Effects of plant essences on physiological characteristics of two cultivars of Iranian commercial pistachio nuts

Mohammad Salmani[1], Hossein Afshari[1]* and Mahdi Mohammadi Moghadam[2]

1. Department of Horticulture, faculty of Agriculture, Damghan Branch, Islamic Azad University, Damghan, Iran
2. Pistachio Research Station, Damghan

Abstract

To study the effects of thyme, savory, mint, and eucalyptus essences on photosynthetic efficiency, chlorophyll fluorescence, changes of transpiration, and leaf temperature of two cultivars of pistachios, namely, Abbasali and Shahpasand, a research was done in one of the commercial gardens of Damghan in 2012. This experiment was implemented in split, split plot as complete randomized block design, by two essences in five levels (4 experimental essences and one control sample). Cultivar type was evaluated at two levels and three replications and effects of treatments on physiological properties of the samples were studied. Type of essence regarding variance analysis table indicates significant effect on the rate of photosynthesis as %1. Also, effects of cultivars, cultivars × essence, concentration of essence, cultivars × concentration of essence, type of essence × concentration of essence and interaction of type and concentration, were not significant. Thyme essence had more pronounced effects on photosynthesis measure than other essences and was at higher rank with average of 12.22 μmolm^{-2}s^{-1}.

Keywords: Abbasali ; photosynthesis; pistachio; plant essential oils; Shahpasand

Salmani, M., H. Afshari and **M. Mohammadi Moghadam.** 2014. 'Effects of essences on physiological characteristics of two cultivars of Iranian commercial pistachio nuts. *Iranian Journal of Plant Physiology* 4 (4), 1159-1166.

Introduction

Using new physiological and genetic techniques has created new opportunities for significant modification of plants and optimal use of these techniques requires a basic understanding of photosynthesis (Rahimiyan et al, 2010). Photosynthesis parameters and water relations are good indexes for detecting the intensity of the stress to pistachio trees and are used as a tool for screening genotypes in breeding programs. Obviously, seeds with more efficiency of photosynthesis under water stress will have higher tolerance to drought stress (Roozban and Arzani, 2005).

Net photosynthesis rate varies during different seasons and different days in a season affected by a number of factors, including the development of branch, leaf age, hormone levels and carbohydrate accumulation in leaves. Also, intensity of photosynthesis varies with changes in

*Corresponding author
E-mail address: h_afshari@ymail.com
Received: April, 2014
Accepted: July , 2014

the light intensity, leaf temperature, air temperature, and humidity (Lasko, 1985).

A series of internal and external factors regulate the process of photosynthesis. Internal factors that may increase the rate of photosynthesis in leaves include existence of young adult leaves with spongy mesophyll and lots of space, lots of big reactive stomata, high chlorophyll content, sufficient turgor pressure, and the ability to use carbohydrates. External factors on the other hand are temperature, light, and humidity (Shaffer et al, 1994). Sanchez Ruiz et al (2000) reported that reduction of photosynthesis at midday shows that stomata conductance and photosynthesis are related and reducing stomata conductance is correlated with minimum xylem water potential and high light assimilates.

Daily photosynthesis of trees increases from flowering and continues to develop a full canopy and reaches a maximum near the middle of summer. Gradually with increasing temperature and leaf senescence at the end of the growing season, photosynthesis decreases (Wiley et al, 1999). When leaves are exposed to sufficient amount of light, comparison of carbon dioxide changes with changes in fruit stored in darkness reveals that the fruits as the main site of carbohydrate consumption control photosynthesis capabilities in leaves (Pavel and Dejong, 1993).

Chlorophyll fluorescence is the excitation energy in photosynthesis that has been widely studied in the photosynthesis research. The chlorophyll fluorescence is used to determine the status of plant physiology and damage to photosynthetic apparatus. Fluorescence properties are true representations of the fluidity, stability, and membrane organization. When photosynthesis II is disturbed by some sources of stress, fluorescence characteristics will change. Therefore, the fluorescence of chlorophyll is an indirect measurement of the green tissue physiology (Brown et al., 2004).

Chlorophyll fluorescence is a sensitive method for the detection and determination of the actual quantities of induction temperature on the photosynthetic systems (krause and Weis, 1991; Govindjee, 1995; Strasser, 1997). Changes in chlorophyll fluorescence are used in evaluation of a system for grading plant stress tolerance (Smillie and Hethrington, 1983; Greaves and Wilson, 1987; Yamada et al., 1996; Hakam et al., 2000). Initially chlorophyll fluorescence (F0) only increases just a little which is maintained in a transient light intensity, but in severe emission, the fluorescence increases rapidly to a maximum value (Fm). The distance between F0 and Fm is known as variable fluorescence (Fv). It seems that there is a good correlation between the ratio of variable to maximum fluorescence and O_2 or CO_2 absorption at low irradiation (Brown et al, 2004).

De Palma (1998) studied the effects of eco-physiological variables on growth and yield of pistachio and stated that as pistachio grows in regions with specific environmental conditions such as hot weather and low humidity, soil and air, high eco-physiological performance can be evaluated as adaptation responses that can increase the amount of carbohydrate in a short time and also the rate of carbon fixation per unit of evaporated water.

The present commercial varieties of pistachio are created based on crop seed traits. Because of pistachio rootstocks and heterozygosis, planting the seeds obtained from a particular tree results in very diverse traits and characteristics. Therefore, because of a long tradition of using pistachio seeds in long-established orchards of pistachio through generations in Iran, Iranian pistachio cultivars and genotypes enjoy a unique diversity. These pistachio cultivars were created and developed by farmers interested in genetic selection and reproduction without many genetic diversity and modifications. Clearly, identification of the varieties and genotypes can potentially increase production of superior cultivars in terms of qualitative and quantitative characteristics of existing varieties (Sajjadi, 2007).

No research studies have so far been done on the eco-physiological indices of pistachio trees in Iran. Therefore, it is necessary to study the eco-physiological characteristics of local varieties of pistachio that show their genetic differences and reactions to agricultural conditions of the region. The present study aims at investigating the effects of foliar spraying some essential oils, namely, thyme, savory, mint, and eucalyptus on the eco-physiological characteristics of pistachio filling time.

Materials and Methods

A split plot experiment was designed based on randomized complete block design with three replications in a village located near Damqan, Iran. 15 to 18 year-old trees from Abbasali and Shahpasand cultivars planted at 4 × 3 meter distances were studied. A total of 288 trees were selected which were similar in terms of growth and strength in spring 2012. Then two similar branches from each tree were designated and labeled in northern and southern position of the tree.

Four concentrations (0, 250, 500, and 750 ppm) of each of the four essential oils of thyme, savory, mint, and eucalyptus were applied in the form of foliar spraying on 6 August, 2012. There were two parameters of the type and concentration of essential oils applied on three trees in each block with 48 trees in each replication and with three replications. A total of 288 trees received different frequency and concentrations of oil essential oils in the form of foliar spraying in the study. Three trees in each block with 48 trees per replicate were treated.

Five mature and healthy leaves were selected d and gas exchanges of leaves were measured in the open air under normal temperature, light, and relative air humidity condition using a portable analyzer, infrared gas (ADC Bio Scientific LCpro + System Serial No. 32989 UK) equipped with infrared gas analysis system (IRGA), and bay leaf PLC4 equipped with sensors for temperature and current density photonic rate. The leaf chamber was measured in a direction that receives the most direct sunlight under field conditions (Novello, 1998). Leaves from the middle parts of the branches were put in the leaf chamber of the device to measure exhaust gases and the data on each index were recorded. Measurements were done at all stages of fruit development in non-cloudy days between 9 a.m. and 11 a.m. and at light intensity of more than 1600 microM photons per m^2 per second during the experiment.

Leaf chlorophyll fluorescence was measured in midday using the relevant

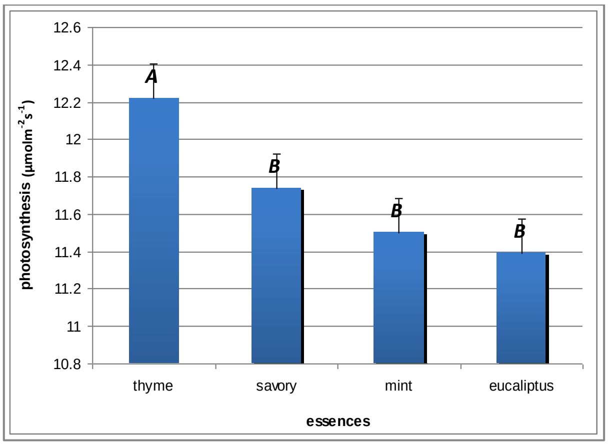

Fig. I. Means comparison of photosynthesis as affected by essential oils in two cultivars

equipment (Opti-Sciences, the United States). The leaves were protected from light for 15 min using special clamps in order to adapt to darkness. Fluorescence parameters such as minimum fluorescence (Fo), maximal fluorescence (Fm), and variable fluorescence (Fv), obtained by subtracting minimum fluorescence from maximum fluorescence value (Fv = Fm - Fo), and also the ratio of variable fluorescence to maximum fluorescence (Fv / Fm) were measured.

Statistical analyses were performed using MSTATC software. For analysis of variance the angular transformation of Arc sin √x was used for data adjustment and mean comparisons were performed with least significant difference test ($p \leq 0.05$).

Results

The effect of essential oils on photosynthesis, leaf temperature, and transpiration

According to Table 1, it is obvious that the rate of photosynthesis in trees treated with essential oils of thyme had the highest level. Although the essential oils of savory, mint, and eucalyptus were put at the same level in terms of their effects on the rate of photosynthesis, the mean for savory was higher than that of mint and eucalyptus.

Results showed that essential oils sprayed on pistachio (cv. Abbasali and Shahpasand) did not have a significant effect on

Table 1
The table of analysis of variance on the effect of different traits

Source of Variation	Degree of Freedom	Mean Squares						
		Photosynthesis	Transpiration	Leaf Temperature	Fo	Fm	Fv	Fv/Fm
Replication	2	44.16**	0.237**	51.75**	1875.33**	61580.5**	70984.16**	0.003 ns
Cultivar	1	0.00ns	0.00ns	0.49ns	6.00 ns	24640.0 ns	35863.34ns	0.006 ns
Types of Oil	3	3.22**	0.009ns	3.56 ns	856.40**	12960.3 ns	11076.92ns	0.005*
Cultivar × Types of Oil	3	0.04ns	0.005ns	0.56 ns	67.15 ns	9385.2 ns	1090.34ns	0.002 ns
Concentration of Oil		0.19ns	0.004ns	0.57ns	59.79 ns	46918.0 ns	3519.94ns	0.001 ns
Concentration of Oil × Cultivar	3	0.03ns	0.010ns	1.02 ns	238.44 ns	827.2 ns	5660.70ns	0.000 ns
Types of Oil × Concentration of Oil	9	0.34 ns	0.003ns	0.75 ns	178.73 ns	8156.6 ns	10167.95ns	0.002 ns
Cultivar × Types of Oil × Concentration of Oil	9	0.36ns	0.001ns	0.32ns	76.83 ns	6439.3 ns	7716.12ns	0.001ns
Error	62	0.59	0.007	3.41	167.97	10106.2	12580.23	0.002
cv%		6.55%	29.07%	5.12%	6.13%	14.46%	22.85%	6.28%

ns, *, ** non-significant or significant at the 5 and 1 percent levels.

leaf temperature and transpiration characteristics.

Minimum chlorophyll fluorescence (Fo)

The results of table of analysis of variance (Table 1) shows that the effects of essential oils on chlorophyll fluorescence were significant only at one percent probability level and the effects of cultivar and interaction between varieties and types of essential oil, concentration, type of essential oil × concentration, amount and type of interaction between the essential oil and the concentration of minimum chlorophyll fluorescence were not significant.

As can be seen in the Figure II, eucalyptus essential oil affected the chlorophyll fluorescence more significantly than other essential oils. In fact, eucalyptus had a more pronounced effect on minimum chlorophyll fluorescence. Mint, fennel, and thyme had the highest level of minimum chlorophyll fluorescence in that order.

Maximum Chlorophyll fluorescence

According to the table of analysis of variance (Table 1) only replication on the maximum chlorophyll fluorescence had a significant effect at $p \leq 0.01$. Effects of oil type, oil concentration, cultivar, and also interaction effects of cultivars × oil concentration, cultivar × oil type, concentration × type of oil essence, and interaction effects of cultivar × oil type × oil concentration on maximum chlorophyll fluorescence were not significant.

Variable chlorophyll fluorescence

As indicated in the table of analysis of variance (Table 1), effects of cultivar, type of essential oil, and essential oil concentration as well as the interaction between the number and type of oil, amount of concentration, type of oil concentration, interaction between variety, type of essential oils, and essential oil concentration on the maximum chlorophyll fluorescence were not significant.

Ratio of variable chlorophyll fluorescence to maximum

Effect of variety, concentration, interaction between number and type of oil, amount of oil concentration, oil type, concentration, and amount and type of interaction between the oil and the highest concentration of chlorophyll fluorescence parameters were not significant.

The effect of oil type on the ratio of variable to maximum chlorophyll fluorescence

According to the table of analysis of variance (Table 1), only type of oil had a significant effect ($p \leq 0.05$) on the ratio of variable to maximum chlorophyll fluorescence. According to Figure (III), all essential oils had significant effects on the ratio of variable to maximum chlorophyll fluorescence. In fact, the essential oils of mint and thyme compared to both savory and eucalyptus showed a more significant impact on the ratio of maximum to variable chlorophyll fluorescence.

Discussion

Reduction in the rate of leaf photosynthesis during the summer could be due to thermal damage to the photosynthetic structures and increasing the amount of light respiration. Anglipous et al. (1996) reported reduction in the rate of photosynthesis at high temperatures in olive. However, Nezyma (1993), Vemmos (1994), and Vemmos et al (1994) stated that the products of photosynthesis and stomatal conductance of fruit-bearing trees in late June were similar to or less than the trees without

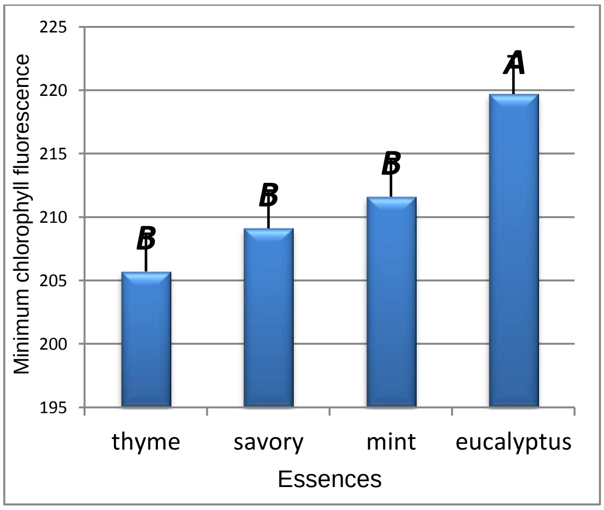

Fig. II. Means comparison of Minimum fluorescence as affected by essential oils in two cultivars

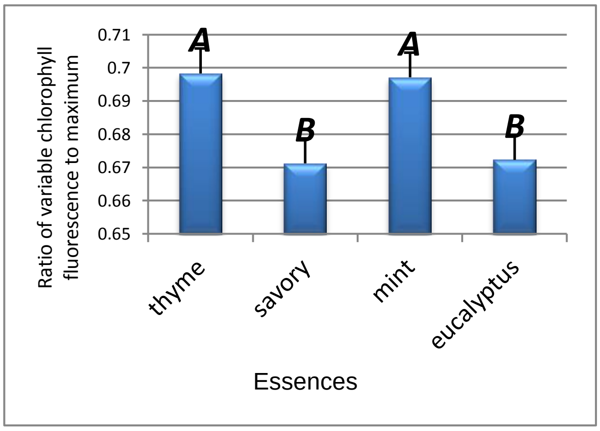

Fig 3. Means comparison of Maximum chlorophyll fluorescence as affected by essential oils in two cultivars

fruit. De Palma in 1998 in Italy studied the photosynthetic characteristics of four different female and two males of pistachio cultivars. The results showed that male Peter cultivar had the highest rate of photosynthesis and the ranking for other cultivars were Larnka female, Ascus male and female, Redalpo, Kerman and Bianca. The changes in stomata conductance, transpiration rate, and the ratio of variable to maximum chlorophyll fluorescence were approximately similar to the variability in photosynthetic rate. In this study higher photosynthesis rate was observed in pistachio trees treated with foliar spraying of thyme essential oil. Photosynthesis rate in the trees treated with the other essential oils showed no significant difference and the best performance was observed with thyme essential oil treatment.

Vmoss (1994) studying the effect of pistachio fruits on net photosynthesis and stomata conductance found that the presence of fruits increased stomata opening and increase in stomata conductance increased photosynthesis rate. Other researchers also confirmed the effect of fruit on the photosynthesis in fruit-bearing trees (Hansen, 1971; De Jong, 1983; wire and Flora, 1983; Fuji and Kennedy, 1985; Danton et al., 1987; Markard, 1987; Roper et al., 1988).

Reduction in stomata conductance of leaves in the middle of the growing season can be caused by normal aging. Also in mid-season, which coincides with the summer fruit, due to temperature rise, vapor pressure deficit, soil moisture deficit, and reduction in the intracellular concentration of CO_2, the stomata conductance decreases?

The direct relationship between stomata conductance and photosynthetic rate shows that reduction in photosynthesis during summer correlates with increase in temperature and reduction in stomatal conductance (Sanchez Ruiz et al 2000). As for the effect of temperature on stomata conductance, Ion and Richter (1991) reported that the cool morning air increased stomata conductance and from midday to sunset, as temperature increased, under the vapor pressure deficit, stomata conductance reduced.

Transpiration rate were measured at different stages of fruit development and it showed that in the early stages of fruit growth, transpiration rate was higher in spring. High transpiration and photosynthesis takes place at low temperature in this process, because at high temperatures the plant closes its window to deal with stress. During the summer, transpiration rate decreased. This decrease can be due to factors such as temperature, soil water loss, and aging of pistachio leaves. Proktor (1981) and Fleksas et al. (2002) stated that fruit effects could be due to an increase in conductivity at high photosynthetic as a result of stomata transpiration.

When a leaf developed in the dark is exposed to intensive light, it emits its characteristic fluorescence pattern. Initially, chlorophyll fluorescence increases immediately a small amount (Fo) which is maintained temporarily at the intense light, but at the intense light saturation, fluorescence rapidly increases to a maximum value (Fm) and the interval between Fo and Fm is known as variable fluorescence (Fv). In fact, Chlorophyll fluorescence is an indirect measure of the physiological state of green tissue. Photochemical quantum yield is commonly known as a QII since it comes mainly from the second photovoltaic optical systems.

Maximum quantum yield: (QII) = Fm-Fo/Fm = Fv / Fm.

It seems that the maximum quantum yield has good correlation with the quantum yield of photosynthesis, which is measured as the ratio of maximum produced O_2 to absorbed CO_2 at low irradiance (Brown et al., 2004). In particular, reduction in quantum yield by inhibition of light, can be evaluated by the ratio of variable to maximum fluorescence (Fv / Fm). If a plant cannot emit the excess energy of the sun, this excess energy is transferred to oxygen via chlorophyll, resulting in photo-oxidation damage. The early signs of damage appear in D1 protein in second optical system. Excessive damage leads to membranes destruction and chlorophyll oxidation. Thus, measuring Fv / Fm helps to better understand the processes associated with photosynthesis in plants (Koochaki et al., 2005).

Hawke et al. (1993) and Johnson and Max (2000) argued that when stress enters the photosynthetic apparatus of plant, variable fluorescence (Fv), which is equal to Fm-Fo, decreases. Nitrogen deficiency and drought stress also reduce the amount of variable fluorescence (Fv). The reason for decrease in the Fv / Fm is increase in Fo that is a result of stress and damage. Gonkalves et al. (2005) found that by increasing the rate of photosynthesis, the quantity of variable to maximum chlorophyll fluorescence (Fv / Fm) will also increase. In general, it can be concluded that in the leaves with high levels of chlorophyll the ratio of Fv / Fm was higher and in old leaves with low chlorophyll content the ratio of Fv / Fm was lower.

The midday decrease in net photosynthetic rates of many C3 plants is a common phenomenon (Huang et al., 2006). The main reasons for this phenomenon are light inhibition, increase in photorespiration, and stomatal closure. Gradual decline in the rate of CO_2 assimilation after a peak in the early morning has also been reported in grapes (Chaumont et al., 1994).

Conclusion

Findings of this study suggest that essential oil type with the specified concentrations did not show significant differences. There is a need to study various concentrations of some other types of essential oils to see the way they enhance the effectiveness and coordination of trees with the environment. In general, the study showed that the stress levels in pistachio trees can be significantly reduced by plant essential oils. This could be due to phenolic compounds present in essential oils that have anti-stress effects. These compounds penetrate into the epidermis of leaves and leaves cell membrane and due to low molecular weight, may reduce sweating and perspiration. It can be hoped that essential oils are used instead of using synthetic compounds which are harmful to consumers and can potentially degrade the environment and pose health risks to users.

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