي بنسب (25.5% TT"، 06.0%" مو 185.15 "TT") . لوحظ ازدياد درجة اللزوجة الظاهرية بازدياد قوة الشد ودرجة الحرارة . كما لوحظ ازدياد درجة اللزوجة الظاهرية عند نفس قوة الشد ودرجة الحرارة . كما لوحظ ازدياد درجة اللزوجة الظاهرية عند نفس قوة الشد ودرجة الحرارة خلال التخزين . اضافة الى ذلك تم استخدام نظام ANFIS التنبؤ بلزوجة العصائر المنتجة. تم تطوير نموذج تحققي (FUZZY) لمقارنة القيم الحقيقية والقيم المتنبة به . كانت نسبة خطأ نظام ANFIS في تنبؤ اللزوجة فقط 9% . لذلك نستنتج انه يمكن تطبيق هذا النموذج التنبؤ عن معايير انتاجية مختلفة في صناعة العصائر .

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Keywords: Beverage Formulations, Whey Protein Isolate (WPI), Carboxymethylcellulose (CMC), Apparent Viscosity, Fuzzy Modeling.

1. Introduction

The dairy industry produces large amounts of whey as a by-product of the cheese industry. Most of whey protein is lost in the drainage without any benefit (Morr and Foegeding, 1990). The environmental pollution and high costs of disposing whey encouraged scientists to explore methods to utilize it in the food industry. Also, the high nutritional value (Damodoran, 1996) and low cost of whey make it an excellent food component (Dickinson, 1997). Several types of proteins are used as emulsifiers in foods since they have a high proportion of non-polar groups and surface active properties (Damodoran, 1996). Whey protein is one of the most important proteins that have the capability to stabilize emulsions (Dickinson, 1997; McClements, 1999). Whey proteins are the soluble proteins in the milk after the precipitations of the caseins at pH 4.6 and 20 °C (Dalgleish, 1996). Whey proteins form about 20% of total milk proteins and are composed mainly of β -lactoglobulin (~66%) and α -lactalbumin (~13%) (Kinsella,1984). The ability of whey proteins to form stable emulsions depends on several factors including pH, and temperature (Kinsella, 1984; Dalgleish, 1996; Singh and Ye, 2000). Because of their ability to adsorb at the oil-

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water interface and their good solubility, whey proteins are considered good stabilizers (Girard et al., 2002). A study investigated the effect of using other types of protein in beverage production such as using soy protein. It was reported that the addition of soy protein imparted significant beverage stability with a good nutritional value. Nonetheless, this system experienced a slight degree of bitterness (Beverage Marketing Corporation of New York, 2005).

Gums are polysaccharides classified according to their origin (Igoe, 1982). The hydrocolloids (gums) have the ability to control both the rheology and texture throughout the stabilization of emulsions, suspensions, foams and starch gelatinization (Rosell et al., 2001). an anionic Carboxymethylcellulose (CMC) is polysaccharide that comes from cellulose. It has a pKa value of about 4.0 and can solubilize whey at an acidic condition by acidification through the formation of insoluble complex (Hidlago and Hansen, 1969; Igoe, 1982). This polysaccharide is widely used in the beverage industry because it is inexpensive; and has the ability to form complexes with whey proteins because of its anionic properties. Interaction between CMC and whey proteins located at the droplet surface of emulsions influences the creaming behavior of the emulsions and stabilizes the emulsion through adsorption of the secondary layer of the CMC (Dickinson, 1998). Some studies investigated the emulsifying properties of WPI/CMC complex (Girard et al., 2002) in a pure system but not in formulating a new beverage product.

Fuzzy inference systems are attractive because they are able to deal with complicated problems without a need for accurate mathematical models (Zadeh, 1965; Zadeh, 1973; Kasabov, 1998). The predictions of properties of beverage viscosity could be considered as a complex system, which may result in significant deviations between simulation results and experimental data if conventional modeling techniques are used. Therefore, fuzzy logic was adopted because it is highly-suited for such complex problems. (Jang and Sun, 1995; Kosko, 1992; Yamaguchi et al, 1991).

Researchers in the food engineering field have recently used several modeling techniques. Tsourveloudis and Kiralakis (2002) described an application of a rotary drying process to olive stones, and modeled the process by using fuzzy and neuro-fuzzy techniques based on available expertise and knowledge for a given, industrial size, rotary dryer. In this study, they used an adaptive neuro-fuzzy inference system based on data taken from an empirical model of the dryer under study. A hybrid approach based on fuzzy logic and genetic algorithms to control a crossflow microfiltration pilot plant was introduced by (Perrot et al., 2003). Simulations and pilot tests showed that it becomes possible to impose dynamics to the process that leads to maintain the state variable at a given reference. Abu Ghoush, Samhouri, Al Holy and Herald (2008) applied neuro-fuzzy modeling technique in predicting the emulsion stability and viscosity of a gumprotein emulsifier in a model mayonnaise system with a prediction accuracy of 96%. In addition, an adaptive neuro-fuzzy inference system (ANFIS) was used by (Samhouri, Abu Ghoush and Herald, 2007) to model color

mayonnaise system. They achieved satisfactory prediction accuracy of 98%.

The main motivation behind this work was to utilize the functional and dietary benefits of whey in making nutritional and good quality beverage. Both, processors and consumers have demanded the use of disposable whey in such products (Damianou et al., 2006). Therefore, the aims of this research were to take the advantage of the WPI/CMC interaction, formulate a beverage with certain properties, evaluate the beverage viscosity and construct a prediction model for the beverage viscosity using fuzzy modeling that can be used as a tool by the food processors to produce a high quality beverage product.

2. Materials and Methods

2.1. Proteins, polysaccharides

Whey protein isolate (WPI) was obtained from Aria food ingredients (Amba-Denmark, LACPRODAN[®] DI-9224, Denamark) and carboxymethylcellulose (CMC) was obtained from TIC-Gums (Belcamp, MD, USA).

2.2. Stages of the Study

Experimental design of this research was divided into two main stages:

2.2.1. Formulation and Evaluation of Emulsions

• Preparation of Emulsions

Three solution combinations were formulated with 6%WPI (preliminary study showed that it exhibited the highest solubility performance) combined with three different concentrations of CMC (0.1, 0.5, and 1%), replicated three times. A total of 9 batches of solutions were produced in random order. Each batch was approximately 1L. Solution combinations were immediately bottled, capped, and placed into refrigerated storage after production. These combinations which were rehydrated in deionized water for 1 h were stored at 4 ° C for 24 h. All solutions were adjusted to pH 4.8. The whey protein isolate/CMC complexes were prepared according to the process patented by Chen et al. (1989).

• Emulsions Evaluations

Emulsion activity index (EAI), and emulsion Stability index (ESI) for all the above combinations were determined by using a turbidimeteric method developed by (Pearce and Kinsella, 1978). The highest EAI and ESI from these combinations were selected for further beverage formulations. This test was used to evaluate the stability and the degree of interaction between the CMC and whey protein isolate at selected concentrations.

2.2.2. Formulation and Evaluation of Beverage

• Beverage Formulations

Beverage formulations (Table 1) were developed using laboratory-scale trials. Production of the beverage was performed according to the processing procedure developed during laboratory-scale trials (Figure 1). Three beverage treatments were formulated with 6%WPI, and 1.0% CMC. This combination exhibited the highest EAI and ESI. WPI-CMC was added to three different ratios of pure orange Juice ("T1" 50:50, "T2", 40: 60, and "T3" 15: 85). A combination of water (without WPI or CMC) and

| TABLE 1. FORMULATION OF |
|--|
| CARBOXYMETHYLCELLULOSE-WHEY PROTEIN ISOATE |
| (WPI) / (CMC) BEVERAGE |

| Ingredients | | Concentrations (%) | | |
|---------------------------------------|----------------|-----------------------|----------------|--------------------|
| | Type 1 (T1) | Type 2 (T2) | Type 3 (T3) | Control (O) |
| Orange Juice | 42 | 52 | 77 | 52 |
| 1% CMC and 6% WPI | 50 | 40 | 15 | 40 (Water only) |
| Sucrose | 5 | 5 | 5 | 5 |
| High Fructose Corn Syrup (HFCS) | 2.5 | 2.5 | 2.5 | 2.5 |
| Natural Orange Essence | 0.01 | 0.01 | 0.01 | 0.01 |
| Natural Orange Color | 0.001 | 0.001 | 0.001 | 0.001 |
| Orange oil | 0.5 | 0.5 | 0.5 | 0.5 |



Figure 1. The production of carboxymethylcellulose (CMC)/ whey Protein Isolate (WPI) beverage process. HFCS: High fructose corn syrup.

orange juice in the ratio 40:60 served as a control. The experiments were replicated three times. A total of 12 batches of beverages were produced in random order, each batch was approximately 1L in size. Beverages were immediately bottled, capped, and placed into refrigerated storage at 4 °C after production. The beverage with 15: 85 was excluded from the study since a preliminary sensory evaluation indicated that the contents of this product precipitated during storage.

Prehydration of WPI and CMC in a small portion of water, with good agitation combined with heat treatment at 80 °C for 30 s resulted in complete mixing without lumping in a relatively short period of time. This temperature was used to minimize denaturation of the heat-sensitive whey proteins. Themlij et al. (2004) reported that the whey protein denaturation was delayed until a temperature of 87 °C.

• Chemical and microbiological properties:

CMC/WPI beverages were stored immediately after production for 8 weeks at 5 °C. Product pH was measured using a pH meter (model 744, CH-9101 Herisau, Switzerland). Triplicate measurements were taken for all samples. Total aerobic plate count was determined according to standard pour plate method (Speck, 1979) on plate count agar (Standard Plate Count Agar, Conda Laboratories, S.A., Spain) incubated at 37 °C for 48 h. Coliform count was determined using Violet Red Bile Agar and Eosin Methylene blue agar (Conda Laboratories, S.A., Spain) incubated at 37 °C for 24 h, respectively.

• Rheological Properties:

Rheological measurements of the developed beverage samples were determined using a Brookfield viscometer (Model LVDV-E, Brookfield Laboratories, Middlebooro, MA, USA). The equipment operates at a rotor speed range 0.3-200 rpm. Shear rate equation was obtained from manufacturer's manual as follows:

$$\nu = 0.22N \tag{1}$$

Where γ : is shear rate in 1/s.

N: is viscometer spindle rotation speed (rpm).

The viscometer can be used to construct rheograms by providing apparent viscosity and shear stress data. A thermostatically controlled water bath was used to maintain the temperature of the beverage constant. Rheological measurements were conducted at 8 °C and at 24 °C (based on consumption preferences – cool (8 °C) and room temperature (24 °C)) at the following rpm values: 100, 60, 50, 30, 20, 12, 10, and 6. The measurements were taken immediately after beverage preparation and repeated after 8 weeks of storage. Prior to measurements, samples were heated in a water bath to reach steady state temperatures of 8 °C and 24 °C to resemble the temperatures based on consumption preferences. All measurements were obtained in triplicate.

2.3. Statistical Analysis

A two-way factorial classification in complete randomized design (CRD) was used. Data were analyzed using statistical analysis software (version 8.2, SAS Institute Inc., Cary, NC). Three batches of beverage were produced for each treatment. Analysis of variance (ANOVA) and means separations were calculated by the general linear model procedure (Proc GLM). Comparisons among treatments were analyzed using Fisher Least Significant Difference (LSD). Treatment means were considered significant at P < 0.05.

2.4. Fuzzy Modeling of Output properties

Neuro-fuzzy is an associative memory system that consists of fuzzy nodes instead of simple input and ouput nodes. Neuro-fuzzy uses neural network learning functions to refine each part of the fuzzy knowledge separately. Learning in a separated network is faster than learning in a whole network. One approach to the derivation of a fuzzy rule base is to use the self learning features of artificial neural networks, to define the membership function based on input-output data. A fuzzy inference system (consisting of rules, fuzzy set membership functions, and the defuzzification strategy) are mapped onto a neural network-like architecture.

Adaptive neuro-fuzzy inference system (ANFIS) is a fuzzy inference system implemented in the framework of an adaptive neural network. By using a hybrid learning procedure, ANFIS can construct an input-output mapping based on both human-knowledge as fuzzy. ANFIS architecture is shown in Figure 2.



Figure 2. General Adaptive Neuro-Fuzzy Interface System (ANFIS) Architecture.

where x and y are the inputs, f is the output, A_i and A_n^2 are the input membership functions, w_i and w_n^2 are the rules firing strengths. Five network layers are used by ANFIS to perform the following fuzzy inference steps: (i) input fuzzification, (ii) fuzzy set database construction, (iii) fuzzy rule base construction (iv) decision making, and (v) output defuzzification. This is a multi-layered neural network architecture where the first layer represents the antecedent fuzzy sets, while the consequent fuzzy sets are represented by the middle layers, and the defuzzification strategy by the output layer. The nodes which have 'square' shape are those containing adaptable parameters, whereas the 'circular' nodes are those with fixed parameters.

ANFIS is more powerful than both the simple fuzzy logic algorithm and neural networks, since it provides a method for fuzzy modeling to learn information about the data set in order to compute the membership function parameters that best allow the associated fuzzy inference system to track the given input/output data (Jang, 1993). ANFIS also employs sugeno-type fuzzy inference system, which is a natural and efficient modeling tool; and is suited for modeling non-linear system by interpolating between multiple linear models. In addition, ANFIS is more powerful than neural network system since it is better than all of them in convergence rates (running time), average training error, root mean square error, and the coefficient of correlation; and it has a built-in ability to validate the modeled system. On the other hand, ANFIS is much more complex than the fuzzy inference systems, and is not available for all of the fuzzy inference system options. It only has a single output, and no rule sharing. In addition, ANFIS cannot accept all the customization options that basic fuzzy inference allows. That is, no possibility to

make our own membership functions and defuzzification functions; the ones provided by ANFIS must be used.

2.4.1. ANFIS Modeling of Viscosity

An adaptive neuro-fuzzy inference system (ANFIS) is an architecture which is functionally equivalent to a Sugeno-type fuzzy rule base. ANFIS is a method for tuning an existing rule base with a learning algorithm based on a collection of training data. This allows the rule base to adapt. Training data is used to teach the neurofuzzy system by adapting its parameters (which in essence are fuzzy set membership function parameters) and using a standard neural network algorithm which utilizes a gradient search, such that the mean square output error is minimized.

The architecture of ANFIS, illustrated in Figure 2, has five layers to accomplish the tuning process of the fuzzy modeling system. The five layers are:

- Layer 1: Every node in this layer is an adaptive node with a node function (i.e., membership function). Parameters of membership functions are referred to as premise or antecedent parameters.
- Layer 2: Every node in this layer is a fixed node, which multiplies the incoming signals and sends the product out. Each node represents the firing strength of a fuzzy rule.
- 3. Layer 3: Every node in this layer is a fixed node which calculates the ratio of the one firing strength to the sum of all rules' firing strengths. The outputs of this layer are called normalized firing strengths.
- 4. Layer 4: Every node in this layer is an adaptive node with a node function (i.e., linear combination of input

variables). Parameters in this layer are referred to as consequent parameters.

5. From the ANFIS architecture Layer 5 : The single node in this layer is a fixed node that computes the overall output as the summation of all incoming signals, shown in Figure 2, it is observed that given the values of premise parameters, the overall output can be expressed as a linear combination of the consequent parameters.

ANFIS applies two techniques in updating parameters. For premise parameters that define membership functions, ANFIS employs gradient descent back-propagation neural networks to fine-tune them. For consequent parameters that define the coefficient of each output equation, ANFIS uses that least squares method to identify them. This approach is called the hybrid learning method. More specifically, in the forward pass of the hybrid learning method, functional signals go forward until layer 4, and the consequent parameters are identified by the least square estimate. In the backward pass, the error rates propagate backward, and the premise parameters are updated by the gradient descent.

ANFIS modeling and prediction of the viscosity output start by obtaining a data set (input-output data points) and dividing it into training and validating data sets. Each input/output pair contains four inputs (i.e., time, shear rate, temperature, and treatment) and one output (i.e., viscosity). The training data set is used to find the initial premise parameters for the membership functions by equally spacing each of the membership functions. A threshold value for error between the actual and desired output is determined. The consequent parameters are computed, using the least squares method. Then, an error for each data pair is found. If this error is larger than the threshold value, the premise parameters are updated, using the back propagation neural networks. This process is terminated when the error becomes less than the threshold value. Then, the testing data points are used to compare the model with actual system for validating purposes.

3. Results and Discussion

3.1. Solution Combinations Evaluation

The EAI of 6%WPI significantly increased with the addition of 0.1, 0.5, and 1 % CMC by 50, 64, and 84 %, respectively (Figure 3). The ESI of 6%WPI significantly increased with the addition of 0.1, 0.5, and 1% CMC by 50, 77, and 97 %, respectively (Figure 4). It is clear that as CMC concentrations increases. This could be due to the formation of a protein-polysaccharide network. The interaction between CMC and whey proteins located at the droplet surface of the emulsions influences the behavior of the solutions and stabilizes the emulsion through adsorption of the secondary layer of the CMC, thus increasing ESI. WPI improves the surface properties of food systems by forming a protective steric barrier around insoluble droplets. At the same time, CMC improves the steric stabilizing properties by forming a thick secondary layer on the outer side of protein. These results are confirmed with the results obtained by others (Dickinson



Figure 3. Emulsion activity index (EAI) of different combinations of carboxymethylcellulose (CMC) and 6% whey protein isolate (WPI). The different letters within each beverage property suggest a significant difference (P < 0.05).



Figure 4. Emulsion stanility index (ESI) of different combinations of carboxymethylcellulose (CMC) and 6% whey protein isolate (WPI). The different letters within each beverage property suggest a significant difference (P < 0.05).

1998; Rosell et al. 2001; Giraard et al. 2002; and Damianou et al. 2006).

3.2. Formulations and Evaluation of Beverages

3.2.1. Chemical and Microbiological Properties

The pH of beverage remained stable for all formulations during the 8-week refrigerated storage period. Beverage pH values were 4.70, and 4.63 for T1, and T2, respectively.

Despite the high water amounts, and nutrients contents, the low pH of the product prevented growth of microorganisms. Beverages aerobic plate count (APC/ml) was 30, and 47 for T1, and T2 treatments, respectively; and remained stable for all formulations during the 8-week refrigerated storage period. This low count is probably spore forming bacteria that do not have the capability to germinate under the acidic conditions of the beverage. The coliform count was undetectable (<10/ml) for T1, and T2 treatments. This is most likely because of relatively high pasteurization temperature (80 °C for 30s) and low pH (~ 4.70) that impede the growth of coliform.

3.3. Rheological Properties

A power law model that is widely used in theoretical analysis of engineering calculations was used to explain the relationship between shear stress and shear rate as follows:

$$\tau = K \gamma^n \tag{2}$$

Where τ : is the shear stress (Pa), K is consistency coefficient, n is the flow behavior index.

For a power law model, the apparent viscosity can be defined as follows:

$$\mu_a = K \gamma^{n-1} \tag{3}$$

Where μ_a : is the apparent viscosity in Pa.s.

A plot of apparent viscosity against shear rate_(a gradient of velocity in a flowing material was used. The SI unit of measurement for shear rate is \sec^{-1}) was obtained for each treatment. The plot was fitted to a power function where K and n were obtained directly from the power law function. The results for n, K, and R² are shown in Table 2.

TABLE 2. VAULES OF n, K, and R² FOR BOTH FORMULATIONS AT 8 AND 24 °C, IMMEDIATELY AFTER FORMULATION FOR TWO TYPES OF DEVELOPED BEVERAGE

| Treatment* | Temperature (°C) | n | K | \mathbb{R}^2 |
|------------|------------------|-------|-------|----------------|
| T1 | 8 | 0.652 | 0.077 | 0.99 |
| T2 | 8 | 0.393 | 0.110 | 0.97 |
| T1 | 24 | 0.620 | 0.058 | 0.97 |
| T2 | 24 | 0.328 | 0.081 | 0.95 |

The results showed that n values were less than one, indicating a shear thinning behavior of the beverage samples. For sample T1, the results showed that n decreased from 0.652 to 0.620 as temperature increased from 8 to 24 °C, and K decreased from 0.077 to 0.058 for the same temperature increment. This decrease was due to the decrease in apparent viscosity as temperature increased. The sample T2 showed a similar trend with n decreasing from 0.392 to 0.328 and K decreasing from 0.11 to 0.081 as temperature increased from 8 to 24° C. These values reflect the nature of shear-thinning fluids.

Equivalent results for measurement taken after 8 weeks are shown in Table 3.

Flow behavior index decreased after 8 weeks. The relationship between apparent viscosity and shear rate for formulation T1 and T2 at different temperatures is shown in Figure 5. The reduction in apparent viscosity with increasing shear rate is most probably due to the structural breakdown of the formulations by hydrodynamic forces generated and the increased alignment of the constituent

molecules, since shearing causes progressive deformation and disruption of droplets resulting in less resistance to flow (Alparslan and Hayta, 2002; Rao, 1999). Obviously, the viscosity increased significantly for all temperatures and formulations after 8 weeks and the relationship

TABLE 3. VAULES OF n, K, and R² FOR BOTH FORMULATIONS AT 8 AND 24 $^{\circ}$ C, AFTER 8 WEEKS FOR TWO TYPES OF DEVELOPED BEVERAGE

| Treatment* | Temperature (°C) | n | K | \mathbb{R}^2 |
|------------|------------------|-------|-------|----------------|
| T1 | 8 | 0.158 | 0.280 | 0.96 |
| T2 | 8 | 0.196 | 0.255 | 0.97 |
| T1 | 24 | 0.048 | 0.325 | 0.93 |
| T2 | 24 | 0.020 | 0.329 | 0.95 |

between apparent viscosity and shear rate became less dependent on temperature and formulation type. This indicates that viscous behavior of both formulations became more stable after 8 weeks, probably due to development of cross polymerization networks (Figure 6) (Girard et al. 2002; and Damianou et al. 2006).

3.3.1. ANFIS Modeling

The fuzzy logic toolbox of Matlab 7.0 was used to obtain the results, and to build a fuzzy model for the viscosity. Figure 7 shows the training curve for building a fuzzy model for viscosity. 160 data points were used for training the system to predict the viscosity. 1500 neural nets learning epochs were used to get a low error of training (i.e., RMSE = 7.48 or 3 percent of the training data range = Maximum - Minimum = 240). A comparison between the actual and ANFIS predicted viscosity after training is shown in Figure 8, which shows that the system is well-trained to model the actual viscosity.

The final fuzzy inference system that predicts the viscosity is shown in Figure 9. As illustrated in Figure 9, a two (Gaussian) type membership functions for each input (4 inputs) resulted in high accurate prediction results.

3.4. Models Validation

The ANFIS prediction model for viscosity was validated by selecting a certain number of data points (i.e., 25 points), different from the other 160 points used for ANFIS training. Each validation data point (i.e., time, shear rate, temperature, and treatment) was fed into the system, and then the predicted properties (i.e., viscosity) were compared to the actual values of the measured viscosity. The average percentage of errors in the modeling of viscosity was 9%, achieving an accuracy of viscosity prediction of 91%. Table 4 shows the data points used in system's validation along with the actual and predicted viscosity values, and the percent errors in the predictions. This table shows that the ANFIS predicted values are close matches of the actual ones.



Figure 5. Apparent viscosity changes with shear rate for two types of developed orange beverage contained different ratios of 6% WPI/1%CMC (50% "T1", and 40% "T2") at 8 and 24 °C before storage.



Figure 6. Apparent viscosity changes with shear rate for two types of developed orange beverage contained different ratios of 6% WPI/1%CMC (50% "T1", and 40% "T2") at 8 and 24 °C after and 8-week storage period.



Figure 7. ANFIS training curve for the viscosity model



Figure 8. Actual and ANFIS-predicted values of viscosity



Figure 9. The final fuzzy inference system (FIS) for predicting the viscosity.

4. Conclusions

High quality and shelf stable beverages were formulated with 6% WPI/1% CMC and pure orange juice combined at 50:50 and 40:60 ratios, respectively. The apparent viscosity decreased as both shear rate and temperature increased. Additionally, the apparent viscosity for the same treatment at certain shear rate/same temperature increased after storage. ANFIS models achieved an average prediction error of viscosity of only 9%. The present study shows that ANFIS is a technique that can be used efficiently to predict the food properties.

It is believed that this approach can be applied to predict many other parameters and properties in food industry.

Table 4. Validation Table

| Time | Shear Rate | Temperature | Treatment | Actual Viscositv | Predicted Viscosity | Error |
|-----------------------|---------------|-------------|-----------|---------------------|------------------------|-------|
| (Weeks) | (1/s) | (°C) | (%)* | (mPa.s) | (mPa.s) | (%) |
| 0 | 11 | 8 | 50 | 33.44 | 34.1 | 1.97 |
| 0 | 4.4 | 8 | 50 | 45.98 | 40.5 | 12 |
| 0 | 1.32 | 8 | 50 | 69.9 | 72.3 | 3.4 |
| 0 | 13.2 | 8 | 40 | 22.97 | 25.8 | 12.5 |
| 0 | 11 | 8 | 40 | 25.66 | 25.8 | 0.54 |
| 0 | 4.4 | 8 | 40 | 44.75 | 37.3 | 16.6 |
| 0 | 2.2 | 8 | 40 | 68.16 | 69.5 | 1.96 |
| 0 | 6.6 | 24 | 50 | 28.3 | 24.5 | 13 |
| 0 | 4.4 | 24 | 50 | 33.03 | 28.6 | 13 |
| 0 | 1.32 | 24 | 50 | 52.19 | 54 | 3.46 |
| 0 | 13.2 | 24 | 40 | 14.3 | 16.1 | 12.7 |
| 0 | 6.6 | 24 | 40 | 17.9 | 22.8 | 27 |
| 0 | 2.64 | 24 | 40 | 42.19 | 42 | 0.45 |
| 0 | 2.2 | 24 | 40 | 47.7 | 49.1 | 2.9 |
| 8 | 11 | 8 | 50 | 37.18 | 37.3 | 0.32 |
| 8 | 4.4 | 8 | 50 | 80.4 | 67.6 | 15.9 |
| 8 | 2.2 | 8 | 50 | 144.1 | 153 | 6 |
| 8 | 13.2 | 8 | 40 | 32 | 36.3 | 13.4 |
| 8 | 6.6 | 8 | 40 | 55.9 | 41.9 | 25 |
| 8 | 2.64 | 8 | 40 | 116.84 | 119 | 1.8 |
| 8 | 1.32 | 8 | 40 | 203.99 | 201 | 1.46 |
| 8 | 4.4 | 24 | 50 | 79.3 | 67.2 | 15.2 |
| 8 | 1.32 | 24 | 50 | 249.5 | 237 | 5 |
| 8 | 4.4 | 24 | 40 | 77 | 64.8 | 15.8 |
| 8 | 2.2 | 24 | 40 | 152 | 166 | 9.2 |
| Average Percent Error | | | | | | 9.22 |

*(50=50:50 "T₁", 40=40: 60 "T₂"; 6 % WPI/1.0% CMC combination: pure orange juice)

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Aspects of Growth, Reproduction, and Feeding Habit of Three Pomacentrid Fish From Gulf of Aqaba, Jordan

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Abstract

Some aspects of growth, reproduction, and feeding habit were investigated in three species of Pomacentridae, Dascyllus timaculatus, Chromis pelloura and Teixeirichthys jordani from the Gulf of Aqaba. A total of 647 fish were collected during March 99 until April 2000. Growth was determined by analyzing the Lengthweight relationship (LWr) and Fulton-type condition factor (K). The gonadosomatic index (GSI) was measured to examine the gonadal maturation and spawning season. The frequency of occurrence method was used to describe the different food items in fish gut. The dietary significance of food items to fish was determined using the index of relative importance (RI). For LWr, results suggested an allometric growth, and the correlation between total length and weight was high in the three fish except T. jordani. However, the regression value (b) was less than 3 in males and females of all fish indicating negative allometric growth except in females of D. trimaculatus that exhibited positive allometric growth (b>3). The condition factor was relatively high in both sexes of all fishes. The spawning months differed according to species. In D. trimaculatus and T. jordani, spawning extends mainly from late summer to early winter while it extends to three months only for C. pelloura during the spring. Feed variability might be related to the composition of available food items in fish habitat. RI results showed that crustacean were the major dietary component in the three fish. However, noticeable difference was observed in composition, consumption, and occurrence of food during the reproductive season for each of the three fishes.

الملخص

تمت دراسة بعض الظواهر الحياتية الأساسية مثل النمو والتكاثر وكذلك نوعية وكمية الغذاء لثلاثة انواع من اسماك عائلة ال (Pomacentridae) آكلة الهوائم من خليج العقبة وذلك خلال دورة سنوية واحدة (1999 ولغاية 2000). اجريت الدراسة على 647 عينة (Inaculatus, Chromis pelloura and Teixeirichthys jordani) ممكية جمعت بشكل شهري شملت الانواع الثلاثة (timaculatus, Chromis pelloura حيث أخذت قياسات مثل الطول الكلي والمعياري والوزن لكل سمكة حيث أخذت قياسات مثل الطول الكلي والمعياري والوزن لكل سمكة على حدة. تم عزل و قياس أوزان الأعضاء التناسلية وكذلك المعد من مجموع العينات السمكية الكلي. من خلال تحليل العلاقة بين الوزن والطول (LWr) وكذلك قياس معامل الحالة (k) تمت دراسة النمو في والتكاثر، كما استخدم معامل الخصوبة لدراسة موسم التبييض والتكاثر، كما استخدمت طريقة التواجد المتكرر لعناصر الغداء في معدات الأسماك وذلك لوصف عناصر الغذاء المختلفة، وان اهميتها لكل نوع من هذه الاسماك قد تم تقديرها عن طريق حساب معامل الأهمية النسبية لهذه العساص.

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Keywords: Pomacentridae, growth, reproduction, feeding habit, Gulf of Aqaba,

1. Introduction

The Pomacentrids, commonly known as Damselfishes, occur in shallow coral reefs and few species penetrate to a depth of 80 m or more (Khalaf and Disi, 1997). Fishes are diurnal; and tend to cluster around coral head for shelter

during night. Many species of this family are plankton feeders, others are omnivores that feed on benthic invertebrates and algae. Pomacentridae is an important plankton feeding fish family in the Red Sea (Khalaf and Disi, 1997). Plankton feeder fish are the most abundant feeding guild on coral reefs, as well as in the sea grass dominating habitats (Khalaf and Kochzius, 2002a). *Labridae* and *pomacentridae* dominated the Jordanian ichthyofauna in terms of species richness (Khalaf and Kochzius, 2002b).

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Along with the forty-five species of the family recorded from the Red Sea, about 29 species were reported from the Gulf of Aqaba (Khalaf and Disi, 1997).

Among the few studies, centering on reproduction of Pomacentridae, is that of Macdonald (1981). He examined seasonal patterns of spawning, food acquisition, and fat storage in two Hawaiian damselfishes; and proposed that both use fat reserves built up during the period of peak food availability to support later spawning. Breeding season of the planktivory Chromis notatus were reported to extend from May to August, and the maturity length is found for both males and females about 60 mm (Go and Jean, 1983a). However, the reproduction of some damselfish species is uniformly high throughout most of the year, but ceases in winter due to fish nesting activities (Stanton, 1985). Thresher (1985) examined the spawning and larval recruitment of eight damselfishes from the Caribbean and Pacific coasts of Panama; and found that the average seasonal pattern of spawning and settlement did not match in any of the eight species. Pomacentrids are very divers in their feeding habits, most are either aggregating planktivores such as the Chromis and Dascyllus or omnivores like the Pomacentrus (Sale, 1990). Richard (1981) reported that the Blacksmith (Chromis punctipinnis) regularly forages on zooplankton during the day and shelters in rocky reefs at night. In the analysis of stomach contents of Chromis notatus, it was found that the fish feed primarily on zooplankton mainly copepods which constitute more than 99 % of the total prey number (Go and Jean, 1983b). Planktivory fishes are very diverse in coral reef ecosystems, and the Red Sea as well. However, little is known on some ecological aspects of these fish in the Gulf of Aqaba, the north eastern extension of the Red Sea . In view of the importance, diversity, and the high abundance of plankton feeding fish in coral reef ecosystem of Gulf of Aqaba, a series of ecological and biological studies were conducted on six different fish species. Fishes selected in the present study (D. trimaculatus, C. pelloura and T. jordani) are representatives of the pomacenridae in Jordanian waters of Gulf of Aqaba. Some aspects of growth, reproduction, and feeding habit were investigated in the three fishes over 13 months period.

2. Materials and Methods

Fish samples were collected at depths between 5-20 m mainly from coral reef habitat and the adjacent sea grass beds at the North Beach of Gulf of Aqaba (Fig. 1). Fish were collected monthly (March 1999 to March 2000), using gill net with different mesh size. The number of collected *D. trimaculatus* is 139, *C. pelloura is* 250, and *T. jordani is* 278.

Fish specimens were measured for total length (TL), standard length (SL), and body weight (Fig. 2). Growth was determined by analyzing LWr and K. The LWr was obtained by using the equation Wt = Log a + b Log L., where, Wt = total fish wet weight in g, L = total length in cm, *a* and *b* are constants (Nielson and Johnson, 1983). K was calculated according to the formula, $K = (Wt / SL^3)$ 100. Where, Wt = total fish weight in g and SL= standard length in cm (Nielson and Johnson, 1983). Reproduction cycle was estimated by using the gonadosomatic index



Figure 1. The locations on Gulf of Aqaba where most of fishes were collected

(GSI) following the formula GSI = (W / Wt) 100 (Gailliet *et al.*, 1986). Where, W; gonad weight in g and Wt; total fish weight in g. Food content analysis was performed, and food items were categorized into major taxonomic groups and the relative importance index (RI) was obtained (Newell, 1993; Smith, 1996). Food content analysis (Hyslop, 1980) was used to describe the importance of the different food items in fish gut. Main items were determined by computing RI following the formula $RI = (AI / \sum AI)$ 100. Where, AI = % frequency of occurrence + % total # + % total weight (George and Hadley, 1979).

3. Results

3.1. LWr and K

LWr results of *D. trimaculatus, C. pelloura,* and *T. jordani* are summarized in Fig. 3. The correlation coefficient (r^2) in *D. trimaculatus* was the highest among other species $(r^2 = 0.91, n = 127)$. K in *D. trimaculatus, C. pelloura,* and *T. jordani,* are shown in Fig. 4. *D. trimaculatus* showed the highest K (0.8-.82) as compared to other two examined fish. Both sexes of *D. trimaculatus* showed maximum K values during early summer with a minimum value during October. *C. pelloura* revealed high K values for both sexes in March with obvious decrease observed in August. Both sexes in *T. jordani* exhibited approximately equal K values with a range of (0.36-0.38) with clear decrease of K occurred in August.

3.2. GSI

Seasonality of gonad development was observed for the three fish (Fig. 5). The GSI of *D. trimacultus* showed that fish females spawn during August through January while males become sexually active during October through February. The GSI of *C. pelloura* suggests spawning activity during January through March. Results of GSI in



Figure 2. Length-frequency histograms of both sexes for the three fish.



Figure 3. Length-weight relationship of both sexes for the three fish.



Figure 4. Monthly changes in the condition factor (mean \pm SE) of both sexes for the three fish.

T. jordani showed that spawning extend from August until December.

3.3. Food Composition and Consumption

Results on gut content analysis are shown in Table 1. RI values showed that crustaceans generally represented the main food item in the three species. The second important food item for D. *trimaculatus* was polychaets. Based on the frequency of occurrence, crustaceans represented the major food item in guts of the three fish. In *D. trimaculatus*, it accounted for 65.6%, *C. pelloura* 64.9% and *T. jordani* 38.3% of all food items.

Seasonal variation in food composition was expressed based on percentage occurrence of the various identifiable food items in guts of *D. trimaculatus, C. pelloura* and *T. jordani* (Table 2). Crustaceans, Molluscs and polychaets were present in the guts of *D. trimaculatus* throughout the year. Crustaceans exhibited highest percentage during March through June. Molluscs were the highest among



Figure 5. Monthly changes in the gonadosomatic index of both sexes for the three fish.

 Table 1. Food composition expressed by frequency of occurrence and the index of relative importance (RI).

 Dascyllus trimaculatus

| Food items | Frequency | | RI |
|---------------------------|-----------|---------------|---------|
| | F | % | |
| Crustacea | 59 | 65.6 | 50.6 |
| Molluscs | 13 | 14.4 | 5.8 |
| Polychaeta | 8 | 8.9 | 3.0 |
| Small fish | 7 | 7.8 | 10.1 |
| Eggs | 52 | 57.8 | 30.5 |
| No. fish examined $= 129$ | No | . fish feedir | ng = 90 |
| (69.8%) | | | |

| Chromis pelloura | | | | | |
|---------------------------|----|---------------|--------------------|--|--|
| Food items | Fr | equency | RI | | |
| | F | % | | | |
| Crustaceans | 24 | 64.9 | 49.7 | | |
| Molluscs | 2 | 5.4 | 2.5 | | |
| Small fish | 3 | 8.1 | 6.8 | | |
| Eggs | 17 | 45.9 | 41.0 | | |
| No. fish examined = 242 | | No. fish feed | ding = 137 (56.6%) | | |

| Teixeirichthys jordani | | | | | |
|---------------------------|----|--------------|--------------------|--|--|
| Food items | Fr | requency | RI | | |
| | F | % | | | |
| Crustacea | 23 | 38.3 | 35.0 | | |
| Molluscs | 2 | 3.3 | 4.1 | | |
| Small fish | 1 | 1.6 | 1.1 | | |
| Eggs | 19 | 31.7 | 59.8 | | |
| No. fish examined $= 284$ | 1 | No. fish fee | ding = 166 (58.4%) | | |

F =frequency of occurrence;

 $RI = 100(AI/\Sigma AI)$. AI = % frequency + % total number + % total weight.

Table 2. Monthly changes in percentage occurrence of various identifiable food items in the stomach of the three fish.

| | Crustacea | | Polychaeta | Mollu | Molluscs | | | Small Fish | | | Eggs | | |
|------------|-----------|-----|------------|-------|----------|-----|-----|------------|-----|----|------|-----|-----|
| | (%) | | | (%) | (%) | | | (%) | | | (%) | | |
| Month | Ср | D t | Тj | Dt | Ср | D t | Тj | Ср | D t | Тj | Ср | D t | Тj |
| Mar (1999) | 100 | 100 | 100 | 14.3 | | | 5.5 | | 57 | 33 | | 100 | |
| Apr | 100 | 25 | 100 | | 100 | 25 | | | | | | 75 | |
| May | 100 | 33 | | | | | | | | | | 33 | |
| Jun | 33 | 71 | | 14.3 | | 43 | | | | | | 71 | |
| Jul | 86 | 50 | 30 | 50.0 | 14 | 50 | 20 | | | 50 | 43 | 50 | 43 |
| Aug | 33 | 40 | 50 | 20.0 | | 20 | | | | | | 33 | |
| Sep | | 78 | 67 | 11.1 | | 17 | | | 11 | | | 72 | |
| Oct | | 75 | | | | | | | 13 | | | 100 | |
| Nov | 100 | 20 | 100 | 20.0 | | | | | | | 100 | 40 | 100 |
| Dec | 100 | 91 | | | | 27 | | 100 | | | 100 | 55 | 100 |
| Jan (2000) | | 90 | 72 | | | | | 67 | | | 33 | 30 | 33 |
| Feb | 60 | 33 | 100 | | | | | | | | 100 | 33 | 100 |
| Mar | 83 | 100 | 31 | 33.3 | | | 6.3 | | | | 67 | 40 | 67 |

other food items of the three fish all year round except in winter. Seasonal variation in food composition and consumption (g food/ g fish) of *D. trimaculatus, C. pelloura* and *T. jordani* are shown in Table 3 and Fig. 6, respectively. Food consumption of *D. trimaculatus* was highest in March while that of *C. pelloura*, it peaked in January. The maximum food consumption for *T. jordani* was observed in August.

4. Discussion

4.1. LWr

An allometric exponential form LWr was observed for the three fish, and that the equation of LWr applies well to *D. trimaculatus, C. pelloura* and *T. jordani.* In *D. trimaculatus,* female however, b value was slightly above 3 suggesting a positive allometric growth (the fish become more rotund as length increases). The isometric growth (b= 3) exists when fish shapes do not change as fish grow (Neilson and Johnson, 1980). Our results indicated that the increase in fish weight is accompanied by an increase in total length and body depth. Results showed also that the three fish are laterally compressed which could be an adaptation to its habitat since these fish live in the vicinity of coral reef. Coral substrata, depth, and location were found among the different factors that affect the growth of a pomacentrid fish, *Dascyllus aruanus*, and *Pomacentrus amboinensis* (Sale, 1990). Growth varies among the different habitats in *Acanthochromis sp.* (Thresher, 1985).

The K values in the three fish changed with sex and season. The increment during periods of low K values for all fish could be related to the seasonal change in seawater temperature, hence attributed to the corresponding variations in food availability. However, K values varied within relatively narrow limits. This might explain appropriate environmental conditions for the three fish in Gulf of Aqaba.

Food consumption increased after the completion of spawning, and that the fish must feed more in order to compensate the energy drawn upon during the period of fasting. The attainment of sexual maturity appears to have an influence on LWr (at sexual maturity the rate of fish growth slows down). Spawning activity may also slow down growth, since many species do not feed properly during nest building and guarding of eggs (Goulet, 1997).

| | | | | , e | | | , , | | | | | | |
|------------|---------------|------|---------------|--------------|------|----------------|--------|------|----------|------|------|------|------|
| Food Item | Crustacea (%) | | Polychaeta(%) | Molluscs (%) | | Small Fish (%) | | | Eggs (%) | | | | |
| Month/Fish | Ср | D t | Тj | D t | Ср | D t | Тј | Ср | D t | Тј | Ср | D t | Тј |
| Mar (1999) | 100 | 48.6 | 45.3 | 3.6 | | | 3.3 | | 54.5 | 49.1 | | 1.2 | 5.2 |
| Apr | 59.9 | 96.7 | 19.4 | | 67.7 | 2.2 | | | | | | | 6.3 |
| May | 98.2 | 92.3 | | | | | | | | | | 7.2 | |
| Jun | 1.2 | 21.4 | | 6.5 | | 18.3 | | | | | | 8.2 | |
| Jul | 74.1 | 7.8 | 5.9 | 14.3 | 0.8 | 72.3 | 27.2 | | | 31.1 | 19.6 | 3.4 | 23.8 |
| Aug | 0.4 | 0.8 | | 1.2 | | 1.8 | | | | | | 6.5 | 28.8 |
| Sep | | 12.6 | 24.3 | 11.4 | | 13.1 | | | 26.2 | | | 10.8 | 65.5 |
| Oct | | 13.2 | | | | | | | 58.2 | | | 4.1 | |
| Nov | 89.9 | 6.5 | 16.2 | 1.4 | | | | | | | 2.7 | 1.1 | |
| Dec | 48.6 | 30.3 | | 8.8 | | | | 54.4 | | | 3.2 | 0.6 | |
| Jan (2000) | 22.1 | 7.8 | 8.2 | | | | | 53.1 | | | 1.4 | | |
| Feb | 5.1 | | 9.3 | | | | | | | 62.2 | 2.9 | | 5.4 |
| Mar | 50.1 | 98.2 | 1.1 | | | | 2.7 | | | | 6.7 | 0.9 | 1.8 |

Table 3 Food item % composition (total monthly food g/total weight in g) in stomachs of the three fish

Cp: C. pelloura, Dt: D. trimaculatus, Tj: T. jordani

4.2. Reproduction

The overall changes in mean GSI for males and females of the three fish were almost similar, but with higher GSI values for females. D. trimaculatus females attained sexual maturity earlier (July) than males (September) which could be as a result of prolonged and complicated courtship of males. Sexual maturation and spawning activity of T. jordani and D. trimaculatus occurred during summer, while that of C. pelloura in winter. It was observed that the three fish enter a resting period during which growth as well as food consumption increased until period of next reproductive season. Diversion from somatic growth to the growth of gonads was indicated by the decrease in K during spawning season (females might be busy in nesting activities) with low food consumption during this period. The duration of spawning could be considered as an adaptation to minimize competition for food and space among larvae. The onset of D. trimaculatus and T. jordani spawning occurred during warm season whereas that of C. pelloura occurred during considerably cold season. The initiation of the reproductive season for the damselfish Amblyglyphidodon leucogaster was regulated by seawater temperature in Gulf of Aqaba (Goulet, 1997). Similar findings were observed in three Hawaiian damselfishes (MacDonald, 1981, Stanton, 1985). The environmental conditions associated with the seasonal changes, particularly in temperature and light intensity, are both important factors in the regulation and timing of spawning among fish (Abu-Hakima, 1987, Wahbeh, 1992).

4.3. Food and Feeding Habits

Certain limitations must be kept in mind when discussing data on feeding habit. The use of gillnets may stress fish severely since fish are hold for long hours, during which much of the diet can be digested. As a result, food items identification becomes more difficult, and sometimes the loss of valuable dietary information may occur. The composition of food in D. trimaculatus showed that the fish feeds mainly on crustaceans, Molluscs, and polychaets. The occurrence of these items in their guts throughout the year suggests food availability in fish habitat (Sarker et al., 1980). Coral reefs are major source of feeding and refuge for fishes (Fishelson, 1977), and that the existence of certain food item in fish guts probably depends on its availability in the natural habitat (Gordon, 1977). Highest zooplankton abundance was recorded in Spring with a peak in June in Gulf of Aqaba (Al-Najjar, 2000). The occurrence of chaetognaths, being a major component of Red Sea zooplankton in addition to polychaets in the gut of D. trimaculatus, suggests that this fish is a generalist predator. Fishes can capture and feed on such benthic forms probably, during their breeding migration from the bottom (Gordon, 1977). The low variety of food items in food of three fish could be related to the fact that these fishes are also consuming benthic invertebrate and algae besides being plankton-feeders (Khalaf and Disi, 1997).

Considering the seasonal changes, data showed that when the occurrence of one food item is scarce, the presence of other item is abundant. Food consumption might be affected by seawater temperature and/or spawning activity. The mean annual range of sea temperature in Aqaba is 20 °C in February and 27 °C in August (Badran, 2001). Consequently, low phytoplankton primary productivity occurs during summer while high productivity occurred during winter in the Gulf of Aqaba, (Badran and Foster, 1998). Maximum food consumption of D. trimaculatus during winter with a minimum in summer can be related to change in water temperature. Major consumption of C. pelloura, was in January and minor in August, and this is probably related to the spawning activity, which exists during the same period. Consumption of T. jordani could be connected to the spawning activity during fall. Such an alteration in food and feeding habits of all investigated fish may be



Figure 6. Monthly change in food consumption % (gut content Wt g/fish Wt g*100) of the three fish

considered advantageous in reducing intra specific competition.

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الملخص

Relative DNA Content of Three Cytotypes of Pohlia Nutants

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Abstract

Relative DNA content of three cytotypes of the moss *Pohlia nutants* have been estimated, using DAPI staining technique. Evidence is presented and showing that the mean relative DNA contents increased from haploid to diploid and from diploid to triploid *P.nutants* cytotypes, but they differ significantly from an expected 1: 2: 3 ratio in haploid, diploid and triploid races. Polyploidy has played a main role in generating the cytotypes. It seems likely that the haploid, diploid and triploid races of *P. nutants* are of long standing autopolyploid.

تم قياس محتوى DNA النسبي في ثلاثة أنواع خلويه لحزاز DNA المعدل nutants باستخدام طريقه الصبغ DAPI أظهرت النتائج أن معدل محتويات DNA النسبي زاد في أنواع خلويه لحزاز P.nutants محتويات DNA النسبي زاد في أنواع خلويه لحزاز DNA من أحادي الصيغة الصبغية ولكنها أختلفت بشكل واضح عن النسبه المتوقعه 2:12 في ألأجناس أحادي الصيغة الصبغية و ثنائي الصيغة الصبغية و ثنائي محد و ثنائي محد و ثنائي الصيغة الصبغية و ثلاثي الصيغة من المحد الصيغة و ألوجان

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Keywords: DNA Content, DAPI Staining Technique, Mosses, Pohlia nutants.

1. Introduction

Genome size variation below the species level is attracting considerable interest among plant biosystematists and cytogeneticists. Measurements of the relative DNA content of nuclei from gametophytes from different populations may provide an efficient mean of differentiating populations where karyotype analysis is difficult or impracticable. The DNA content of nuclei of gametophytes may also provide a useful taxonomic criterion and may provide evidence of evolution of one taxon from another (Grellhuber and Obermayer, 1998).

The estimation of nucleic acid contents by chemical means requires a large amount of material and is relatively time consuming. The DNA content of single nuclei can be estimated by measuring the density of Feulgen staining. The light absorbed by the stained nucleus is proportional to the DNA content. A more sensitive measure can be made by using a fluorochrome that stains DNA in a quantitative manner. Such a fluorochrome is DAPI. Other methods depend upon the relationship between nuclear volume and DNA content. These relationships show that nuclear volume and interphase volume are directly proportional to DNA content per cell and per chromosomes, respectively. Therefore when the nuclear volume of meristematic cells is known, an estimate of DNA content can be made (Sparrow et al., 1972). This method appears to be inadequate specially when the

interphase chromosome volume is obtained by dividing the average of the interphase nucleus by somatic chromosome number; and represents the volume occupied by the average chromosome at interphase, neglecting other nuclear components such as the nucleolus or volume changes during replication of chromosomes. Clearly, the accuracy of measurements with very small nuclei is questionable. To assess the DNA content in nuclei, Feulgen stain was used, but there are many difficulties, for example excess or too little stain affect the results as does the influence of the background. The fluorescence of the DAPI/DNA complex has been used as a quantitative estimate of the DNA (Brunk et al., 1979; Lin et al., 1977). Many workers are satisfied that DAPI can be successfully applied to measure DNA and detect intraspecific variation (Rayburn et al., 1989; Biradar and Rayburn, 1993). Moreover, the simplicity of the staining procedure coupled with the brightness of the DAPI/DNA complex provide a convenient technique of cell cycle studies and comparative estimates of DNA contents of nuclei.

So to obtain satisfactory results, the fluorochrome 4,6-diamidino-2-phenylindole (DAPI), which has been shown to bind specifically to DNA, will be applied.

To date, comparatively few reports on DNA content of bryophytes are available. Isolation of nuclear, chloroplast and mitochondrial DNA from the moss *Physcomilrella patens* has been reported (Marienfeld et al., 1989 and Knight, 1994). DNA content of a wild type strain and a somatic hybrid *Physcomitrella patens* was estimated, using flow cytophotometry (Reski et al., 1994). Relative changes in DNA content in the hornwort *Anthoceros punctatus*, using DAPI stain, have been demonstrated by Izumi and

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Ono (1994). Nuclear DNA contents of 17 species of bryophytes have been studied by Renzaglia *et al.* (1995). Thoni and Schnepf (1994) studied the nuclear DNA content in spore nuclei of *Funaria hygrometrica*. Moreover, numerous reports of intraspecific polyploidy in mosses were also published (Abderrahman and Smith, 1983; Abderrahman, 1998 and Abderrahman, 2004).

It is now well established that there are three cytotypes of the moss *Pohilia nutants* with (n=11, 22 and 33) respectively (Smith, 1978; Fritsch, 1982, 1991). They are indistinguishable morphologically. In view of their morphological similarity, it was decided to investigate possible cytological differences, and to this end relative DNA contents of the cytotypes will be investigated using DAPI staining technique.

2. Materials and Methods

Previous studies indicate that factors such as latitude, altitude, and time of the day may affect mitotic activity and cell cycle duration, thus having impact on the c-value and intraspecific genome size. So, materials were collected from different localities in Jordan by ourselves (Table 1) and kept under uniform conditions for at least four weeks in polythene bags at room temperature out of direct sunlight in the laboratory. Gametophyte shoot apices were fixed in a glacial acetic acid and ethyl alcohol (1:3) solution for 2h. Cells, then were transferred into Feulgen stain for cytological studies. Chromosome number of each sample will be determined i.e. haploid diploid and triploid cytotypes.

Gametophyte cells from each cytotype samples were measured, using DAPI staining technique described by Lin et al. (1977) and Brunk et al. (1979). Materials were fixed in 5% gluteraldehyde EM in Tris buffer, pH 7 for 5 min. The stain was made up as stock solution of 100 μ g/ml in buffer containing 100m M NaCl, 10 mM EDTA and 10m M Tris, pH 7.

Shoot apices stained with DAPI were squashed on slide, and a microscope fitted with an incident U.V. light source measured fluorescence of nuclei, and photomultiplier coupled microscope to a pen recorder. Filters were used giving a final wavelength of 350 nm. The DAPI / DNA complex fluoresces at 450 nm. As the variation in fluoresces is proportional to the mean value in the first peak G1 of the cytotypes, transformation of data by means of Log_{10} will be used to overcome this proportional variation. Measurements of fluorescence were transformed by Log_{10} , and are expressed as arbitrary units.

A one-way analysis of variance was carried out. Tukey's test was applied, and found to be suitable for this sort of data. Tukey's interval estimate was also applied to calculate the 95% confidence intervals for the differences between means of pairs of groups of samples (Neter and Wasserman, 1974). The statistic q' was calculated by:

$$q' = q_{v,r} \sqrt{\frac{MSE}{2} \left(\frac{1}{n1} + \frac{1}{n2}\right)}$$
 (1)

Where $q_{v,r}$, was derived from tables for v= number of groups (i.e. cytotypes) and r= number of degrees of freedom of error term in the analysis of variance. MSE is the mean square error from the analysis of variance Both nl and n2 are sample sizes of the two groups. 95% confidence intervals were then calculated as:

$$(\mathbf{X}_{i} - \overline{\mathbf{X}}_{j}) - \mathbf{q}' \leq (\boldsymbol{\mu}_{i} - \boldsymbol{\mu}_{j}) \leq \mathbf{q}' + (\overline{\mathbf{X}}_{i} - \overline{\mathbf{X}}_{j})$$
(2)

Where x_i , x_j were the means of Log_{10} transform data of the two groups and (μ i- μ j) was the expected difference *Pohilia nutants* between gatherings means. The DNA content in haploid diploid and triploid was assumed to be in the ratio 1: 2:3.

3. Results

As may be seen from Figs. 1a, 1b, and 1c there are biomodality in DNA content within each category of *Pohlia nutants* corresponding to G1 and S, G2 and M phases of the cell cycle. Estimates of DNA content for G1 nuclei in arbitrary units of the three cytotypes of *Pohlia nutants* showed that the mean value of the first peak was estimated to be 49 ± 2.70 in the haploid, the mean value of the diploid cytotype was estimated to be 86 ± 1.95 , and the mean value of the triploid cytotype was estimated to be 120 ± 2.20 (Table 2).

Differences between diploids and haploids and triploid and diploid were compared using Tukey's interval estimates (Neter and Wesserman, 1974). The mean relative contents increased from haploid to diploid and from diploid to triploid cytotypes, as expected. These results are outside the 95% confidence intervals (i.e the differences in means between diploids and haploids and those between triploids and diploids are outside the 95% confidence intervals). Therefore, the increase in the DNA content was not proportional to the increase in chromosome number in the three cytotypes of *Pohlia nutants*.

Table 1. Localities and habitat of Pohlia nutant cytotypes.

| | | 5 51 |
|---|---------------------|----------------------|
| Locality | Habitat | Cytotype |
| Salt: 10 Km. West of Amman | Wet soil | Haploid and diploid |
| Om-Qais: 10 Km. North of Irbid | Shady moist soil | Diploid and triploid |
| Sweileh: 6 Km. North west of Amman | Shaded walls | Haploid and diploid |
| Ain AL-Basha: 20 Km. North of Amman | Damp soil | Haploid and triploid |
| Wadi AL-Sair: 20 Km. North west of Amman | Damp soil | Diploid and triploid |
| Fuhais: 10 Km. west of Amman | Wet soil | Haploid and triploid |
| Jubaiha: University of Jordan campus, Amman | Wet shaded | Haploid and triploid |



Figure 1. Histogram of DNA quantities in Pohlia nutants: a, haploid; b, diploid and c, triploid cytotypes. (Vertical axis-number of readings; horizontal axis-arbitrary units)

Table 2. Mean DNA content of G1 nuclei expressed as arbitrary units of the three cytotypes of *Pohlia nutants*.

| Cytotype | Mean DNA content expressed in arbitrary units |
|------------------|--|
| Haploid (n=11) | 49 ± 2.70 |
| Diploid (n= 22) | 86 ± 1.95 |
| Triploid (n= 33) | 120 ± 2.20 |

4. Discussion

There are only few publications on DNA amounts of mosses; the only relevant publications dealing with absolute DNA contents of Bryatae are those of Abderrahman and Smith (1983), Reski et al. (1994), Renzaglia et al. (1995), Zouhair and Lecocq (1998), Lamparter et al. (1998), Temsch et al. (1998, 1999), Volglmayr (2000) and Abderrahman (1998, 2004).

Although the DAPI technique provides a quick and reliable measure of DNA content, the results from different organisms are not necessarily comparable, as DAPI results vary depending upon DNA base constitution which may differ from one group or organism to another (Schweizer and Nagl, 1976). Thus, it is only possible to provide relative DNA contents of the three cytotypes rather than estimate absolute quantities by comparison of DAPI data from an organism of known DNA content such as *Drosophila*.

There are numerous examples of intraspecific polyploidy in bryophytes (Smith, 1978; Fritsch, 1982, 1991). Among 289 accessions of 138 different moss taxa, Volglmayr (2000) found only three species of intraspecific polyploidy. These are *Aerichum undulatum*, *Fontinalis antipyretica* and *Amblystegium serpens*. The most prominent of these is *A. undulatum* with a DNA content ratio (max. /min.) of 2.7 suggesting triploidy.

In the present study, biomodality in DNA content within each cytotype corresponding to G1 and S, G2 and M phases of the cell cycle was present (Fig. 1. a, b and c).On the other hand, one single peak of fluorescence was generated by Reski et al. (1994) in studying four *Physcomiterella patents* genotypes, suggesting an arrest in the cell cycle during day time.

If, now appear to be the case, the chromosome races of *Pohlia nutants* (n=11, 22 and 33) series (Smith, 1978; Fritsch, 1982, 1991), it might be expected that DNA quantities in the three cytotypes would be present in a 1:2:3 ratio.

Reference to Table 2, it is clear that the mean relative DNA contents increased from haploid to diploid and from diploid to triploid as would be expected. Applying Tukey's interval estimate showed that the ratio between haploid and diploid is significantly less than 1: 2, and that between diploid and triploid is also significantly less than 2:3. Therefore, the increase in the DNA content is not proportional to the increase in chromosome number. It would appear that there are differences in DNA content between haploid and diploid and diploid and triploid plants. These results are in accord with the findings of Abderrahman and Smith (1983) studying chromosome length and relative DNA content of three cytotypes of Atrichum undulatum in which the three cytotypes differed significantly from an expected 1: 2: 3 ratio in haploid, diploid and triploid races. These results are also consistent with those obtained by Abderrahman (1998) studying two types of Funaria hygrometrica in which the mean relative DNA contents increased from haploid to diploid plants, but they also differ from an expected 1: 2 ratio. The presence of positive correlation between nuclear DNA content and chromosome number was also reported by Lobachevska and Demkiv (1990) in their comparative study of Plagiothecium platyphyllum and Brachythecium

velutinum. Moreover, these results are also consistent with those reported by Abderrahman (2004) in a comparative investigation into nuclear DNA content of the moss *physcometruim pyriforme*. In contrast, the mean of DNA content in haploid and diploid *Sphagnum* (peat moss) were close to the expected 1:2 ratio, namely 1: 2.049 (Temsch et al., 1999).

It is evident that C-value variation within mosses is remarkably small, when compared with angiosperms (Bennet et al., 1998). As mosses can be supposed to be a very old group of plants (Kenrick and Crane, 1997), with the main clade already differentiated in the Palaezoic (Stewart and Rothwell, 1993). This constancy in C-values evidently needs an explanation, especially if compared with the phylogenetically young angiosperms with their vast heterochromatin and repetitive DNA accumulation. This implies the presence of a strong selection pressure towards the maintenance of small DNA amounts, which could be correlated with basic feature of moss biology. A possible mechanism which could contribute to maintenance of small C-values in mosses which has been recently recorded for Physcomiterella patens following transformation experiments (see Reski, 1998).

Although there have been numerous cytological observations of Pohlia nutants (see Smith, 1978 and Fritch, 1982, 1991) there have been no reports of abnormalities cytological suggestive of recent autopolyploidy. However, autopolyploidy is of frequent occurrence in mosses and there may be some mechanism promoting normal meiosis in recent autopolyploids. Chromosomal divergence between the three cytotypes, at least in the plants were studied, suggests that they are of long standing. In view of the morphological uniformity of the cytotypes (Smith, 1978) and on the basis of our observations, it is suggested that the haploid, diploid and triploid races of P. nutants are of long standing autopolyploid origin. As we have three cytotypes of Pohlia nutants, the possible role of aneuploidy has been ruled out.

There is abundant evidence of aneuploidy in bryophytes which may affect genome size. Some instances of aneuploidy in mosses are the result of variation in numbers of m-chromosomes. The occurance and distribution of m-chromosomes in mosses is not strong(Smith,1978). Moreover, *Pohlia nutants* posses three cytotypes, and there is no report in the literature to support the presence of aneuploidy in this plant. Thus, it seems more likely that the role of aneuploidy in *Pohlia nutants* is ruled out.

It is known that estimating genome size using DAPI as the flourochrome "Further research, including more samples of. *Pohlia nutants* and the use of flow cytometry and molecular techniques, is required to elucidate causes of this variation in genome size.

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Effects of Cinnamon on Blood Glucose and Lipids Levels in Diabetic Patients (Type2)

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Abstract

The present study was designed to investigate the effects of supplementation of cinnamon on levels of blood glucose and lipids among type 2 diabetics. The samples consisted of 75 subjects of both sexes (40 males and 35 females) with type 2 diabetes, and the doses of cinnamon 6g were equally administered orally in the form of capsules with breakfast, lunch, and dinner. The doses were given for 4 weeks. Blood samples were taken on the starting day of the experiment and at the end of 4 weeks. The fasting blood glucose and lipids levels of subjetcs were determined. From the results types 2 obtained, the mean value of fasting blood glucose level on the starting day before cinnamon intake was found to be 210.5mg/dl, and the mean values for lipids were triglyceride (205.5mg/dl), total cholesterol (290 mg/dl) and low-density lipoprotein (LDL) (170mg/dl). When diabetic subjects consumed the dose of cinnamon for 4 weeks, their mean fasting blood glucose level dropped to 120.5 mg/dl, triglycerides (160.2 mg/dl), total cholesterol (215.4 mg/dl) and LDL (122.5 mg/dl). The reduction in the mean fasting blood glucose and lipids levels were significant at P<0.001 and P<0.05, respectively. Conclusion: This study provides evidence that cinnamon is effective in decreasing glucose level and lipids level among type 2 diabetic individuals.

الملخص

إن الدر اسـة الحاليـة صـممت حتـى تتحقـق مـن تـأثير القرفـة فـي نـسبة الـسكر والدهون في الدم لمرضى السكري نوع 2.

شملت الدراسة 75 مريض سكري نوع 2 من كلا الجنسين(35 انثى و40 ذكر) تم إعطاؤهم جرعة مقدارها 6غم على شكل كبسولات مع الفطور والغداء وكذلك العشاء, وهذه الجرعة أعطيت لمدة أربع أسابيع, حيث تم أخذ عينة من الدم في اليوم الأول قبل اعطائم القرفة وكذلك عينة أخرى في نهاية الأسبوع الرابع، حيث تم فحص مستوى السكر والدهون لمرضى السكري نوع 2 وتم تحليل النتائج باستخدام برنامج إحصائي.

كانت النتائج كتالي الوسط الحسابي لمستوى السكر في بداية استخدام القرفة كان 2015 وكان الوسط الحسابي لشحوم الثلاثية 2.505 والكوليسترول 290 والبوتينات الشحمية منخفصة الكثافة 170 وعندما استخدم المرضى القرفة لمدة أربع أسابيع كان الوسط الحسابي للسكر 20.55 ولـشحوم الثلاثية 2.52 والكوليسترول 2.514 والبوتينات الشحمية منخفصة 2.221 . وكان النزول في السكر ذو أهمية 20.00 P و الدهون 20.05 P.

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Keywords: Cinnamon, Blood Glucose, Lipids Level, Type 2 Diabetes.

1. Introduction

During 2004, approximately 400,000 (15%) Jordanian adults had diabetes (increasing 7% than in 1996), and an estimated 350,000 (12%) had impaired fasting glucose, and approximately 23% had high blood cholesterol — an increase from 9% in 1996.(Mokdad AH.2007 and Zindah M *et al.* 2004)

Diabetes mellitus is a chronic disorder of glucose metabolism resulting from dysfunction of pancreatic beta cells and insulin resistance. It is still a serious global health problem. The disease prevails in both genders and all age groups, so the general public has a concern about its control and treatment. Botanical products can improve glucose metabolism and overall condition of persons with diabetes not only by hypoglycemic effect, but also by improving lipid metabolism, antioxidant status, and capillary function (Broadhurst, 1997). (Broadhurst *et al.* 2000) and (Jarvill etal.,2001) re-evaluated the extract of cinnamon on insulin function in the insulin-dependent utilization of glucose using a rat epididymal adipocyte assay.

Cinnamon was the most bioactive product. The glucose oxidation enhancing bioactivity was lost from cinnamon

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by polyvinylpyrrolidone (PVP) treatment, indicating that the active phytochemical were likely to be phenolic in nature. They concluded that the extract of cinnamon had improved the glucose and insulin metabolism.

However, those studies were conducted in vitro. There is a general view that the results of animal studies may not be applied to human. Therefore, this study was designed to see the effect of cinnamon on blood glucose in Type 2 diabetic individuals.

Cinnamon has been shown to be generally safe when ingested and to have many pharmacological properties, such as antioxidants activity and antibacterial effects (Lopez et al, 2005, Jellin 2006).

Altschuler et al. (2007) and Solomon et al, 2007 have investigated the impact of cinnamon on glucose and plasma lipid concentrations in patients with diabetes but yielded conflicting results and had modest sample sizes. These findings led to widespread cinnamon use, although no study has yet evaluated the effects of cinnamon in Arab diabetic populations with likely differences in diet, Body Mass Index (BMI), baseline glucose levels, and prescribed medication. Therefore, report here the first Arab study examining the effects of cinnamon on glucose and lipids levels in subjects with type 2 diabetes.

2. Materials and Methods

2.1. Data Collection

The study design was utilized to show the impact of cinnamon supplementation on blood glucose and lipids level among type 2 diabetic. The study was conducted in Jordan at Al Mafraq Governmental Hospital; from January 2008 to March 2009

Seventy five individuals with type2 diabetes of both sexes (40 males and 35 females) of age 40 years or older were recruited for participating in the current study. Only those diabetic subjects using Glibenclamide drug and who were not taking medicine for other health conditions and whose fasting blood glucose were in the range of 160-300mg/dl, and subjects with high lipids level were included in the study. The study was approved by Medical Ethical Committee of the Zarqa Private University. Data for the present study were collected through utilizing the following tools:

Fasting Blood Glucose and lipids level were measured two times once at baseline before cinnamon intake (as a control) and the second measured after cinnamon intake for 4 weeks.

The treatment was conducted for 4 weeks. Type 2 diabetic individuals were allowed to take their routine diet and usual diabetic medicine. The individuals were told to take 4 capsules each (500mg) 2g of whole cinnamon powder immediately after breakfast, lunch and dinner for 4 weeks. The research did not suggest any alterations in other aspects of the subject's medical care, diet, or exercise. Compliance was monitored by contact with the subjects.

2.2. Biochemical Analysis

Biochemical analysis was done by collection of blood samples, approximately 7ml blood samples were taken before breakfast from the vein directly into lithium heparin vacuum tubes for measurements of fasting blood glucose level, triglyceride, total cholesterol and LDL on the starting day and at end of week 4. The samples were transferred into the laboratory of the Zarqa Private University. All biochemical measurements were carried out by the same team of laboratory technicians.

Prior to implementation of the training program, an official permission was obtained from the supervisors of the selected units. This was intended to facilitate data collection and to explain study purpose. At the beginning of the study, participants were invited to participate in the study. The researcher explained the study purpose and procedures for the randomly selected sample. Potential subjects were further informed that the participation was voluntary and that study findings would be presented group wise and no individual would be recognized.

2.3. Statistical Analysis

Collected data were tabulated and needed statistical analyses were done using descriptive statistic, means, and standard deviation (SD) of the means were calculated utilizing the computer data processing (SPSS, version 12). A probability value (P) of <0.05 was considered to be statistically significant.

3. Results

Seventy five subjects of type 2 diabetes were randomized into the study. The sample had a mean age of 59 years (SD = 10) with 40 (53.3%) patients aged ≤ 60 years and Thirty-five patients (46.7%) > 60. The males were 40 subjects (53%) and female subjects were 35(47%). The mean length of time since diabetes was diagnosed was 16 years (SD = 9).

Repeated measure ANOVA was used to assess the effectiveness of Cinnamon among type 2 diabetic individuals by examining blood glucose and lipid levels changes across the time between starting day and end of week 4.

The effect of cinnamon on FBG levels and lipid levels of diabetic individual is shown in table 1. The FBG and lipid levels values on day 0 indicate the FBG and lipid levels of diabetic individuals before the start of cinnamon intake. So these levels were the control values for the study.

| Test | before cinnamon intake | after cinnamon intake | Percentage of reduction | |
|---------------------|------------------------------|--------------------------|-------------------------|--|
| | Mean± | Mean± | % | |
| | SD(mg/dl) | SD(mg/dl) | | |
| Baseline (FBG) | 210.5 ± 33.70 | $120.5 \pm 6.9*$ | 47% | |
| Triglyceride | 205.5 ± 22.65 | $160.2 \pm 5.2*$ | 22% | |
| Cholesterol | 290 ± 25.50 | $215.4 \pm 8.5*$ | 26% | |
| LDL | 170 ± 20.30 | $122.5 \pm 7.1*$ | 28% | |
| * Significant at (F | P<0.05) | | | |

Table 1. Fasting Blood Glucose and lipids levels for Type 2 Diabetic Study Participants.

(FBG) fast blood glucose, LDL- low- density lipoprotein On the starting day of the experiment (day 0), the mean FBG level of the diabetic individuals was 210.5 mg/dl, and lipid level of Triglyceride was 205.5 mg/dl, Cholesterol 290 mg/dl , LDL 170 mg/dl. When the diabetic individuals of these groups used the doses of cinnamon for 4 weeks, their mean FBG dropped to 120.5 mg/dl and lipid levels Triglyceride dropped to 160.2 mg/dl, Cholesterol dropped to 215.4 mg/dl, LDL dropped to 122.5 mg/dl. The reduction in the mean FBG and lipid levels were significant at (P<0.05). This conclusion was supported by the repeated measure ANOVA (F) test.

4. Discussion

Human studies demonstrating beneficial effects of cinnamon supplementation on glucose regulation have examined subjects with type 2 diabetes (Khan *et al*, 2003; Mang *et al*. 2006). The present study shows that 4 weeks of cinnamon supplementation does improve plasma glucose and lipids levels in patients with type 2 diabetes.

A number of spices and herbs have a long history of traditional use in treating elevated blood sugar levels Broadhurst *et al.* (2000). One such compound that has recently been the subject of intense research is cinnamon. Over the past two decades, **in** *vitro* and **in** *vivo* data have been accumulating and supporting the role of cinnamon on glycemic control.

Recently, Khan *et al.* (2003) presented the first data on the effects of cinnamon supplementation in *vivo* in humans. In their study, 10 patients with type 2 diabetes (aged 52.2 ± 6.3 y) consumed 1, 3, or 6 g of cinnamon or placebo daily for a period of 40 days. Cinnamon consumption led to a major reduction in fasting serum glucose (18–29%), reduction in triglyceride (23–30%), reduction in LDL (7–27%), and total cholesterol (12–26%) concentrations in each of the cinnamon supplementation trials. The present study shows that 4 weeks of cinnamon supplementation does improve triglyceride, LDL, and total cholesterol. Consequently, the authors concluded that small amounts of cinnamon likely represent a safe and effective means to reduce the risk factors for the development of co-morbidities associated with diabetes.

In the present study, we investigated the effects of short-cinnamon use (6 g/d) on fasting blood glucose. Consumption of cinnamon for 4 weeks significantly at (P <0.001) lowered the mean fasting blood glucose levels(47%) of diabetic individual as compared to their mean corresponding blood glucose values at the start of the experiment (day 0), and also lower the mean of triglyceride(22%), LDL(28%), and total cholesterol(26%).

This trend was justified as cinnamon was potentiating the function of insulin in carbohydrate metabolism. Khan et al.(1990) have reported that an unidentified factor is present in cinnamon that potentiates the action of insulin in carbohydrate metabolism. They termed this factor as insulin potentiating factor (IPF). Broadhurst, et al. (2000) reconfirmed the presence of this factor in cinnamon. This hypoglycemic effect of cinnamon may or may not be like other hypoglycemic drugs. This unidentified factor increased the activity of insulin 3 fold in glucose metabolism in rat epididymal rat fat cell. Anderson et al.(2006) characterized this unidentified factor present in cinnamon as methylehydroxy chalcone polymers (MHCP). They explained that MHCP made fat cells more responsive to insulin by activating the enzyme that causes insulin to bind to cells (insulin-receptorkinase) and inhibiting the enzyme that blocks this process (insulin-receptorphosphatase) leading to maximal phosphorylation of the insulin receptor, which is associated with increased insulin sensitivity.

Ziegenfuss *et al*,(2006) trial with diabetic adults in Germany showed less pronounced, but still noteworthy, results with a water-soluble cinnamon extract that was equivalent to 3 g/day of whole cinnamon powder. Their findings indicate that consuming cinnamon for 12-weeks leads to significant improvements in several features of the metabolic syndrome (i.e., fasting blood sugar, systolic blood pressure, and body composition).

Previously shown trials revealed a marked insulinmimetic effect of cinnamon powder, resulting in improved blood glucose regulation. Other trials showed somewhat different results, and sometimes to contradictory results, matters that may depend on how the many variables involved affect one another. Vanschoonbeek *et al.*(2006) reported no effect of 1.5 g/d x 6 weeks cinnamon powder on indices of glycemic control in 25 postmenopausal women from the Netherlands. This study is different from that of Khan *et al.* (2003) and Mang *et al.*(2006), as well as the current study, in that only postmenopausal females were included as subjects. Whether differences in hormonal affect the potential interaction between cinnamon supplementation and glucose control is unknown at this time.

Moreover, Altschuler et al. (2007) explained their negative results in the light of mechanistic differences between type 1 and type 2 diabetes, i.e., the lack of endogenous insulin production in the former. They were the first to suggest that cinnamon may act by stimulating endogenous insulin production. If this was true, it would explain our results. However, this contention does not fit well with the majority of published research, which instead suggests a mechanism focused on the insulin receptor. Moreover, Altschuler et al.(2007) further added that it is also possible that participants were not given cinnamon for a long enough duration. Because 90 days is less than the full 120-day lifespan of red blood cells, perhaps this shorter duration contributed to a false-negative result. However, we believe that 90 days is a sufficient time in which to demonstrate an effect, and also pointed out that these results are consistent with other recent observations (Mang et al. 2006.; Vanschoonbeek et al, 2006).

In summary, this study provides evidence that cinnamon is effective in decreasing glucose level and lipids level among type 2 diabetic individuals. Coupled with other recent research, our results demonstrate positive effect on decreasing fasting blood glucose and lipids levels , it introduces significant remarks regarding the efficacy of cinnamon in diabetic subjects. In the light of this research, it is recommended that diabetic individuals should use cinnamon in their food preparations on regular basis. This will keep their sugar level and lipids levels near to normal values.

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Culturable Whitefly Associated Bacteria and Their Potential as Biological Control Agents

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Abstract

Bio-pesticides play an important role in reducing the deleterious effects associated with using conventional insecticides. For this reason, the potential of eleven whitefly-associated bacterial isolates as biological control agents was studied under lab conditions. These bacteria were three gram negatives; Erwinia persicinus, Pseudomonas plecoglossicida and Pseudomonas putida, and 8 gram positives; Brevibacterium casei, Staphylococcus gallinarum, Bacillus pumilus, Bacillus licheniformis, Bacillus subtilis, Exiquobacterium acetylicum, Exiguobacterium undae, and Micrococcus caseolyticus. Results revealed that Erwinia persicinus. Bacillus pumilus and Exiquobacterium acetylicum were the most effective in reducing Bemisia tabaci 2nd nymphal instar populations. Erwinia persicinus was the most promising bacterial isolate to be developed as a biological control agent

الملخص

يلعب استخدام المبيدات الميكروبية دورا هاما في التقليل من الآثار الضارة المصاحبة لاستخدام المبيدات الحشرية التقليدية. لذلك فقد كان الهدف من هذا البحث هو تجربة إمكانية استخدام إحدى عشرة عزلة بكتيرية تم عزلها من جسم حشرة الذبابة البيضاء بهدف استخدامها كمبيدات بكتيرية على نفس الحشرة التي عزلت منها. جربت ثلاث عزلات بكتيرية سالبة لصبغة غرام هي Pseudomonas و putida و ثماني عزلات موجبة لصبغة غرام هي:

Brevibacterium casei, Staphylococcus gallinarum, Bacillus pumilus, Bacillus licheniformis, Bacillus subtilis, Exiquobacterium acetylicum, Exiguobacterium undae

و Micrococcus caseolyticus.

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Keywords: Bio-insecticide, IPM, Mortality, Aleurodidae, Bemisia tabaci, Erwinia persicinus.

1. Introduction

The sweet potato whitefly, Bemisia tabaci Gen. (Homoptera: Aleurodidae), is a key pest of vegetables in Jordan (Al-Musa et al., 1987). It is also a serious polyphagous economic pest attacking more than 600 plant species worldwide of agronomic, horticultural, and ornamental crops (Gennadius, 1989; Byrne et al., 1990; Brown, 1994). Whitefly management has not traditionally relied on neonicotinoid use (McKenzie et al., 2005), it currently relies on these pesticides. The adverse effects are likely, regardless of the chemical used, and are not particular trait of neonicotinoids (Palumbo et al., 2001). However, the increasing resistance of Bemisia species to insecticides provides an impetus to use integrated pest control measures, including biopesticides and biological control to combat this pest (Atevvat, 2009b). To date, no bacterial insecticide has been discovered with sufficient activity against whiteflies to warrant commercial production. Insect-associated bacteria may be promising to control whiteflies. For example Enterobacter cloacae

exhibited a mild pathogenicity with 34% mortality towards the silver leaf whitefly, *Bemisia argentifolii* (Davison et al., 2000). Thus, the present study is attempted to investigate the toxic potential of eleven whiteflyassociated bacterial isolates that were isolated from adults and nymphs of *B. tabaci* collected from different host plants grown in different regions of Jordan in 2007 (Ateyyat et al., 2009a) towards the sweet potato whitefly, *B. tabaci*.

2. Materials and Methods

2.1. Bacterial Isolates

The tested bacteria were three gram negatives; Erwinia persicinus,Pseudomonas plecoglossicida and Pseudomonas putida, and 8 gram positives; Brevibacterium casei, Staphylococcus gallinarum, Bacillus pumilus, Bacillus licheniformis, Bacillus subtilis, Exiquobacterium acetylicum, Exiguobacterium undae, and Micrococcus caseolyticus. Designation, bacterium name and source of isolated bacteria from Bemisia tabaci whitefly are presented in Table 1.

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| | | Source | | | | | | |
|-------------|-----------------------------|-----------------|----------------|--------------|--|--|--|--|
| Designation | Бастегит - | B. tabaci stage | Host plant | Location | | | | |
| MAZ-1 | Bacillus licheniformis | nymphs | Cotton | Homrat Sahen | | | | |
| MAZ-2 | Micrococcus caseolyticus | nymphs | Snake-Cucumber | Salt | | | | |
| MAZ-3 | Brevibacterium casei | adults | Cotton | Homrat Sahen | | | | |
| MAZ-4 | Staphylococcus gallinarum | adults | Cotton | Homrat Sahen | | | | |
| MAZ-5 | Bacillus pumilus | nymphs | Snake-Cucumber | Salt | | | | |
| MAZ-9 | Bacillus subtilis | adults | Snake-Cucumber | Baqa' | | | | |
| MAZ-30 | Exiquobacterium acetylicum | nymphs | Cucumber | Salt | | | | |
| MAZ-36 | Pseudomonas putida | nymphs | Snake-Cucumber | Salt | | | | |
| MAZ-40 | Erwinia persicinus | nymphs | Cauliflower | Ghor | | | | |
| MAZ-B2 | Exiguobacterium undae | adults | Cotton | Homrat Sahen | | | | |
| MAZ-C4 | Pseudomonas plecoglossicida | adults | Cotton | Homrat Sahen | | | | |

Table 1. Designation, bacterium name, and source of isolated bacteria from Bemisia tabaci whitefly.

2.2. Enrichment of Bacterial Isolates

The bacterial isolates were enriched by inoculation into 100 ml of nutrient broth (Oxoid Ltd, Cambridge, UK); amended with 0.1% Tween 20; and incubated in an orbital shaker incubator (Orbital 4535, Farma Scientific, Canada) at 28°C with shaking at 150 rpm for overnight. After incubation, bacterial culture broths were adjusted at 600 nm to get an optical density of (0.5) where $A_{600nm}^{=0.5}$ was equivalent to 1 x 10⁸ CFU/ml.

2.3. Bemisia Tabaci Culture

Bemisia tabaci was cultured in a controlled greenhouse compartment at $24 \pm 2^{\circ}$ C and a 16h photoperiod on potted cotton plants, *Gossypium hirsutum* L. The plants were grown in 15 cm diameter pots filled with a mixture of 1:1 sand and peat moss. To obtain *B. tabaci* 2nd instars of uniform ages, tomato plants (variety Guardian, Enza Zaden, Jordan) with 4–5 leaves were exposed to oviposition by placing within the infested cotton plants in the culture. After a 24–48h oviposition period, plants were removed from the culture and all adult *B. tabaci* were aspirated from them. The plants were then placed in a *B. tabaci* free greenhouse compartment at $24 \pm 2^{\circ}$ C and a 16h photoperiod where *B. tabaci* eggs were allowed to hatch and the nymphs develop for 9–10 days to second instar nymphs.

2.4. Mortality effects on B. tabaci 2nd instar nymphs

The effect of the bacterial isolates on the sweet potato whitefly was studied under laboratory conditions using a

leaf-dip bioassay. A leaf cage was prepared from two 9 cm Petri plates by attaching the bottom of the upper plate to the cover of the lower plate. A four mm hole was made through the two plates. Tomato leaflets infested with 2nd instar nymphs of the sweet potato whitefly (as described above) were inserted through those holes following treatment. Tomato leaflets were treated by immersion in each isolate broth for 5 s. The treated leaflets were placed under a laminar hood until air dried after they were transferred to the leaf cages. A two cm opening covered with whitefly proof muslin was cut in the cover of the upper plate. The bottom of the lower plate was filled with water to prevent the wilting of the tomato leaflet. A 9 cm filter paper was placed in the bottom of the upper plate to provide additional humidity. Each leaf cage was assigned randomly to one of the isolates and to a control treatment. The control treatment used non-inoculated broth. There were 7 replicates (leaf cages) for each isolate. The leaf cages were incubated at 24°C ±2 and a 16h photoperiod, and whitefly mortality was recorded on 1, 3, and 5d post application. A whitefly nymph was considered dead if it was shriveled or if its color changed to brown when compared to the normal pale yellow color. In order to confirm pathogenicity of bacteria showed toxic activity against whiteflies, these bacterial isolates were recovered from the dead inoculated whiteflies with these bacterial isolates. The liquid culture of these bacterial isolates showing toxic activity against the whiteflies was centrifuged into the supernatant and pellet. If the bioassay with the supernatant caused high mortality, then the bacteria is considered toxic to whiteflies.

2.5. Effects of bacterial concentration on B. tabaci 2^{nd} instar nymphs.

Based on the results from the previous bioassay, three bacterial isolates *Erwinia persicinus, Bacillus pumilus*, and *Exiquobacterium acetylicum* were selected for further studies. Broths of these bacterial isolates were enriched as described above and measured by a spectrophotometer at 600 nm to get three different optical densities (0.5, 1, and 2) where $A_{600nm}^{=1}$ was equivalent to 2 x 10⁹ CFU/ml. The bacterial isolate and density combinations were bioassayed as above except that mortality was recorded 5d post application.

2.6. Statistical Analysis.

Arcsine-transformed percentage data were subjected to a one-way ANOVA, followed by a Least Significant Differences test at 95% confidence level (SAS Institute, 2005).

3. Results

Bemisia tabaci 2^{nd} nymphal instars showed no significant changes in the shape and color when treated with the non-inoculated broth. Twenty four hours post treatment, all tested bacterial isolates, produced significantly (*F* =262.15; *df*=11,72; *P*<0.001) greater mortality towards *B. tabaci* 2^{nd} nymphal instars when compared with the control treatment (Fig.1).



Figure 1.Percentage mortality of *Bemisia tabaci* 2^{nd} nymphal instars (± SE), 1 day post application with bacteria. Percentages were arcsine transformed before analysis. Means with the same letter are not significantly different using LSD at 95 % confidence level.

Pathogenicity of these bacteria was confirmed as these bacteria were recovered from the dead inoculated whiteflies with these bacterial isolates. *Erwinia persicinus, Bacillus pumilus,* and *Exiquobacterium acetylicum* gave levels of insect mortality that was significantly greater than those produced by the other bacterial isolates over the same period (Fig. 1). After 3 days, *Erwinia persicinus, Bacillus pumilus,* and *Exiquobacterium acetylicum* continued to produce significantly higher levels of mortality (F = 134.17; df = 11,72; P < 0.001) towards the *B. tabaci* 2nd nymphal instars in comparison with the other bacterial isolates (Fig. 2). Even though an increase in the mortality of *B. tabaci* 2nd nymphal instars after using the





Figure 2. Percentage mortality of *Bemisia tabaci* 2^{nd} nymphal instars (± SE), 3 days post application with bacteria. Percentages were arcsine transformed before analysis. Means with the same letter are not significantly different using LSD at 95 % confidence level.

bacterial isolates was recorded 5 days post treatment when compared with that recorded after 1 and 3 days, the treatments showed the same scenario within these three periods (Figs. 1- 3). Using *Erwinia persicinus* resulted in significantly (F = 58.8; df = 11,72; P < 0.001) higher mortality compared with other isolates (Fig. 3), and this was the only isolate that resulted in more than 50% mortality five days post application (Fig. 3).



Figure 3. Percentage mortality of *Bemisia tabaci* 2^{nd} nymphal instars (± SE), 5 days post application with bacteria. Percentages were arcsine transformed before analysis. Means with the same letter are not significantly different using LSD at 95 % confidence level.

Further studies using the three most promising isolates *Erwinia persicinus, Bacillus pumilus,* and *Exiquobacterium acetylicum* showed that mortality caused by these isolates did not increase remarkably with increasing densities of the bacterial cultures (Fig. 4). These three bacterial isolates resulted in mortalities ranging between 30 and 52% (Figs 1-3). For these isolates, mortality of 2nd nymphal instar *B. tabaci* increased with



Figure 4. Mortality of *Bemisia tabaci* 2nd nymphal instars treated with three bacterial isolates at three different concentrations.



Figure 5. Time response mortality of *Bemisia tabaci* 2nd nymphal instars exposed to three bacterial isolates, isolated from whitefly adults.

time, and the most notable increase in mortality was observed with *Erwinia persicinus* from 36 to 52% (Fig. 5).

4. Discussion

Screening candidate biological control agent (BCAs) from a variety of microorganisms and environments is both difficult and laborious (Enva et al., 2007). Microorganisms that can grow on the phyllosphere may be better candidate BCAs than those that cannot (Andrews, 1992). Some of these bacteria are originally reside in plant tissues, mainly inside vascular tissues without doing harm to the plant, and they transfer to the whiteflies as they probe the vascular tissues of their host plants (Fukui et al., 1999; Kobayashi and Palumbo, 2000). This finding implies that cultivable whitefly-associated bacteria may be effective as BCAs against B. tabaci. In the present study, mortality towards *B. tabaci* 2nd nymphal instars was obtained, which exceeded 50% using Erwinia percisinus that was isolated from whiteflies collected from cauliflower plants (Ateyyat et al., 2009a). Another species of Erwinia, Erwinia aphidocola isolated from the homopteran pea aphid showed mild pathogenicity against aphids (Harada and Ishikawa, 1997).

Among the tested bacteria, *Exiquobacterium* acetylicum (43 % mortality) and *Bacillus pumilus* (40% mortality) exhibited mild pathogenicity towards *B. tabaci* 2^{nd} nymphal instars. The mild pathogenecity of the tested bacteria may have resulted from the production of antimicrobial metabolites that affect the mutualistic bacteria such as the well-known *Buchnera* spp. For example, the antibiotic gallidermin is produced by the Gram-positive bacterium, *Staphylococcus gallinarum* (Kempf et al., 2000). Gallidermin exhibits a powerful bacteriocidic activity against Gram-positive bacteria (Hörner et al., 1990). Although competition between BCAs and other associated microorganisms is generally considered an important factor in reducing the suppression activity of BCAs (Weller, 1988), the interactions between BCAs and resident bacteria on *B. tabaci* are poorly understood because the structure of the bacterial community on whiteflies is also poorly understood.

In this study, the effect of the tested bacterial isolates against *B. tabaci* 2^{nd} nymphal instars was evaluated using the highly susceptible stage of whitefly in a leaf dip bioassay. This technique is expected to be better than suspending bacteria isolates in sucrose and feeding them to adults through parafilm sachets used by Davidson et al. (2001). We observed mortality after 1, 3 and 5 days post treatment. After that, we stopped getting the mortality because it exceeded 20 % in the negative control treatment. The highest increase in mortality from the first to the fifth day was obtained by *Micrococcus caseolyticus* (from 5% to 25 %), followed by *Erwinia percisinus* (from 36% to 52 %). The trials to increase the concentration of the three bacteria species to enhance their effectiveness as biological control agent did not give a valuable increase.

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هيئة التحرير

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الأعضاء:

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