Benghazi University

Faculty of Science - Department of Botany

The effect of temperature levels on water permeability of different fruit cuticular layers

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by:-

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بسم الله الرحمن الرحيم

﴿قل اعملوا فسيرى الله عملكم ورسوله والمؤمنون﴾

سورة التوبة 105

الإهداء

إلى مَنْ يَسعدُ قَلْبِي بلقياها

إلى رَوضَة الْحُبّ التّي تَنْبُتُ أزْكَى الأَزْهَار

أُمي

إلى رَمْزُ الْرُجُولَةُ وَالتَضْحِيَةُ

إلى مَنْ دَفَعَنِي إلى الْعَلْمُ وَبِهِ أَزْدَاد افْتِخَارَ

أَبِي

إلى مَنْ هُوَ أَقْرَبُ إليّ مِنْ رُوحِي

إلى مَنْ شَارَكَنِي حَضْنَ أَلامُ وَبِهُمُ أَسْتَمِدُ عَزْتِي وَإِصْرَارِي

أَخْوَاتِي

إلى مَنْ أَنَسنِي فِي دِرَاسَتِي وَشَارَكْنِي هُمُومِي

تِذْكَاراً وَتَقْدِيراً

أصدقائي

الشكر والتقدير

الحمد لله ذي المن والفضل والإحسان ،حمدا"يليق بجلاله وعظمته وصل اللهم على خاتم الرسل من لانبى بعده صلاة تقضى لنا بها الحاجات وترفعنا بها أعلى الدرجات ،وتبلغنا بها أقصى الغايات من جميع الخيرات في الحياة وبعد الممات ، لله الشكر أولا وأخيرا، على حسن توفيقه وكريم عونه وعلي ما من وفتح به علي من أنجاز هذا العمل المتواضع

كما أدين بعظيم الفضل والشكر والعرفان بعد الله سبحانه وتعالى في انجاز هذا البحث وإخراجه بهذه الصورة إلى الدكتور/**سالم عبد العالي الشطشاط** الذي منحى الكثير من وقته وجهده وتوجيهاته وارشادته وآرائه القيمة .ومد يد العون لي دون ضجر للسير قدما بالدارسة نحو الأفضل سائله المولى القدير أن يجزيه عنى خير جزاء ويثبته الأجر أن شاءالله.

كما اشكر كل من تقدم وأفادني وقدم لي النصائح والإرشادات والى أعضاء هيئه التدريس بقسم علم النبات والعاملين به ،وإلى إدارة كلية العلوم ، و العاملين بمكتب الشؤون القانونية وإدارة جامعة بنغازي .

كما أتقدم بالشكر والتقدير إلى زوجي **أبوبكر عبد الحميد** علي دعمه المعنوي ووقوفه إلي جانبي وتشجعيه لي حتى أتمكن من أتمام دراستي العليا.

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ABSTRACT

The plant cuticle forms the interface between the aerial environment and the living cells of the plant. Therefore, the cuticle has to manage multiple physiological and ecological functions like controlling water loss.

One of the physical factors influenced water permeability of cuticles is temperature. This study was conducted on fruits included tomatoes*(Lycopersicon esculentum*.L) ,yellow and red grape (*Vitis vinifera.* L) , and plume *(Prunus domestica*.L) .which collected from local market in Benghazi. Libya , These fruits has been collected carefully and accurately so that they were almost equal in size ,where they were visually examined to ensure that they are free from any damages or injuries caused by micro-organisms through dermis layer of fruits, where the measure and calculate the area of each fruit separately and control water loss through the sensitive balance weight and embrace the whole night. And recording of these weights in private tables and then measure the water loss through the dermis layer, where the change of temperature of fruits starts at 15 C° to 45 C° and every time we weigh and calculate the proportion of water permeability of fruits where fruits change.

The fruits change starts with external shape size ,and atrophy tissues with increase in temperature, and thus calculate the total average for each type of fruits and statistically a analyzed by one way using(spss) program.

The findings indicated that these fruits are affected to varying different temperatures, the higher the temperature , the higher percentage of water permeability through the dermis layer. Also, the results showed that the average of permeability of fruits vary

according to the temperature where starting from 15C°, to 45 C° to increase in water loss. The temperature above 25 C° and the loss of water in all kinds of fruits was very high. This difference is due to the difference in the composition of cuticular of fruits and ecophysiological adjustment which was genetically proved.

INTRODUCTION

Water is essential for life and it plays a major role in all physiological processes of the plant cell. Thus, both shortage and excess of water can cause physiological problems for plants. To control or avoid negative environmental conditions, plants, like all other living organisms, have developed a suite of physiological, anatomical and morphological adaptations. Most plant species possess specific adaptations to their habitats. One basic adaptation of plants for their survival on the mainland is the plant cuticle. Studies of Silurian and Devonian plant fossils showed that cuticles are very resistant and the oldest known cuticles are over 400 million years old (Woodward 1998, Edwards *et al*. 1996*)*. Early studies on the nature of cuticles were started in the 20th century (Kolattukudy 1981).

The cuticle is defined as a heterogeneous, extracellular biopolymer (Schönherr and Huber 1977, Kirsch *et al.* 1997),which is synthesized by epidermal cell(Marge *et al.*2001).

The cuticle covers all primary above-ground parts of the plants, such as leaves and fruits (Schönherr 1976a *;* Marga *et al.* , 2001, Round *et al.* 2000, Jetter and Schäffer, 2001, Neinhuis *et al.* 2001; Niederl *et al.* 1998*)* but not woody stems and wounds (Kerstiens 1996). It forms the interface between the plant cell and the atmosphere (Niederl *et al.* 1998, Luque *et al.* 1995, Jetter and Schäffer 2001). The cuticle forms an effective barrier against desiccation(Marga *et al.* 2001) and thus the main function of the cuticle is the reduction of water loss It forms the interface between the plant cell and the atmosphere (Niederl *et al.,* 1998, Luque *et al.* 1995, Jetter and Schäffer 2001).

The cuticle forms an effective barrier against desiccation(Marga *et al.* 2001) and thus the main function of the cuticle is the reduction of water loss from plants when the stomata are closed (Schönherr 1976a). The cuticle also acts as the first protective barrier against UV radiation (Mariani and Wolters-Arts, 2000) and it reduces leaching, e.g. it protects leaves from an excessive loss of ions and nutrients (Niederl *et al.*, 1998**).** It is clear from different studies and researches that the temperature has important effects on plant life, not only the low temperature but also the high lest one.

The aim of the study

A number of studies showed different effects of different factors on water permeability of isolated cuticular membranes , but a little is known about the effect of like these factors on water permeability of intact organs like leaf and fruit.

Therefore, the aim of this study is to test the effect of different temperature levels on water permeability of some fruit of different plant species using the whole fruit.

LITERATUR REVIEW

The plant cuticle is a hydrophobic, continuous and flexible thin (from 0,1 to 10 µm); (Vogg *et al.* 2004) membrane consisting of two lipid fractions; the polymer matrix (cutin polymer or cutin-containing layer) and cuticular waxes which are deposited on the outer surface and embedded in the matrix (Luque *et al*. 1995).

The cutin polymer, which makes up the bulk of the cuticular membrane (Schönherr 1976b), forms the mechanically stable polymer matrix (Round *et al.* 2000), which is attached to the epidermal cell wall with a pectinaceous layer (Kolattukudy 1981) and presumably other cell wall carbohydrates. It is a lipophilic, amorphous polymer membrane (Holloway 1982).

Cutin is composed of mainly C16- and C18-hydroxy fatty acids cross-linked by ester bonds(Kolattukudy 1981, Riederer and Schreiber 2001). Polysaccharides, such as pectin, crystalline cellulose and hemicelluloses are also embedded in the polymer matrix (Jeffree 1996, Schönherr and Baur 1996). In addition, polyuronic acids, proteins and phenolic compounds can be found in cutin (Schönherr 1976b). Cutin amounts range from 20 % to 84 % by weight of the isolated cuticles (Schönherr 1976b). The second important fraction of the cuticle is composed of soluble lipids.

These represents a complex mixture of aliphatic and cyclic compounds and they are often called cuticular waxes (Schönherr and Riederer 1989). These lipids consist of intracuticular waxes, which are embedded within the cutin polymer matrix and of epicuticular waxes, which are deposited as thin films and aggregates on the leaf and fruit surfaces. The structure is summarized in(Figure 1).

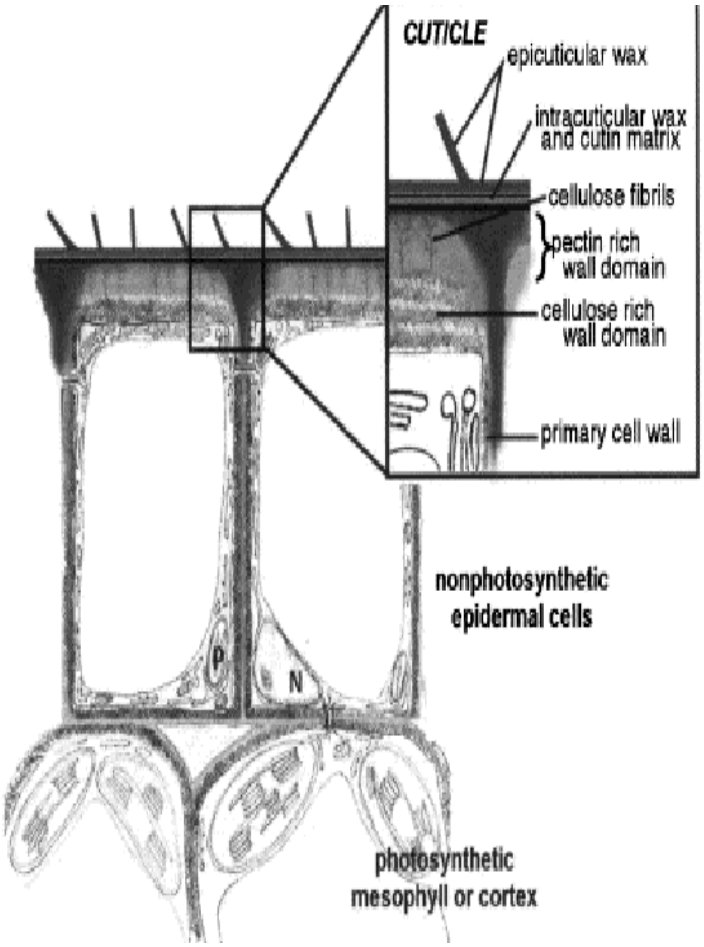


Figure 1. Schematic drawing of the structure of the cuticular membrane showing the components of the cuticle: the cuticle proper (cutin) forms an electron dense layer over the epidermal cells; both, intracuticular waxes and epicuticular waxes form the surface lipids (Kunst and Samuels, 2003).

Cuticular wax is a general term for a complex heterogeneous mixture of very long-chain (C20 - C34) fatty acids and their derivatives (Rhee *et al.*, 1998). They are synthesized from C16- and C18-precursors that are produced in the plastids (Bird and Gray, 2003). In addition varying proportions of cyclic compounds such as pentacyclic triterpenoids and hydroxycinnamic acid derivatives (Riederer and Markstädter ,1996) are part of the wax. The proportion of these compounds differs among plant species and even among the different tissues of an individual plant (Mariani and Wolters-Arts, 2000). Although these waxes represent a low amount of the total mass of the cuticle, from 1 to 10 % (Walton ,1990), they are responsible for 90 to 99, 9 % of the total resistance of the cuticular membrane to water loss (Riederer and Schreiber, 1995). Removing them from the cuticle using organic solvent such as chloroform has demonstrated their efficiency in forming a barrier. The correlation between the chemical composition of cuticular waxes and their function as a transpiration barrier is still unsolved (Vogg *et al.*, 2004). The upper leaf side has usually more epicuticular wax crystals compared to the lower side. The formation of cuticular waxes has always been discussed with the problems of their movement through the cuticle (Neinhuis *et al.*, 2001). Neinhuis *et al.*, (2001) suggested that the molecules, which finally form the cuticular waxes diffuse through the cuticle as molecules dissolved in water.

Knowledge on amounts and chemical composition of cuticular waxes is necessary in order to understand their functions. These features (amounts and composition) depend on endogenous and exogenous factors (Riederer and Markstädter ,1996). A number of studies have shown that environmental factors such as light, humidity and temperature may influence the amount and composition of cuticular waxes (Riederer and Markstädter, 1996). Dynamic changes of epicuticular waxes during leaf development (aging factor) were also reported (Jetter and Schäffer, 2001).

There are two physical properties of particular interest, which have been recently revised: the rheological and thermal characteristics. They concern the water relationship with the cuticle and, consequently, with the cutin. The role of plant cuticles, and more specifically the cuticles waxes, as barriers against the transport and diffusion of water, has been extensively studied (Kerstiens and Wolters, 2000).

However, questions such as the exact relationship between the molecular transport properties and the mechanical characteristics, in other words, the rheological properties, of the plant cuticles are still unraveled Connected with these research lines are the studies on the thermal properties of plant cuticles The debate on the existence of polar pores in the cuticles membranes that may contribute to the permeability of water and polar solutes still remains open.(Riederer and Schreiber,2001) have recently reviewed this controversial topic and they have concluded that the bulk of water diffuses as single molecules across the lipophilic barrier that constitutes the cutin and waxes, while a only minor fraction moves through the more polar pores present in the cutin matrix.

The rheology of the plant cuticle and cutin is of particular interest. It is known that the diffusion and sorption across polymers is influenced by the mechanical properties of the polymer itself. Some factors that affect these properties are the presence of fillers and plasticizers polymer density, in the polymer matrix and the temperature Two important physiological problems are related to these properties. One of them is the use of foliar applied chemicals, which could modify the permeability of the biopolymer. The other physiological tissue is the fruit cracking as a consequence of an insufficient flexibility of the cutin. Cuticle cracking is a persistent and widespread problem in some greenhouse grown fruits, that causes degradation of fruit appearance and subsequently serious economic losses(Aloni *et al* ,1998) Despite the importance

of cuticle in the potential elucidation of these physiological problems, there are only very few studies on cuticular rheology. From stress-strain studies, (Petracek and Bukova, 1995) described the cuticle as a viscoelastic polymer network. These authors also reported that isolated tomato fruit cuticles expanded and became more elastic and susceptible to fracture after hydration, suggesting that water plasticizes the cuticle.

Some authors have used atomic force microscopy and solid-state (NMR) to investigate the effect of water sorption on the elastic properties of isolated tomato fruit cutin (Round *et al* 2000)the interesting conclusion can be formulated from this singular study: water absorbed by the cutin acts as a plasticizer promoting molecular flexibility and softening the polymer network. One can visualize that water disrupts Hydrogen bonded cross-links between chains and also diminishes chain–chain methylene hydrophobic interactions. Temperature-dependent changes in isolated plant cuticles, waxes and cutin have also been performed Isolated plant cuticles and cutins from several species showed a significant high specific heat.

This high value means that he cuticular material requires greater amount of heat to raise their temperature by 1j of temperature. Specific heat value of cutin was around 2–2.5 J K^{-1} g . whereas cellulose, main component of plant cell wall, has a specific heat of 1.5 J K^{-1} g. Although the cuticular material contributes only as a minor mass fraction to the whole leaves and fruits, it could play an important role as a thermoregulatory in the course of the biophysical interaction between the plant and the environment.

When analysing the permeation of solutes and water molecules across the plant cuticle, it can be treated as a homogeneous solubility/mobility membrane (Riederer and Schreiber, 1995). In this case, the transport across the plant cuticle is simply occurring along the chemical potential that is caused by the difference of the

concentrations of the permeating molecules between the inside leaf and the outside of the leaf. The mechanism of foliar penetration consists of two phases; surface adsorption (an initial phase), and cuticular penetration. It is initiated when a droplet of water containing some solute comes in contact with the cuticle (Schönherr and Riederer , 1989). The permeating molecules are sorbed by the membrane on one side, kipenetrate it, dissolved as single molecules within the membrane phase, and they leave the membrane on the other side. However, this model can be only used with lipophilic solutes and it reaches its limits when polar compounds are considered (Riederer and Schreiber, 2001). Alternatively, a model suggesting two parallel paths of diffusion across the plant cuticle was suggested (Schönherr 2000, Riederer and Schreiber, 2001). The first pathway, similar to that described above, is formed by the amorphous phases of cutin and wax, which can be used only by lipophilic solutes.

The second path is formed by polar pores of molecular dimensions filled with water, which can be penetrated by water, and polar charged organic as well as inorganic compounds (Riederer and Schreiber, 2001). The diameter of polar pores in isolated cuticular membranes devoid of cuticular waxes was determined using organic molecules of known diameter. The pore radius was estimated to be around 0.45 nm for Citrus and Alliums (Schönherr, 1976 b).(Schönherr ,1976a) argued that these pores are dynamic structures and they arise only on hydration of polar functional groups in the polymer matrix. Due to very small radii of the pores, the molecule size is one of the important properties that determine mobility of polar solutes in the cuticle. Thus, only small molecules can diffuse in these pores (Schönherr and Riederer ,1989).

The barrier properties of the cuticle depend to a large extent on cuticular waxes. Therefore, the transport across the plant cuticle mainly depends on the wax layer,

which consists of crystals that are embedded within a cutin matrix of amorphous material. The crystals or impermeable flakes; Riederer and Schreiber, 1995) reduce the volume of the barrier available for diffusion and lead to a highly tortuous paths across it (Figure. 2)

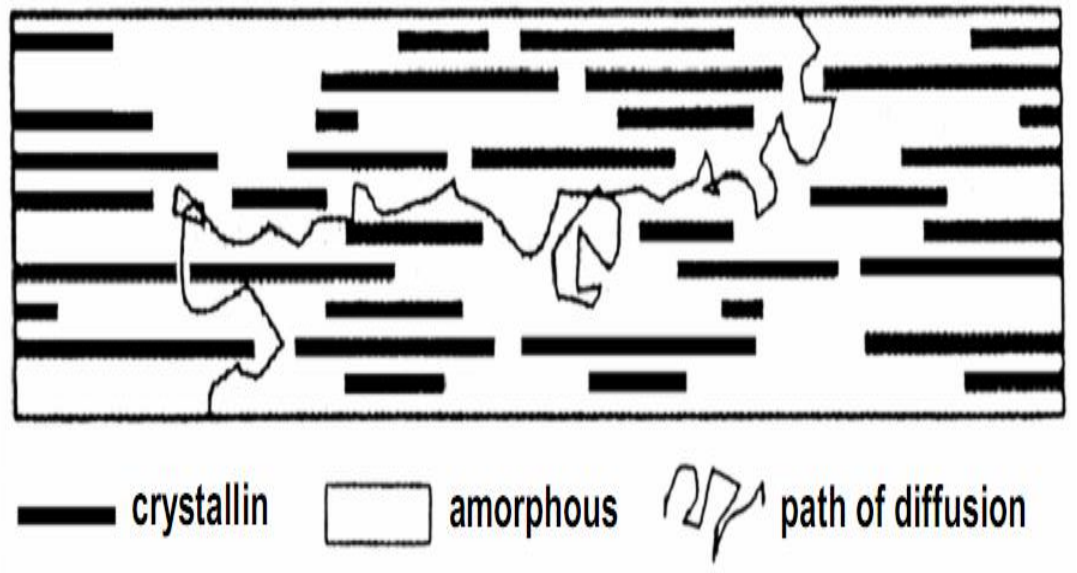


Figure 2. Tortuosity of the pathway through the cuticular membrane; The solute molecule move through the amorphous wax and jump from vacancy to vacancy. Dependent on crystalline wax formation and their distribution, crystalline waxes reduce the volume of the amorphous phase available for diffusion (Riederer and Schreiber, 1995).

The plant cuticle forms the interface between the aerial environment and the living cells of the plant. Therefore, the cuticle has to manage multiple physiological and ecological functions. It is an effective barrier to the transport of solutes and gases in and out of the cell(White *et al.*, 2002) and it plays an important role during the foliar uptake of agrochemicals (Burghardt *et al*. 1998). It reduces leaching and thus prevents leaves from an excessive loss of ions and nutrients(Tyree *et al*., 1992, Niederl *et al.*, 1998).

It also presents the major barrier to penetration of plant tissues by a variety of environmental chemicals such as sulfuric and nitric acid, when the plants are exposed to these acids (Hauser *et al*. 1993). Furthermore, it forms the primary barrier against bacterial and fungal attacks and reduces the infection of plants by pests and pathogens. The cuticle can also protect the photosynthetic tissues from excess light by reflecting and scattering and subsequently attenuating the light to such an extent that it causes no damage to the tissues.

The permeability is a parameter that is characteristic for a given type of cuticle, a given solute(or solvent) and at a given temperature (Schönherr and Riederer, 1989). The permeability is a useful parameter for describing permeability of cuticular membranes and it is defined as follows:

$$\mathbf{P = F / (A \cdot \Delta c)}$$

(F) represents the flow rate, (A) the exposed area of the cuticle and (Δc) the concentration difference between donor and receiver compartments also called the driving force for diffusion.

Water permeability of isolated cuticular membranes has been studied extensively in the last years, especially from an ecophysiological point of view. Water permeabilities of plant cuticles from different species are highly variable. They differ not only among different species, but also differ within the same species. They can even vary within the isolated crystalline amorphous path of diffusion for cuticles obtained from the same organ (leaf or fruit).

Interspecific variability varies over 2.5 orders of magnitude (Riederer and Schreiber, 2001). Cuticular water permeability is not correlated to the thickness or to wax coverage of the cuticle (Riederer and Schreiber, 2001).

The differences of water permeability are caused by ecophysiological adaptations that are genetically fixed. In adaptation to their habitats, ever green epiphytic or climbing plants growing naturally in tropical climates and species adapted to dry climates exhibited the lowest water permeability. In contrast the highest water permeability were observed with the deciduous plants growing in temperate climates(Schreiber and Riederer ,1996). Studies of fruit cuticles indicated that their water permeability were about 10 times higher than those of leaf cuticles with highest water permeability (Riederer and Schreiber, 2001). Cuticular permeability is influenced by physical (temperature, humidity, pH) and chemical (adjuvants, pollutants) factors. Many studies and investigations of cuticular permeability showed that water permeability was increased by increasing temperature (Schönherr and Baur, 1996), relative humidity (Schreiber *et al.* 2001) and by increasing pH (Schönherr, 1976a).

it is obvious that cuticular waxes play an important and a decisive role in determining permeabilities of cuticles. they form the transport barrier even though they make up only a small percentage of the total mass of the cuticle. extracting the

waxes from the cuticle reveals their efficiency as a barrier. the correlation between wax chemical composition and their function as transpiration barrier is poorly understood (vogg *et al.* , 2004).

The effect of epicuticular wax on cuticular permeability is not completely known at this time because of the difficulties in removing epicuticular waxes without affecting intracuticular waxes. therefore, only the effect of the complete wax extraction has been studied (Schönherr and Riederer,1989). Polymer matrix membranes are membranes where wax has completely been extracted. Their permeability of water and solutes are one to three orders of magnitude higher than those of cuticular membranes (CMs) (Schönherr, 1982). As described above, two parallel pathways in cuticular

membranes for permeating molecules were hypothesized. There are estimations, that the pores occupy about 6 ppm of the surface area of the cuticle (Tyree *et al*., 1992).

Increasing water permeability of (MXs) up to three orders of magnitude, suggest that 100 to 1000 times more pores were exposed by removing cuticular wax (Tyree *et al.* 1992).

Water permeability of cuticles increases also with increasing air humidity. This was demonstrated by using isolated cuticular membranes by a number of investigators *(*Schönherr and Schmidt 1979, Schönherr and Merida 1981, Schreiber *et al.* 2001*).* The effect of humidity is caused by water molecules sorbing to the polar sites of the cuticle, which leads to the formation of polar pores, and eventually, increasing water permeability. permeation of some kinds of cations to cuticular membranes increases also water permeability. With increasing humidity, rates of salt penetration increase, due to dissolution of salt residues on the surface of the cuticle *(*Schönherr 2000, 2001*).* This process is controlled by the point of deliquescence (POD) of the salt

(Schönherr and Luber 2001), which is defined as the conversion of a solid substance into a liquid as a result of absorption of water vapor from the air. The salt residue could sorb the moisture from the air is above the POD, depending on humidity and hygroscopicity of the salt. When the humidity the salt residues on the cuticle dissolve and penetration occurs, while below a solid crystalline residues are formed and the uptake process stops (Schönherr and Luber 2001).

The membrane permeability may be affected by solution pH in three ways (Schönherr and Riederer 1989): direct effect of pH, effect on the driving force via electrical potentials, and change of the properties of the solutes by dissociation. The cuticles are polyelectrolytes and their isoelectric point (IEP) is around pH 3 (Schönherr and Huber, 1977).Above this point, when pH increases, the cuticles carry fixed negative charges. These charges are an important characteristic affecting the water content of the polymer matrix via swelling (Şahin *et al*,. 2002). Unionized carboxyl groups are little hydrated (Schönherr and Riederer ,1989), and when the pH increase, the ionization degree of these functional groups will increase, they become able to attract more water molecules to the polymer matrix (swelling) and subsequently water permeability will be increased The radius of the water filled pores is not pH dependent. With increasing pH level , the number of pores increased but not their radii. (Schönherr, 1976a) reported that the number of pores per cm^2 was increased from 5·1010 to around 16·1010 when the pH level was increased from 3 to 9.(Beyer *et al* 2002) reported that pH gradients between donor and receiver solutions are also very important to sorption of cations to plant cuticles, which reduced water uptake of the cuticles.

Rate of plant growth and development is dependent upon the temperature surrounding the plant and each species has a specific temperature range represented

by a minimum, maximum, and optimum. These values were summarized by (Hatfield *et al.* 2008,2011) for a number of different species typical of grain and fruit production . the expected changes in temperature over the next 30-50 years are predicted to be in the range of 2-3c°(In tergovernmental panel climate change (IPCC)(2007). heat waves or extreme temperature events are projected to become more intense, more frequent, and last longer than what years (Meehl *et al*. ,2007).extreme temperature events may have short-term durations of a few days with temperature events may have short- term duration of a few days with temperature increases of over 5c°a above the normal temperature. extreme events occurring during the summer period would have the most dramatic impact on plant productivity; however, there has been lithe research conducted to a recent review by (Barlow *et al*, (2015). On the effect of temperature extreme s ,frost and heat, in wheat revealed that frost caused sterility and a abortion of formed grains while excessive heat ,caused reduction in grain number and reduced duration of the gravelling period . analysis by (Meehl *et al*, .(2007). revealed that daily minimum temperature will increase more rapidly than daily temperatures and a greater likelihood of extreme events and these changes cloud have detrimental effects on grain yield.

If these changes. In temperature are expected to occur over the next 30 years then understanding the potential impacts on plant growth and development will help develop adaptation strategies to offset these impacts.

MATERIALS AND METHODS

Plant material: Mature fruits of tomato *Lycopersicon esculentum* Mill., grape *Vitis vinifera* L., and plume *Prunus domestica* L. were purchased on the market of Benghazi city, Libya, in 2014. They were selected for their size uniformity of each fruit type and were visually investigated to exclude any damages or infections by microorganisms. The area of each fruit cuticle calculated using the fruit radius which determined manually by venire caliper (Table 1)

	FRUIT RADIUS (cm)			
No.	Tomato	Grape R	Grape Y	Plume
1	2.6	2.4	2.5	3.3
2	2.7	2.4	2.6	3.3
3	2.7	2.4	2.3	3.2
4	2.5	2.5	2.3	3.5
5	2.5	2.5	2.2	3.7
6	2.5	2.4	2.4	3.6
7	2.5	2.4	2.3	3.4
8	2.3	2.5	2.5	3.9
9	2.4	2.4	2.3	
10	2.4	2.4	2.3	
MEAN	2.51	2.43	2.37	3.49
S.D	0.13	0.05	0.13	0.24

Table 1: The uniformity of the fruit size. The radius was determined manually using Venire caliper and subsequently used to find out the exposed area of fruit cuticle to silica gel.

Measurement of water loss: Water permeability of fruit cuticular membranes was determined using a gravimetric method. 10 fruits of each plant species were placed in closed polyethylene boxes above silica gel. In order to prevent damage of the membranes; a flat metal net was placed between the fruits and silica gel granules. The boxes prepared in this way were incubated in an incubator (Binder, Tuttlingen, Germany) at 15, 25 ,35 , and 45C°, respectively. The incubation period was overnight in all fruits. After incubating fruits at each temperature level, Water loss was monitored by weighing the fruits every 1 to 2 hours for 4 to 5 times. Water loss was determined with a microbalance (Sartorius Analytic BP 221S, Göttingen, Germany).Amounts of water diffused across the fruit membranes were sum ad up and plotted as a function of time. Rates of water loss were calculated from linear regression lines fitted to the plotted data.

Calculations of fruit cuticular water permeability: water permeability of each isolated fruit species was determined using the equation :

$$P = F / (A \cdot \Delta c)$$

Where P is permeability, F ($g \cdot s^{-1}$) represents the flow rate, A (cm^2) the area of the fruit cuticle and Δc ($g \cdot m^{-3}$) the driving force for diffusion. The water permeability of each individual fruit was calculated. After that, the mean of total permeability of each fruit species was determined.

Regression equations were fit to transpiration kinetics and means of permeability of 10 fruits were calculated. Results are given as means with standard error.

Statistical analysis: the data were collected and the mean of 7-10 fruits with standard deviation calculated .one way analysis of variation was also used to find out the differences between treatments (spss version).

THE RESULTS

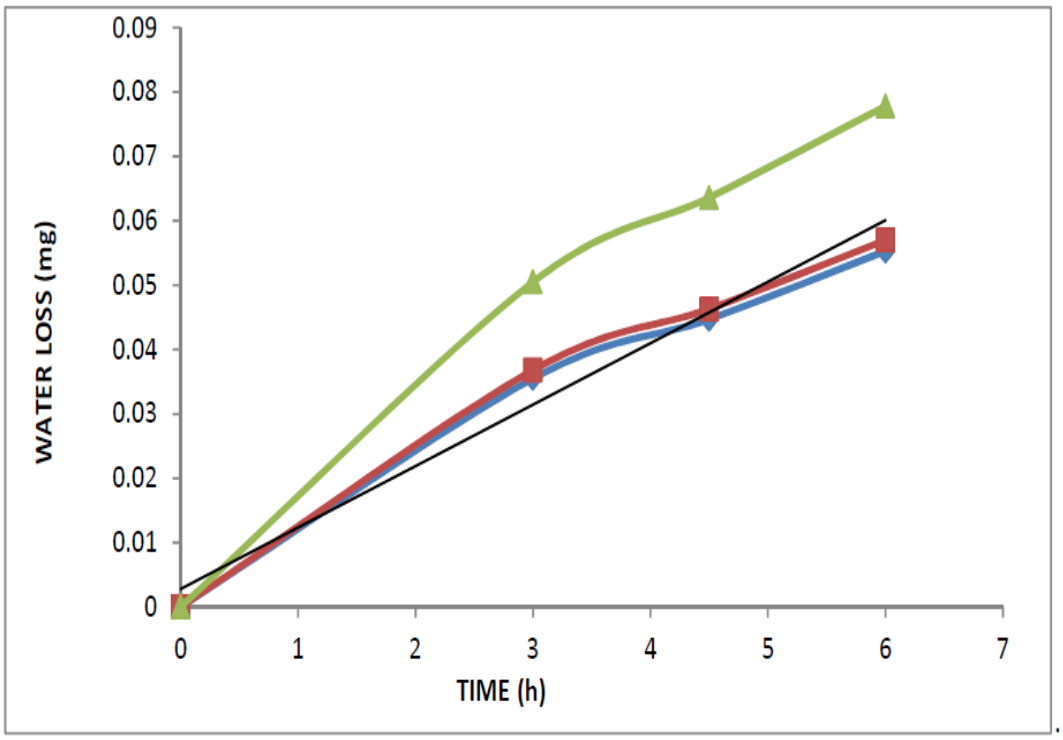
The correlation factor (r^2) of water loss through fruit cuticles

The water loss of all type fruits was determined depending on the correlation between weight loss of water and time in seconds. The correlation factor was very high between these factors and r^2 was more than 0.97 for all fruit types (Figure3).

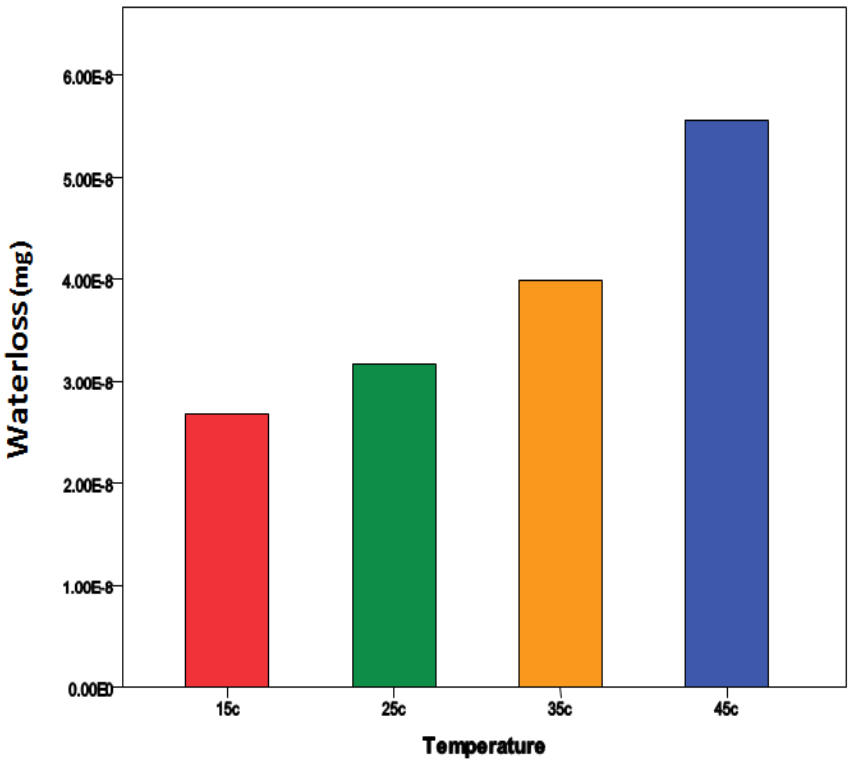
Tomato fruit (*Lycopersicon esculentum* L.) **permeability**

It is clear from the results of tomato fruits that increasing temperature levels was increased water permeability through the fruit cuticle (Figure4). But there was no significant effect between the temperature levels using statistical analysis of one way classification when ($p < 0.01$)or ($p \leq 0.05$) .The mean of permeability of tomato fruits were (1.29E-08 ± 2.35E-09), (9.46E-08 ± 2.02E-07), (4.67968E8 ± 9.44E-09) and (6.49503E.08 ± 1.36E-08) for the temperature of 15 C°, 25 C° , 35 C°, 45 C° respectively.

Calculations of temperature effect which determined by divided the permeability after treatment by that before treatment **(P_{after}/P_{before})** showed that the effect was increased in all treatments and it was (1) , (2.00) , (3.12) and (4.10)f for the temperature of 15 C°, 25 C° , 35 C°, 45 C° respectively. (Figure5)



Figure(3) Water loss through fruit cuticles per time



Figure(4) Water permeability of tomato (*Lycopersicon esculentum* L.) at different temperature levels.

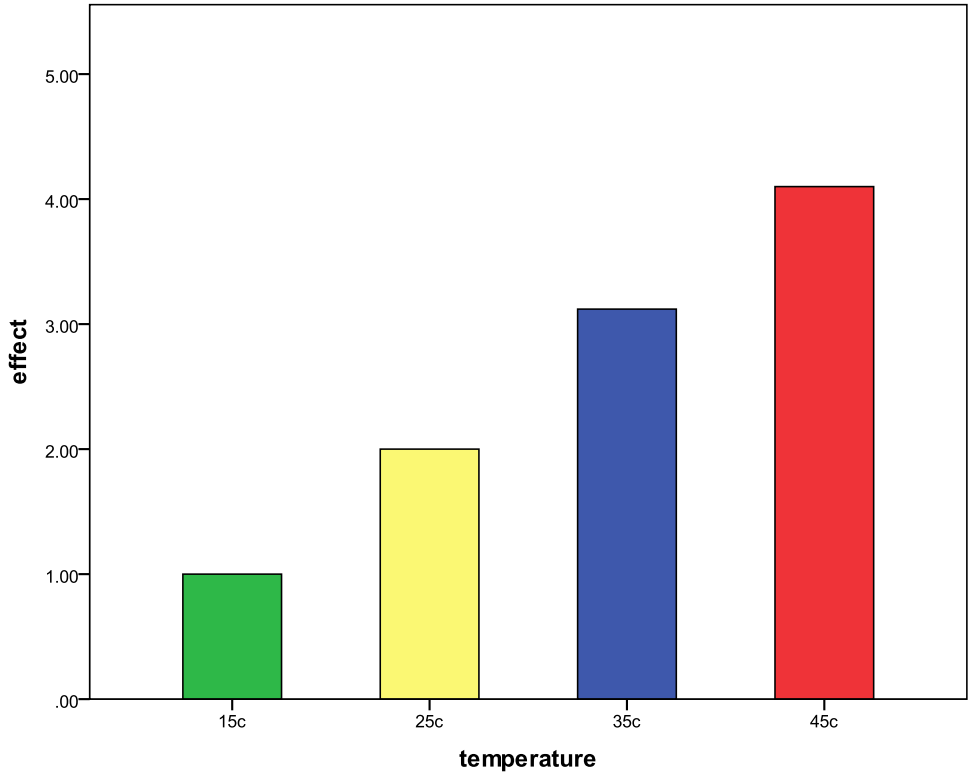
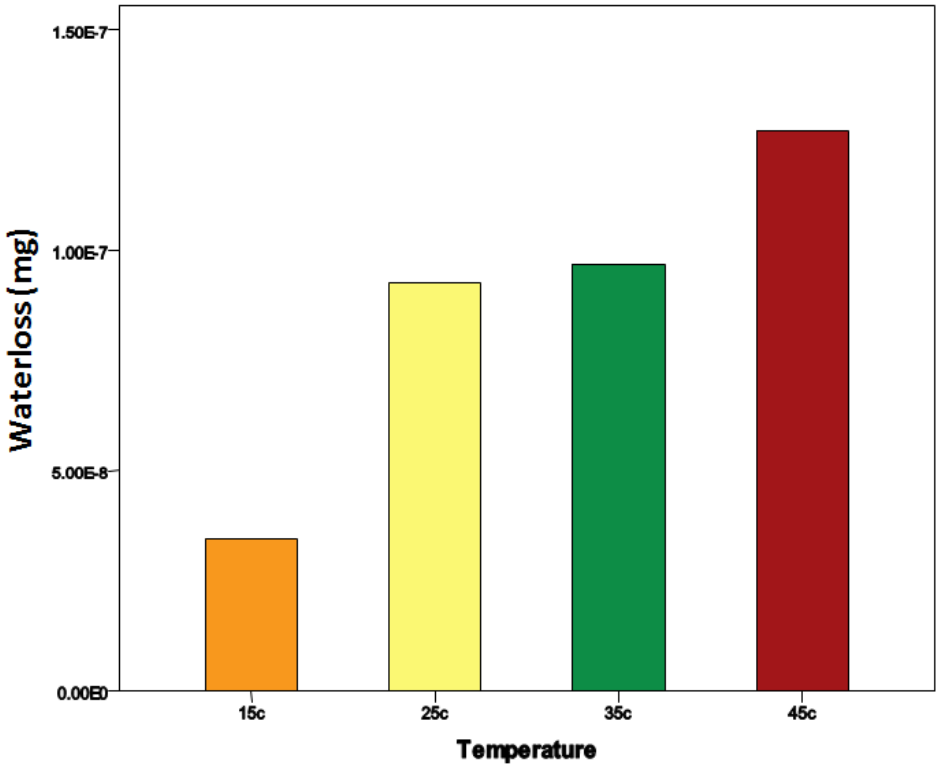


Figure (5) The effect of temperature treatment on water permeability of tomato fruit cuticle of (*Lycopersicon esculentum* L.).

Yellow grape(*vitis vinifera* L.) **fruit permeability**

from the results of yellow grape fruits ,increasing temperature level was increased water permeability through the fruit cuticle (Figure 6). But there was no significant effect between the temperature levels using statistical analysis of one way classification when($p < 0.01$)or ($p \leq 0.05$) .The mean of permeability of yellow grape fruits were (3.45E-08 ± 7.39E-09) , (9.26E-08 ± 4.14E-08) ,(9.66E-08± 2.03E-08),and (2.17E-08± 1.27E-07). for the temperature of 15 C°, 25C ° , 35C °, 45C ° respectively. In addition the results showed that the effect was increased in all treatments and it was (1) ,(2.68) ,(2.80) and (3.68)for the temperature of 15 C°, 25C° , 35C °, 45C ° respectively.(Figure7)



Figure(6) Water permeability of at Yellow grape (*vitis vinifera* L.) at different temperature levels.

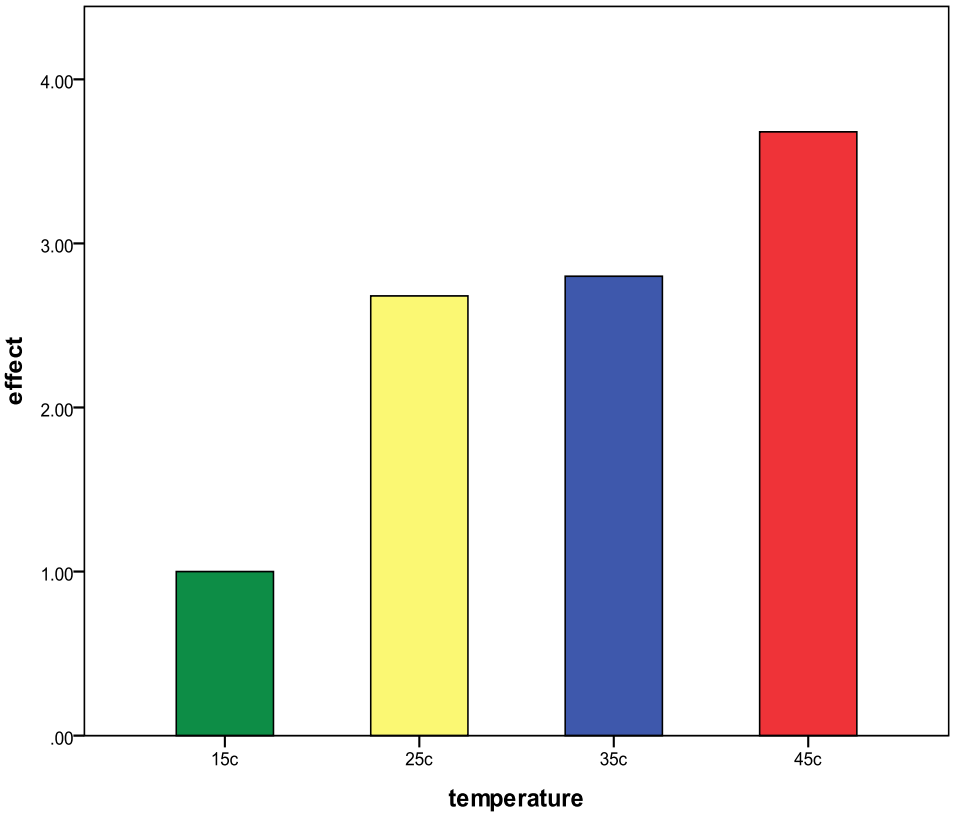
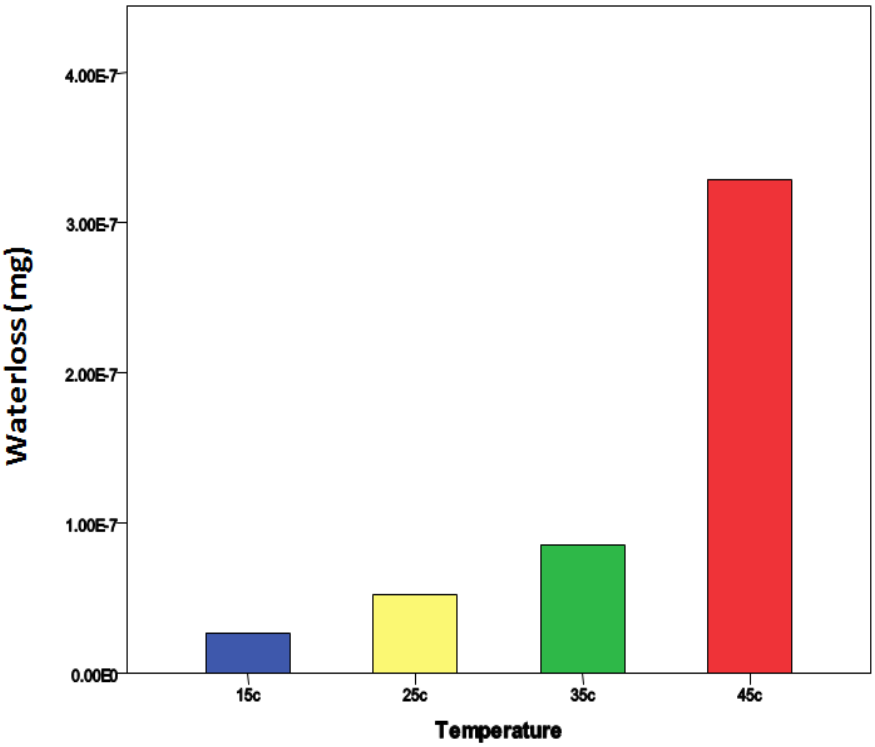


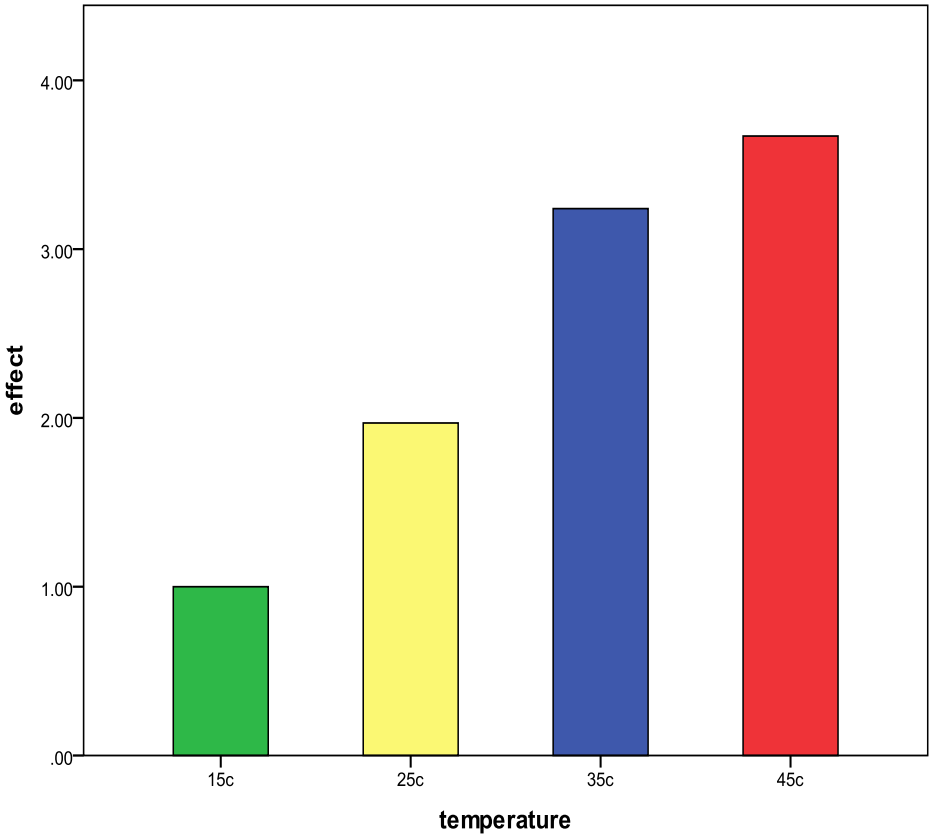
Figure (7) The effect of temperature treatment on water permeability of fruit cuticle of Yellow grape*(vitis vinifera* L.)

Red grape (*vitis vinifera* L.) **fruit permeability**

The results of red grape fruits showed that increasing temperature levels was increased water permeability through the fruit cuticle (Figure8). But there was no significant effect between the temperature levels using statistical analysis of one way classification when ($p < 0.01$)or ($p \leq 0.05$). The mean of permeability of red grape fruits were (2.63E-08 ± 9.56E-09) ,(5.19E-08 ± 8.7E-07) ,(8.54E-08± 7.39E-09) ,and **(**3.29E-07± 7.92E-09). for the temperature of 15 C°, 25C ° , 35C °, 45C ° Respectively Calculations of temperature effect which determined by divided the permeability after treatment by that before treatment (P_{after}/P_{before}) showed that the effect was increased in all treatments and it was (1) ,(1.97) ,(3.24) and (3.67) for the temperature of 15 C°, 25 C° , 35 C°, 45 C° respectively.(Figure 9).



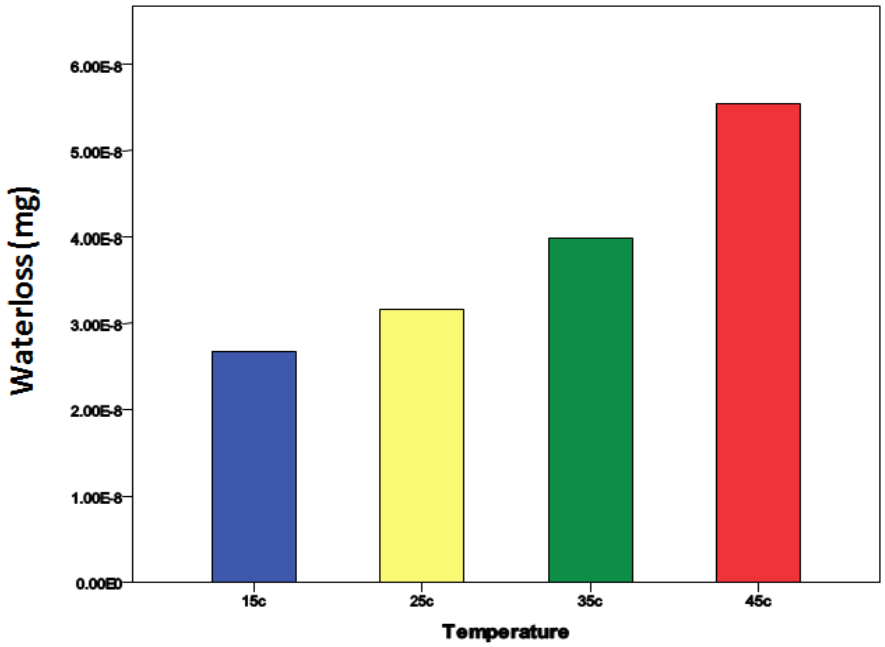
(Figure8) Water permeability of at Red grape(*vitis vinifera* L.) at different temperature levels.



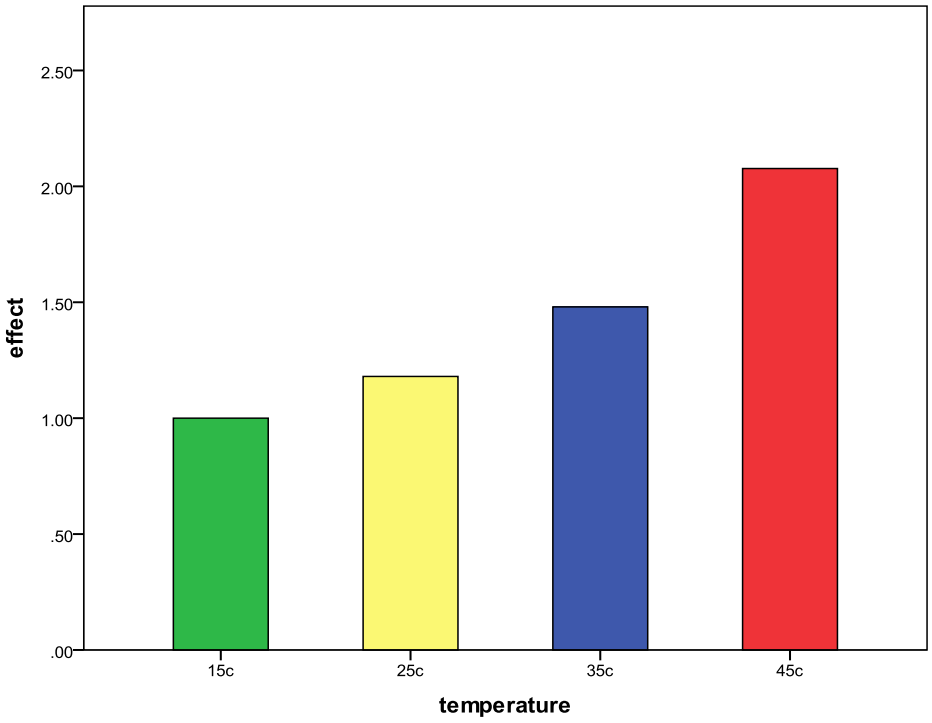
(Figure9) The effect of temperature treatment on water permeability of fruit cuticle of Red grape (*vitis vinifera* L.)

Plume (*Prunus domestica*) **fruit permeability**

It is clear from the results of plum fruits that increasing temperature levels was increased water permeability through the fruit cuticle (Figure10). But there was no significant effect between the temperature levels using statistical analysis of one way classification when ($p < 0.01$)or ($p \leq 0.05$). The mean of permeability of plum fruits were (2.67E-08 ± 1.3E-08) ,(3.16E-08 ± 1.62E-08**) ,(** 3.98E-08 ±1.13E-08), and (5.55E-08± 1.21E-08 **).** for the temperature of 15C °, 25C ° ,35 C°,45C ° respectively Calculations of temperature effect which determined by divided the permeability after treatment by that before treatment **(P_{after}/P_{before})** showed that the effect was increased in all treatments and it was(1), (1.18), (1.48) and (2.07) for the temperature of 15 C°, 25 C° , 35 C°, 45 C° respectively.(Figure11).



(Figure10) Water permeability of plume (*Prunus domestica* L.) at different temperature levels.



(Figure11)T he effect of temperature treatment on water permeability of fruit cuticle of plume (*Prunus domestica* L.)

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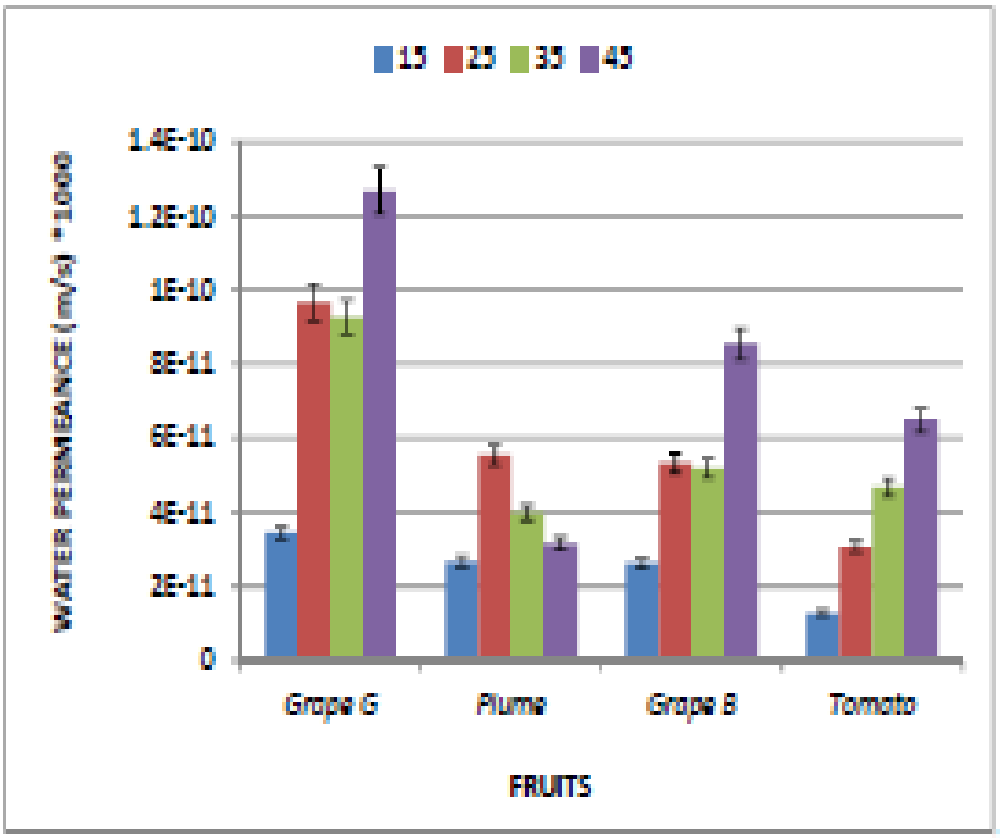

(Figure12) Increasing water permeability of different fruits at different temperature levels started from 15c° to 45c°

DISCUSSION

From the results, it is clear that all fruit species appeared high water permeability through the cuticular membrane. Even though some of these fruits reflected less water loss than others, but they are still in the level.The plant cuticle forms the interface between the aerial environment and the living cells of the plant. Therefore, the cuticle has to manage multiple physiological and ecological functions. It is an effective barrier to the transport of solutes and gases in and out of the cell(White *et al*., 2002) and it plays an important role during the foliar uptake of Agrochemicals (Burghardt *et al*., 1998). It reduces leaching and thus prevents leaves from an excessive loss of ions and nutrients(Tyree., 1992, Niederl *et al*., 1998).

Water permeability of plant cuticles from different species are highly variable. They differ not only among different species, but also differ within the same species. They can even vary within the cuticles obtained from the same organ (leaf or fruit). Interspecific variability varies over 2.5 orders of magnitude (Riederer and Schreiber 2001). Cuticular water permeability is not correlated to the thickness or to wax coverage of the cuticle (Riederer and Schreiber, 2001). The differences of water permeability are caused by ecophysiological adaptations that are genetically fixed. In adaptation to their habitats, ever green epiphytic or climbing plants growing naturally in tropical climates and species adapted to dry climates exhibited the lowest water permeability.

In contrast the highest water permeability were observed with the deciduous plants growing in temperate climates (Schreiber and Riederer. ,1996). Studies of fruit cuticles indicated that their water permeability were about 10 times higher than those of leaf cuticles with highest water permeability (Riederer and Schreiber,

2001).Knowledge on amounts and chemical composition of cuticular waxes is necessary in order to understand their functions. These features (amounts and composition) depend on endogenous and exogenous factors (Riederer and Markstädter, 1996). A number of studies have shown that environmental factors such as light, humidity and temperature may influence the amount and composition of cuticular waxes (Riederer and Markstädter, 1996). Dynamic changes of epicuticular waxes during plant development (aging factor) were also reported (Jetter and Schäffer ,2001).

There are two physical properties of particular interest, which have been recently revised: the rheological and thermal characteristics. They concern the water relationship with the cuticle and, consequently, with the cutin. The role of plant cuticles, and more specifically the cuticles waxes, as barriers against the transport and diffusion of water, has been extensively studied (Kerstiens and Wolters ,2000). There is no question that creating a good water barrier, and hence allowing a plant to control water loss through regulation of its stomata conductance, represents the major physiological role of plant cuticles. However, permeabilitiy for water (Kerstiens, 1996b; Riederer and Schreiber, 2001) and other compounds (Buchholz *et al*., 1998; Niederl *et al*., 1998) differ by up to about three orders of magnitude between different types of cuticles. It is likely that this huge variation is due, at least in part, to the cuticle's involvement in many other processes, in past and present environments, is poor. Without it and an appreciation that the biosynthesis of many cuticular components is tied up with other metabolic processes that have been subjected to further pressures and constraints, it will remain difficult to determine why different plants have such vastly different cuticles, in terms of ultra structure and chemical composition, and how to improve them in crop plants with regard to desirable traits

such as drought or pest resistance without impairing others. Recent progress in the more applied areas relevant to cuticular permeability, particularly the study of foliar uptake of lipophilic, hydrophilic, and ionic Agrochemicals, has been impressive, but there is still quite a poor understanding of some of the most basic physiological differing not just between species but between different organs of a species (i.e. stems and leaves, which may respond quite differently to manipulation of the same gene)

It is obvious that cuticular waxes play an important and a decisive role in determining permeability of cuticles. They form the transport barrier even though they make up only a small percentage of the total mass of the cuticle. The barrier properties of the cuticle depend to a large extent on cuticular waxes. Therefore, the transport across the plant cuticle mainly depends on the wax layer, which consists of crystals that are embedded within a cutin matrix of amorphous material. The crystals (or impermeable flakes).(Riederer and Schreiber, 1995) reduce the volume of the barrier available for diffusion and lead to a highly tortuous paths across it. The structural and compositional variability is of particular importance for the cuticular permeability to water, as this compound is likely not only to use the lipophilic pathway (i.e. random diffusion in the lipophilic polymer and accessible wax domains) but, to some unknown extent probably depending on circumstances, aqueous pores as Cuticular permeability to water is usually characterized by the variable permeability (P), which is the ratio of the water flow rate density to driving force, the latter being expressed as a concentration). In the case of water, the concentration is often expressed as density of liquid water, but there are advantages in using the equivalent concentration of water vapor in the gas phase,, in particular with respect to temperature effects (Kerstiens, 1996b). When liquid water is present on one side of the cuticle .the rate of plant growth and development having dependent upon the temperature surrounding

the plant and each species a specific temperature range represented by a minimum, maximum, and optimum. it is clear from different studies and researches that the temperature has important effect on plant life, not only the low temperature but also the high one. If these changes. In temperature are expected to occur over the next 30 years then understanding the potential impacts on plant growth and development will help develop adaptation strategies to offset these impacts. In general, increasing temperature reduces the amounts of any diffusions sorbed by the cuticle but increases their mobility, with the overall effect being positive in the case of water. The temperature dependence of permeation is quantified by its activation energy; the stronger the temperature dependence, the higher is the activation energy. Temperature is the predominant physical factor influencing the permeability of a barrier. Two terms contributing to permeability are temperature-dependent: the diffusion coefficient of a molecule diffusing in the membrane increases with temperature while its partition coefficient between the membrane and the adjacent phases (generally) decreases.

Enhanced cuticular water permeability by approximately one order of magnitude a strong dependence of cuticular permeability on temperature has also been reported for the penetration of organic solutes across plant cuticular membranes . It should be noted that these data are corrected for the temperature dependence of the water saturation deficit and thus describe the temperature effect on cuticular transport properties exclusively, under real conditions, the combination of decreasing cuticular resistance and increasing driving force will lead to drastically elevated flow rates of water across the cuticle.

The effect of temperature (P_{after}/P_{before}) was increased by increasing the temperature level from 15 C° to 45 C°. This might increase solubility of wax flakes and subsequently, the crystals became more permeable. It is clear from the results of species fruits that increasing temperature levels was increased water permeability through the fruit cuticle. But there was no significant effect between the temperature levels using statistical analysis of one way classification when ($p < 0.01$)or ($p \leq 0.05$). The mean of permeability of species fruits were different. results showed that the percentage of water permeability was differed a among the species . it is clear that the leves of 15 C°,25 C°,35 C°,45 C°.

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الملخص بالعربي

تشكل طبقة الكيوتكيل للنبات الوصلة بين البيئة الجوية والخلايا الحية للنبات.هذه الطبقة تمتلك العديد من الوظائف الفسيولوجية والبيئية التي تربط النبات بالعوامل البيئية، لذلك بعض النباتات تتميز بسمك هذه الطبقة والبعض آلاخر بقلة هذه الطبقة. والسبب الرئيسي يرجع ألي التكيف البيئي للنبات . هذه الطبقة تختلف في التركيب الكيميائي من نبات إلي نبات،لذلك أجريت هذه الدراسة علي مجموعه من ثمار النبات منها الطماطم ، العنب الأصفر، العنب الأحمر، البرقوق . تم تجميعها من سوق مدينة بنغازي ليبيا في(2014) حيث تم اختيار هذه الثمار بعناية ودقه بحيث تكون متساوية في الحجم تقريبا وخالية من آي إصابات أو هجمات ميكروبية، حيث يتم حساب مساحه أقطار هذه الثمار بأداة القدم ذات الورنية وتدوينها في جداول خاصة واحتضانها لمدة ليلة كاملة في الحضانة وحساب وزنها عن طريق ميزان الحساس ومراقبة خسارة الماء من خلال طبقة الكيوتكيل وتسليط مستويات مختلفة من درجات الحرارة تبد أمن 15 إلي 45 وحساب هذه الأوزان وقياس نسبة خسارة الماء ومعالجتها إحصائيا لاستخراج الفروقات المعنوية بين هذه الثمار ومدي التأثير هذه الطبقة بعامل مؤثر مثل(درجة الحرارة) وقياس نسبة خسارة الماء وتأثير درجة الحرارة عليه كان الغرض من هذه الدراسة هو اختبار تأثير مستويات مختلفة من درجات الحرارة علي نفاذية الماء لطبقة الكيوتكيل لثمار المستخدمة في هذه الدراسة .دلت النتائج علي أن هذه الثمار تتأثر بدرجات الحرارة المختلفة ،حيث أن كلما زادت درجة الحرارة زادت نسبة نفاذية الماء من خلال طبقة الكيوتكيل، أظهرت النتائج أن متوسط نفاذية هذه الثمار تختلف باختلاف درجات الحرارة وتبدأ الخسارة عند درجة حرارة 25 وفي كل الأنواع المستخدمة في هذه الدارسة كانت نسبة النفاية عالية جدا وكان السبب يرجع إلي اختلاف في(تركيب الكيوتكيل لهذه الثمار).

APPENDIX

ANOVA

Tomato

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	43.012	.000
Within Groups	.000	22	.000		
Total	.000	25			

Multiple Comparisons

(I) temperature	(J) temperature	Mean Difference (I-J)	Std. Error	
15c	25c	-1.51124333333E-8*	5.30566331291E-9	.0
	35c	-3.38873214286E-8*	5.15184317993E-9	.0
	45c	-5.02268500000E-8*	4.93930373953E-9	.0

Multiple Comparisons

	25c	15c	1.51124333333E-8*	5.30566331291E-9	.0
		35c	-1.87748880952E-8*	4.57290668094E-9	.0
		45c	-3.51144166667E-8*	4.33205595455E-9	.0
	35c	15c	3.38873214286E-8*	5.15184317993E-9	.0
		25c	1.87748880952E-8*	4.57290668094E-9	.0
		45c	-1.63395285714E-8*	4.14223777129E-9	.0
	45c	15c	5.02268500000E-8*	4.93930373953E-9	.0
		25c	3.51144166667E-8*	4.33205595455E-9	.0
		35c	1.63395285714E-8*	4.14223777129E-9	.0

*. The mean difference is

Tomato

LSD

(I) temperature	(J) temperature	95% Confidence Interval	
		Lower Bound	Upper Bound
15c	25c	-0.0000000261	-0.0000000041
	35c	-0.0000000446	-0.0000000232
	45c	-0.0000000605	-0.0000000400
25c	15c	0.0000000041	0.0000000261
	35c	-0.0000000283	-0.0000000093
	45c	-0.0000000441	-0.0000000261
35c	15c	0.0000000232	0.0000000446
	25c	0.0000000093	0.0000000283
	45c	-0.0000000249	-0.0000000077
45c	15c	0.0000000400	0.0000000605
	25c	0.0000000261	0.0000000441
	35c	0.0000000077	0.0000000249

GrapeR

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
15c	10	0.0000000263	9.06569087776E-9	2.86682317367E-9	0.0000000198	0.0000000328

25c	8	0.0000000529	1.04504026546E-8	3.69477529159E-9	0.0000000441	0.0000000616
35c	6	0.0000000527	4.29551165877E-9	1.75363529136E-9	0.0000000482	0.0000000572
45c	9	0.0000000949	7.92215863070E-9	2.64071954357E-9	0.0000000889	0.0000001010
Total	33	0.0000000563	2.77824194865E-8	4.83629846099E-9	0.0000000464	0.0000000661

Descriptives

	Minimum	Maximum
15c	1.67713000E-8	4.68123000E-8
25c	3.58386000E-8	6.69625000E-8
35c	4.75584000E-8	5.90990000E-8
45c	8.21980000E-8	1.07939000E-7
Total	1.67713000E-8	1.07939000E-7

ANOVA

GrapeR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	104.111	.000
Within Groups	.000	29	.000		
Total	.000	32			

Multiple Comparisons

Multiple Comparisons

GrapeR

LSD

		35c	1.92029166667E-10	4.59408248696E-9	.967
		45c	-4.20586708333E-8*	4.13345869385E-9	.000
	35c	15c	2.63759333333E-8*	4.39278305765E-9	.000
		25c	-1.92029166667E-10	4.59408248696E-9	.967
		45c	-4.22507000000E-8*	4.48336543416E-9	.000
	45c	15c	6.86266333333E-8*	3.90850737089E-9	.000
		25c	4.20586708333E-8*	4.13345869385E-9	.000
		35c	4.22507000000E-8*	4.48336543416E-9	.000

*. The mean difference is significant at the 0.05 level.

(I) temperature	(J) temperature	95% Confidence Interval	
		Lower Bound	Upper Bound
15c	25c	-0.0000000348	-0.0000000183
	35c	-0.0000000354	-0.0000000174
	45c	-0.0000000766	-0.0000000606
25c	15c	0.0000000183	0.0000000348
	35c	-0.0000000092	0.0000000096
	45c	-0.0000000505	-0.0000000336
35c	15c	0.0000000174	0.0000000354
	25c	-0.0000000096	0.0000000092
	45c	-0.0000000514	-0.0000000331
45c	15c	0.0000000606	0.0000000766
	25c	0.0000000336	0.0000000505
	35c	0.0000000331	0.0000000514

Descriptives

GrapeY

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
15c	10	0.0000000345	7.38735893718E-9	2.33608801347E-9	0.0000000292	0.0000000397
25c	9	0.0000000889	3.53719636656E-8	1.17906545552E-8	0.0000000617	0.0000001160
35c	10	0.0000000926	2.02558857027E-8	6.40547348447E-9	0.0000000781	0.0000001071

45c	9	0.0000001248	2.19291735722E-8	7.30972452407E-9	0.0000001080	0.0000001417
Total	38	0.0000000841	3.97818708393E-8	6.45347162279E-9	0.0000000710	0.0000000971

Descriptives

GrapeY

	Minimum	Maximum
15c	2.57017000E-8	5.32629000E-8
25c	4.48603000E-8	1.41470000E-7
35c	6.56415000E-8	1.38253000E-7

45c	8.25492000E-8	1.49637000E-7
Total	2.57017000E-8	1.49637000E-7

ANOVA

GrapeY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	25.453	.000
Within Groups	.000	34	.000		
Total	.000	37			

Multiple Comparisons

GrapeY

LSD

(I) temperature	(J) temperature	Mean Difference (I-J)	Std. Error	Sig.
15c	25c	-5.43951666667E-8*	1.05837347335E-8	.000
	35c	-5.81735500000E-8*	1.03014509361E-8	.000
	45c	-9.03868666667E-8*	1.05837347335E-8	.000
25c	15c	5.43951666667E-8*	1.05837347335E-8	.000
	35c	-3.77838333333E-9	1.05837347335E-8	.723
	45c	-3.59917000000E-8*	1.08586827208E-8	.002
35c	15c	5.81735500000E-8*	1.03014509361E-8	.000
	25c	3.77838333333E-9	1.05837347335E-8	.723
	45c	-3.22133166667E-8*	1.05837347335E-8	.004
45c	15c	9.03868666667E-8*	1.05837347335E-8	.000
	25c	3.59917000000E-8*	1.08586827208E-8	.002
	35c	3.22133166667E-8*	1.05837347335E-8	.004

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

GrapeY

LSD

(I) temperature	(J) temperature	95% Confidence Interval	
		Lower Bound	Upper Bound
15c	25c	-0.0000000759	-0.0000000329
	35c	-0.0000000791	-0.0000000372
	45c	-0.0000001119	-0.0000000689
25c	15c	0.0000000329	0.0000000759
	35c	-0.0000000253	0.0000000177
	45c	-0.0000000581	-0.0000000139
35c	15c	0.0000000372	0.0000000791

	25c	-0.0000000177	0.0000000253
	45c	-0.0000000537	-0.0000000107
45c	15c	0.0000000689	0.0000001119
	25c	0.0000000139	0.0000000581
	35c	0.0000000107	0.0000000537

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Int	
					Lower Bound	
15c	8	0.0000000267	1.30367034882E-8	4.60917072042E-9	0.0000000158	0.
25c	8	0.0000000555	1.61825968906E-8	5.72141199929E-9	0.0000000420	0.
35c	8	0.0000000398	1.12829224897E-8	3.98911550202E-9	0.0000000304	0.
45c	8	0.0000000316	1.20636040708E-8	4.26512812202E-9	0.0000000216	0.
Total	32	0.0000000384	1.67945124368E-8	2.96887840770E-9	0.0000000324	0.

Descriptives

Plume

	Minimum	Maximum
15c	1.30050000E-8	5.47315000E-8
25c	3.52229000E-8	8.01362000E-8
35c	3.01644000E-8	5.73635000E-8
45c	8.32301000E-9	4.58908000E-8
Total	8.32301000E-9	8.01362000E-8

ANOVA

Plume

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	7.211	.001
Within Groups	.000	28	.000		
Total	.000	31			

Multiple Comparisons

Plume

LSD

(I) temperature	(J) temperature	Mean Difference (I-J)	Std. Error	Sig.
15c	25c	-2.87800875000E-8*	6.63639097632E-9	.000
	35c	-1.30712375000E-8	6.63639097632E-9	.059
	45c	-4.92633875000E-9	6.63639097632E-9	.464
25c	15c	2.87800875000E-8*	6.63639097632E-9	.000
	35c	1.57088500000E-8*	6.63639097632E-9	.025
	45c	2.38537487500E-8*	6.63639097632E-9	.001
35c	15c	1.30712375000E-8	6.63639097632E-9	.059
	25c	-1.57088500000E-8*	6.63639097632E-9	.025
	45c	8.14489875000E-9	6.63639097632E-9	.230
45c	15c	4.92633875000E-9	6.63639097632E-9	.464
	25c	-2.38537487500E-8*	6.63639097632E-9	.001
	35c	-8.14489875000E-9	6.63639097632E-9	.230

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Plume

LSD

(I) temperature	(J) temperature	95% Confidence Interval	
		Lower Bound	Upper Bound
15c	25c	-0.0000000424	-0.0000000152
	35c	-0.0000000267	0.0000000005
	45c	-0.0000000185	0.0000000087
25c	15c	0.0000000152	0.0000000424
	35c	0.0000000021	0.0000000293
	45c	0.0000000103	0.0000000374
35c	15c	-0.0000000005	0.0000000267
	25c	-0.0000000293	-0.0000000021
	45c	-0.0000000054	0.0000000217
45c	15c	-0.0000000087	0.0000000185
	25c	-0.0000000374	-0.0000000103
	35c	-0.0000000217	0.0000000054

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for	
						Lower Bound	Upper B
temperature15	Tomato	5	0.0000000124	1.12422525456E-9	5.02768818246E-10	0.0000000110	0.000000
	grapeR	10	0.0000000263	9.06569087776E-9	2.86682317367E-9	0.0000000198	0.000000
	GrapeY	10	0.0000000345	7.38735893718E-9	2.33608801347E-9	0.0000000292	0.000000
	Plume	8	0.0000000267	1.30367034882E-8	4.60917072042E-9	0.0000000158	0.000000
	Total	33	0.0000000268	1.12587252263E-8	1.95989249645E-9	0.0000000228	0.000000
temperature25	Tomato	6	0.0000000275	3.96029740571E-9	1.61678464561E-9	0.0000000234	0.000000
	grapeR	8	0.0000000529	1.04504026546E-8	3.69477529159E-9	0.0000000441	0.000000
	GrapeY	9	0.0000000889	3.53719636656E-8	1.17906545552E-8	0.0000000617	0.000000
	Plume	8	0.0000000555	1.61825968906E-8	5.72141199929E-9	0.0000000420	0.000000
	Total	31	0.0000000591	3.00292238348E-8	5.39340781647E-9	0.0000000481	0.000000
temperature35	Tomato	7	0.0000000463	5.95885809047E-9	2.25223665790E-9	0.0000000408	0.000000
	grapeR	6	0.0000000527	4.29551165877E-9	1.75363529136E-9	0.0000000482	0.000000
	GrapeY	10	0.0000000926	2.02558857027E-8	6.40547348447E-9	0.0000000781	0.000000
	Plume	8	0.0000000398	1.12829224897E-8	3.98911550202E-9	0.0000000304	0.000000
	Total	31	0.0000000608	2.60924979036E-8	4.68635096660E-9	0.0000000512	0.000000
temperature45	Tomato	9	0.0000000626	1.21954177310E-8	4.06513924365E-9	0.0000000533	0.000000
	grapeR	9	0.0000000949	7.92215863070E-9	2.64071954357E-9	0.0000000889	0.000000
	GrapeY	9	0.0000001248	2.19291735722E-8	7.30972452407E-9	0.0000001080	0.000000
	Plume	8	0.0000000316	1.20636040708E-8	4.26512812202E-9	0.0000000216	0.000000
	Total	35	0.0000000799	3.76100695380E-8	6.35726205813E-9	0.0000000669	0.000000

ANOVA

		Sum of Squares	df	Mean Square	F
temperature15	Between Groups	.000	3	.000	6.499
	Within Groups	.000	29	.000	
	Total	.000	32		
temperature25	Between Groups	.000	3	.000	10.193
	Within Groups	.000	27	.000	
	Total	.000	30		
temperature35	Between Groups	.000	3	.000	28.598
	Within Groups	.000	27	.000	
	Total	.000	30		

temperature45	Between Groups	.000	3	.000	65.450
	Within Groups	.000	31	.000	
	Total	.000	34		

Multiple Comparisons

Dependent Variable	(I) factor	(J) factor	Mean Difference (I-J)	Std. Error	Sig.
temperature15	Tomato	grapeR	-1.39490400000E-8*	5.00921571705E-9	.009
		GrapeY	-2.20931400000E-8*	5.00921571705E-9	.000
		Plume	-1.43457775000E-8*	5.21375702108E-9	.010
	grapeR	Tomato	1.39490400000E-8*	5.00921571705E-9	.009
		GrapeY	-8.14410000000E-9	4.09000750610E-9	.056

		Plume	-3.96737500000E-10	4.33810806400E-9	.928
	GrapeY	Tomato	2.20931400000E-8*	5.00921571705E-9	.000
		grapeR	8.14410000000E-9	4.09000750610E-9	.056
		Plume	7.74736250000E-9	4.33810806400E-9	.085
	Plume	Tomato	1.43457775000E-8*	5.21375702108E-9	.010
		grapeR	3.96737500000E-10	4.33810806400E-9	.928
		GrapeY	-7.74736250000E-9	4.33810806400E-9	.085
temperature25	Tomato	grapeR	-2.53687791667E-8*	1.17061543731E-8	.039
		GrapeY	-6.13400833333E-8*	1.14240369066E-8	.000
		Plume	-2.79776416667E-8*	1.17061543731E-8	.024
	grapeR	Tomato	2.53687791667E-8*	1.17061543731E-8	.039
		GrapeY	-3.59713041667E-8*	1.05324416143E-8	.002

		Plume	-2.60886250000E-9	1.08377930096E-8	.812
	GrapeY	Tomato	6.13400833333E-8*	1.14240369066E-8	.000
		grapeR	3.59713041667E-8*	1.05324416143E-8	.002
		Plume	3.33624416667E-8*	1.05324416143E-8	.004
	Plume	Tomato	2.79776416667E-8*	1.17061543731E-8	.024
		grapeR	2.60886250000E-9	1.08377930096E-8	.812
		GrapeY	-3.33624416667E-8*	1.05324416143E-8	.004
temperature35	Tomato	grapeR	-6.40186190476E-9	7.48654680616E-9	.400
		GrapeY	-4.63435785714E-8*	6.63147220820E-9	.000
		Plume	6.50609642857E-9	6.96443884569E-9	.358
	grapeR	Tomato	6.40186190476E-9	7.48654680616E-9	.400
		GrapeY	-3.99417166667E-8*	6.94894508046E-9	.000

		Plume	1.29079583333E-8	7.26738071925E-9	.087
	GrapeY	Tomato	4.63435785714E-8*	6.63147220820E-9	.000
		grapeR	3.99417166667E-8*	6.94894508046E-9	.000
		Plume	5.28496750000E-8*	6.38301363666E-9	.000
	Plume	Tomato	-6.50609642857E-9	6.96443884569E-9	.358
		grapeR	-1.29079583333E-8	7.26738071925E-9	.087
		GrapeY	-5.28496750000E-8*	6.38301363666E-9	.000
temperature45	Tomato	grapeR	-3.23130333333E-8*	6.85629947043E-9	.000
		GrapeY	-6.22173666667E-8*	6.85629947043E-9	.000
		Plume	3.09905237500E-8*	7.06731172937E-9	.000
	grapeR	Tomato	3.23130333333E-8*	6.85629947043E-9	.000
		GrapeY	-2.99043333333E-8*	6.85629947043E-9	.000

		Plume	6.33035570833E-8*	7.06731172937E-9	.000
	GrapeY	Tomato	6.22173666667E-8*	6.85629947043E-9	.000
		grapeR	2.99043333333E-8*	6.85629947043E-9	.000
		Plume	9.32078904167E-8*	7.06731172937E-9	.000
	Plume	Tomato	-3.09905237500E-8*	7.06731172937E-9	.000
		grapeR	-6.33035570833E-8*	7.06731172937E-9	.000
		GrapeY	-9.32078904167E-8*	7.06731172937E-9	.000

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