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Research Article

EFFECT OF METHANOL AND ASCORBIC ACID FOLIAR APPLICATION ON PHOTOSYNTHETIC PIGMENTS OF PEANUT (*ARACHIS HYPOGAEA* L.) UNDER RAINFED CONDITION

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ABSTRACT
In order to evaluate the effect of foliar application of methanol and ascorbic acid on chlorophyll and carotenoid content of peanut (*Arachis hypogaea* L. var.NC2) an experiment was conducted in agricultural research farm of Astaneh Ashrafiyeh (north of Iran) in 2013-2014. A completely randomized block design with three replications on a factorial experiment with two factors including four levels of methanol (0 (Control), 10, 20, and 30 volumetric percentage) and four levels of ascorbic acid (0 (Control), 1000, 2000, and 3000 mg/lit) was used. Methanol and ascorbic acid foliar applications were done two times during the growing season with 15 days intervals and spraying started in 73 code stage of BBCH-scale. The photosynthetic pigments were measured after 7 days of each foliar application. Most significant effects were obtained by spraying 20-30 (v/v) methanol and (2000-3000 mg/lit) ascorbic acid at the two application dates. Comparisons between chlorophyll contents and meter readings showed that the chlorophyll meter readings (SPAD) were positively correlated to actual chlorophyll content. Thus, the chlorophyll meter readings (SPAD) can be a good tool to diagnose the integrity of the photosynthetic system in peanut leaves.

Keywords: *Peanut, Methanol, Ascorbic Acid, Photosynthetic Pigments*

INTRODUCTION
Peanut is one of the most important and economical oilseeds in tropical and subtropical regions and is mostly cultivated owing to its oil, protein and carbohydrate content (Panhwar, 2005). Peanut cultivation in Iran is done in Guilan, Golestan and Khuzestan provinces. In Guilan province, it is mainly cultivated in Astaneh Ashrafieh county along Sepidroud River.

Most higher plants produce and emit methanol as a result of pectin demethylation. This volatile organic compound produced especially during the early stages of leaf expansion and is released from leaves via stomata (Nemecek-Marshal *et al.,* 1995). Plant tissue, however, can also metabolize methanol. Although there is no methanol oxidase in higher plants, they can convert methanol to CO2 (Cossins, 1964). According to Gout *et al.,* (2000), assimilation of methanol by plants takes place before its oxidation. The role of methanol as a plant growth regulator (Devlin *et al.,* 2001) or an agent to enhance fruit quality (colour and composition) and to advance maturity would need to be studied more in detail. Methanol molecule is smaller than carbon dioxide one, so easily absorbed by plant and converted to formaldehyde by methanol oxidase then to format (Methanoeic acid). The format is converted to CO2 by format dehydrogenase and increases intracellular CO2 assimilation (Nonomura and Benson, 1992). Methanol is an alternative fuel that can be produced from domestic resources, both fossil and renewable. Methanol or "wood alcohol" is a colorless, toxic liquid. Many cultivated area are situated in arid zone, where crop photosynthesis and productivity is limited by drought. Thus any treatment, such as methanol, that improves plant water relation and reduces stress impacts, could be of benefit. Recent reports indicate that vegetative growth and yield of C3 crops were enhanced by foliar methanol application and that overall crop water use was reduced by methanol sprays. It has been suggested that methanol may act as a C source for the plant and a photorespiration inhibitor. However foliar application of methanol solutions on

crops would improve their accelerate ripening, reduce impacts of drought and decline crop water requirements. Methanol also appeared to improve the efficiency of water use in C3 plants, especially under water stress situations. On the other hand, methanol leads to increase of plants resistance to drought stress because these compounds play primary role in preventing increasing photorespiration induced in stressed plants (Tavassoli and Galavi, 2011).

Ascorbic acid (also named vitamin C) has important antioxidant and metabolic functions in both plants and animals, but humans, and a few other animal species, have lost the capacity to synthesize it. Plant-derived ascorbic acid is thus the major source of vitamin C in the human diet. Although the importance of ascorbic acid in human health has been realized for three centuries, the final identification of this essential nutrient molecule came at twentieth century after continuous efforts in medicine, philology and chemistry (Zhang, 2013). Consistent with its multi-function in human and animals, ascorbate in plants has beneficial influences on various aspects in plants.

Through modifying gene expression, ascorbate not only act to regulate defense and survival but also act via phytohormones to modulate plant growth (Pastori *et al.,* 2003). Emerging research results indicate that ascorbate, existing widely in plants as the abundant micromolecule substance, fulfils its essential roles in series of physiological processes such as plant defense against oxidization, co-factor of key enzymes, plant cell division, cell expansion, growth and development, and senescence (Smirnoff, 1996; Horemans, 2000).

Ascorbate, at least in some plant species, is also the substrate for the biosynthesis of oxalate and tartrate (Loewus and Loewus, 1987; Loewus, 1999). The objectives of this study were to (i) Evaluate the effect of methanol and ascorbic acid foliar application of on photosynthetic pigments of peanut under rainfed condition, (ii) to establish the ability of a portable chlorophyll meter (SPAD) to estimate chlorophyll (chlorophyll a, chlorophyll b and total chlorophyll) in peanut and correlates these data with extractable chlorophyll obtained by a conventional method.

MATERIALS AND METHODS

Field Experiment

In order to evaluate the effect of foliar application of methanol and ascorbic acid on chlorophyll and carotenoid content of peanut (*Arachis hypogaea* L. var.NC2) an experiment was conducted in agricultural research farm of Astaneh Ashrafiyeh (Township located in 37° 16' latitude and 49° 56' longitude, north of Iran) in 2013-2014. A completely randomized block design with three replication on a factorial experiment with two factors including four levels of methanol (0 (Control), 10, 20, and 30 volumetric percentage) and four levels of ascorbic acid (0 (Control), 1000, 2000, and 3000 g/lit) was used. To each one of these methanol application practices, 1 g/lit tetrahydrofolate was added as catalysts. The Methanol and ascorbic acid foliar application was done two times during the growing season with 15 days intervals and spraying start in 73 code stage of BBCH-scale [Meier, 2001]. Foliar application of methanol and ascorbic acid were made with a backpack sprayer between 17:00 and 19:00 p.m. at the beginning of peanut pod and seed growth stages, in both years.

Chlorophyll Extraction

The chlorophyll and carotenoid content was determined according to the method of Lichtenthaler and Wellburn (1983). Fresh young peanut leaves (0.5 g) were extracted in 80% acetone. The amounts of chlorophyll a, b and carotenoid were determined spectrophotometrically, by reading the absorbance at 663, 646, and 470 nm and their contents calculated by use of following equations. The chlorophyll and carotenoid content results are expressed as unit's mg per gram-fresh weight (mg/g fw).

Chlorophyll a= (12.21 (A 663) - 2.81 (A 646)) × V/W × 1000

Chlorophyll b= (20.13 (A 646) - 5.03 (A 663)) × V/W × 1000

Carotenoid= ((1000 (A 470) - 3.27 (Chlorophyll a) - 104 (Chlorophyll b))/ 227

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Where, A = Absorbance at specific wave lengths, V = Final volume of chlorophyll extract in 80% acetone, W = Fresh weight of leaves extracted.

The chlorophyll and carotenoid content were measured twice a day, for 7 days, after the each foliar application of methanol and ascorbic acid.

Chlorophyll Meter Readings (SPAD)

Chlorophyll meter readings (SPAD) were taken with a hand-held dual wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan). After cleaning surface dust from the selected leaves, on each leaf, three SPAD readings were taken on each side of the midrib. This procedure was followed for averaging heterogeneity of chlorophyll distribution in the leaf surface. Before taking individual measurements, the meter was set to zero without any sample in the sample box slot by pressing the same button that is used for data collection. While recording SPAD readings, care was taken to ensure that SPAD meter sensor fully covered the leaf lamina and that interference from veins and midribs was avoided. The 30 youngest fully expanded leaves from each plot were used for sampling 7 days after the each foliar application of methanol and ascorbic acid. The instrument was stored and readings were automatically averaged to generate one reading per plot.

Statistical Analyses

The SAS software package was used to analyze all data (SAS 9.2) and means were compared by the least significant differences (LSD) test at 0.05 probability level. Relationships between the photosynthetic pigments were analyzed using both SPSS and Excel software.

RESULTS AND DISCUSSION

Results

Chlorophyll Meter Readings (SPAD)

With attention to results of data variance analysis table (Table 1), the effect of methanol and ascorbic acid foliar application showed significant differences at 1% and 5% probability level respectively, on chlorophyll meter readings $(SPAD)^1$. But effect of year, interaction effect of methanol × ascorbic acid foliar application and other interaction effect treatments were no significant (Table 2). With attention to results of data variance analysis table (Table 1), the effect of methanol and ascorbic acid foliar application and theses interaction effect showed significant differences at 1%, 1% and 5% probability level respectively, on chlorophyll meter readings $(SPAD)^2$. But effect of year and other interaction effect treatments were non significant (Table 1). Results showed that, with increasing concentration of methanol foliar application on plants the chlorophyll meter readings $(SPAD)^1$ positively increased (Table 2). Between methanol foliar application levels, the highest amount of chlorophyll meter readings $(SPAD)^1$ were obtained from M20 and M30 treatments (20-30 v/v) with 44.01 and 44.22 respectively. Also, the lowest chlorophyll meter readings $(SPAD)^1$ with 41.07 was found from M0 treatment (control). Results showed that, with increasing concentration of methanol foliar application on plants the chlorophyll meter readings $(SPAD)^2$ positively increased (Table 2).

Between methanol foliar application levels, the highest amount of chlorophyll meter readings $(SPAD)^2$ were obtained from M20 and M30 treatments (20-30 v/v) with 43.83 and 43.67 respectively. Also, the lowest chlorophyll meter readings $(SPAD)^2$ with 40.87 was found from M0 treatment (control). Results showed that, with increasing concentration of ascorbic acid foliar application on plants the chlorophyll meter readings $(SPAD)^1$ positively increased (Table 2). Between ascorbic acid foliar application levels, the highest amount of chlorophyll meter readings $(SPAD)^1$ were obtained from AsA1000, AsA2000 and AsA3000 treatments (1000-3000 g/lit) with 43.10, 43.60 and 43.23 respectively. Also, the lowest chlorophyll meter readings $(SPAD)^1$ with 42.09 was found from AsA0 treatment (control). Results showed that, with increasing concentration of ascorbic acid foliar application on plants the chlorophyll meter readings $(SPAD)^2$ positively increased (Table 2). Between ascorbic acid foliar application levels, the highest amount of chlorophyll meter readings $(SPAD)^2$ were obtained from AsA2000 and AsA3000 treatments (2000-3000 g/lit) with 43.27 and 44.05 respectively. Also, the lowest chlorophyll meter readings $(SPAD)^2$ with 40.90 was found from AsA0 treatment (control). With attention to interaction effect of methanol × ascorbic acid foliar application on chlorophyll meter readings $(SPAD)^2$ (Figure 1), the highest amount of chlorophyll meter readings $(SPAD)^2$ were obtained from M20AsA1000,

M20AsA2000, M20AsA3000, M30AsA2000 and M30AsA3000 treatments. The lowest chlorophyll meter readings (SPAD)^2 was recorded from M0AsA0 treatment.

Positive and significant correlations ($p<0.01$) were found among chlorophyll meter readings (SPAD)^2 ($r= +0.285^{**}$), chlorophyll a^1 ($r= +0.718^{**}$), chlorophyll a^2 ($r= +0.505^{**}$), chlorophyll b^1 ($r= +0.797^{**}$), chlorophyll b^2 ($r= +0.493^{**}$), total chlorophyll^1 ($r= +0.782^{**}$), total chlorophyll^2 ($r= +0.505^{**}$), carotenoid^1 ($r= +0.512^{**}$), carotenoid^2 ($r= +0.385^{**}$), with chlorophyll meter readings (SPAD)^1 according to the two-years results of the research, as seen in table 3. Positive and significant correlations ($p<0.01$) were found among chlorophyll meter readings (SPAD)^1 ($r= +0.285^{**}$), chlorophyll a^1 ($r= +0.468^{**}$), chlorophyll a^2 ($r= +0.699^{**}$), chlorophyll b^1 ($r= +0.454^{**}$), chlorophyll b^2 ($r= +0.782^{**}$), total chlorophyll^1 ($r= +0.468^{**}$), total chlorophyll^2 ($r= +0.709^{**}$), carotenoid^1 ($r= +0.353^{**}$), carotenoid^2 ($r= +0.528^{**}$), with chlorophyll meter readings (SPAD)^2 according to the two-years results of the research, as seen in table 3.

Table 1: Analysis of variance (mean square and significance) for effect of methanol and ascorbic acid foliar application on photosynthetic pigments of peanut under rainfed condition

S.O.V	Df	Chlorophyll Meter (SPAD)^1	Chlorophyll Meter (SPAD)^2	Carotenoid^1	Carotenoid^2	Total Chlorophyll^1
Year (Y)	1	9.6203	6.2067	0.000472	0.00072	0.02600
Y (R)	4	2.7351	0.9448	0.072779	0.078047	0.00123
Methanol (M)	3	50.3952**	47.7937**	0.077196**	0.086842**	4.373672**
Y×M	3	1.1636	0.7679	0.000054	0.000006	0.00008
Ascorbic (AsA)	3	9.9974*	44.8446**	0.000544	0.002281**	0.10674**
Y×AsA	3	0.1038	1.5342	0.000019	0.000010	0.00041
M×AsA	9	3.5570	5.8987*	0.000213	0.000617	0.02792**
Y×M×AsA	9	6.0332	4.1236	0.000346	0.000319	0.01803
Error	60	4.5539	2.5352	0.000425	0.000413	0.00791
Cv (%)		4.96	3.73	6.99	6.60	4.36
S.O.V	Df	Total Chlorophyll^2	Chlorophyll b^1	Chlorophyll b^2	Chlorophyll a^1	Chlorophyll a^2
Year (Y)	2	0.04420*	0.00062	0.000828	0.01926	0.0330*
Y (R)	4	0.00597	0.000034	0.000140	0.00101	0.00461
Methanol (M)	3	3.90804**	0.045659**	0.04494**	3.5252**	3.11614**
Y×M	3	0.00269	0.000006	0.000059	0.00011	0.00192
Ascorbic (AsA)	3	0.02246*	0.002228**	0.000531*	0.07903**	0.01698*
Y×AsA	3	0.00097	0.000005	0.000011	0.00026	0.00067
M×AsA	9	0.00927	0.000541**	0.000214	0.021007**	0.00690
Y×M×AsA	9	0.01430	0.000375	0.000338	0.01310	0.01053
Error	60	0.01019	0.000182	0.000240	0.00595	0.00739
Cv (%)		5.15	4.37	5.09	4.45	5.19

** and ** significant at level of 5 and 1%, respectively. Values that do not have any symbol are non-significant. (1: First foliar application, 2: Second foliar application).*

Chlorophyll a

With attention to results of data variance analysis table (Table 1), the effect of methanol and ascorbic acid foliar application and theses interaction effect showed significant ($p\leq0.01$), on chlorophyll a^1. But effect of year and other interaction effect treatments were non significant (Table 1). With attention to results of data variance analysis table (Table 1), the effect of year, methanol and ascorbic acid foliar showed

significant differences at 5%, 1% and 5% probability level respectively, on chlorophyll a^2. But interaction effect of methanol × ascorbic acid foliar application and other interaction effect treatments were non significant (Table 1). The highest chlorophyll a^2 of peanut obtained in the second year with 1.67 mg/g fw. Results showed that, with increasing concentration of methanol foliar application on plants the chlorophyll a^1 positively increased (Table 2). Between methanol foliar application levels, the highest amount of chlorophyll a^1 were obtained from M20 and M30 treatments (20-30 v/v) with 2.04 and 2.03 mg/g fw respectively. Also, the lowest chlorophyll a^1 with 1.25 mg/g fw was found from M0 treatment (control). Results showed that, with increasing concentration of methanol foliar application on plants the chlorophyll a^2 positively increased (Table 2). Between methanol foliar application levels, the highest amount of chlorophyll a^2 were obtained from M20 and M30 treatments (20-30 v/v) with 1.94 and 1.95 mg/g fw respectively. Also, the lowest chlorophyll a^2 with 1.19 mg/g fw was found from M0 treatment (control). Results showed that, with increasing concentration of ascorbic acid foliar application on plants the chlorophyll a^1 positively increased (Table 2). Between ascorbic acid foliar application levels, the highest amount of chlorophyll a^1 were obtained from AsA2000 and AsA3000 treatments (2000-3000 g/lit) with 1.76 and 1.79 mg/g fw respectively. Also, the lowest chlorophyll a^1 with 1.66 and 1.71 mg/g fw were found from AsA0 (control) and AsA1000 treatments respectively. Results showed that, with increasing concentration of ascorbic acid foliar application on plants the chlorophyll a^2 positively increased (Table 2). Between ascorbic acid foliar application levels, the highest amount of chlorophyll a^2 were obtained from AsA1000, AsA2000 and AsA3000 treatments (1000-3000 g/lit) with 1.66, 1.68 and 1.67 mg/g fw respectively. Also, the lowest chlorophyll a^2 with 1.62 mg/g fw was found from AsA0 treatment (control). With attention to interaction effect of methanol × ascorbic acid foliar application on chlorophyll a^1 (Figure 2), the highest amount of chlorophyll a^1 were obtained from M20AsA1000, M20AsA2000, M20AsA3000, M30AsA2000 and M30AsA3000 treatments. The lowest chlorophyll a^1 were recorded from M0AsA0, M0AsA1000, M0AsA2000 and M0AsA3000 treatments.

Positive and significant correlations ($p<0.01$) were found among chlorophyll meter readings $(SPAD)^1$ ($r= +0.718^{**}$), chlorophyll meter readings $(SPAD)^2$ ($r= +0.468^{**}$), chlorophyll a^2 ($r= +0.929^{**}$), chlorophyll b^1 ($r= +0.990^{**}$), chlorophyll b^2 ($r= +0.889^{**}$), total $chlorophyll^1$ ($r= +0.995^{**}$), total $chlorophyll^2$ ($r= +0.926^{**}$), $carotenoid^1$ ($r= +0.672^{**}$), $carotenoid^2$ ($r= +0.633^{**}$), with chlorophyll a^1 according to the two-years results of the research, as seen in table 3. Positive and significant correlations ($p<0.01$) were found among chlorophyll meter readings $(SPAD)^1$ ($r= +0.505^{**}$), chlorophyll meter readings $(SPAD)^2$ ($r= +0.699^{**}$), chlorophyll a^1 ($r= +0.929^{**}$), chlorophyll b^1 ($r= +0.892^{**}$), chlorophyll b^2 ($r= +0.989^{**}$), total $chlorophyll^1$ ($r= +0.926^{**}$), total $chlorophyll^2$ ($r= +0.995^{**}$), $carotenoid^1$ ($r= +0.625^{**}$), $carotenoid^2$ ($r= +0.689^{**}$), with chlorophyll a^2 according to the two-years results of the research, as seen in table 3.

Chlorophyll b

With attention to results of data variance analysis table (Table 1), the effect of methanol and ascorbic acid foliar application and theses interaction effect showed significant ($p\leq0.01$), on chlorophyll b^1. But effect of year and other interaction effect treatments were non significant (Table 1). With attention to results of data variance analysis table (Table 1), the effect of methanol and ascorbic acid foliar showed significant differences at 1% and 5% probability level respectively, on chlorophyll b^2. But effect of year, interaction effect of methanol × ascorbic acid foliar application and other interaction effect treatments were non significant (Table 1). Results showed that, with increasing concentration of methanol foliar application on plants the chlorophyll b^1 positively increased (Table 2). Between methanol foliar application levels, the highest amount of chlorophyll b^1 were obtained from M20 and M30 treatments (20-30 v/v) with 0.344 and 0.343 mg/g fw respectively. Also, the lowest chlorophyll b^1 with 0.253 mg/g fw was found from M0 treatment (control). Results showed that, with increasing concentration of methanol foliar application on plants the chlorophyll b^2 positively increased (Table 2). Between methanol foliar application levels, the highest amount of chlorophyll b^2 were obtained from M20 and M30 treatments (20-30 v/v) with 0.338 and 0.339 mg/g fw respectively. Also, the lowest chlorophyll b^2 with 0.248 mg/g fw was found from M0 treatment (control). Results showed that, with increasing concentration of ascorbic acid foliar application on plants the chlorophyll b^1 positively increased (Table 2). Between ascorbic acid foliar application

levels, the highest amount of chlorophyll b^1 were obtained from AsA2000 and AsA3000 treatments (2000-3000 g/lit) with 0.314 and 0.312 mg/g fw respectively. Also, the lowest chlorophyll b^1 with 0.298 and 0.305 mg/g fw were found from AsA0 (control) and AsA1000 treatments respectively. Results showed that, with increasing concentration of ascorbic acid foliar application on plants the chlorophyll b^2 positively increased (Table 2). Between ascorbic acid foliar application levels, the highest amount of chlorophyll b^2 were obtained from AsA1000, AsA2000 and AsA3000 treatments (1000-3000 g/lit) with 0.305, 0.309 and 0.306 mg/g fw respectively. Also, the lowest chlorophyll b^2 with 0.298 mg/g fw was found from AsA0 treatment (control). With attention to interaction effect of methanol × ascorbic acid foliar application on chlorophyll b^1 (Figure 3), the highest amount of chlorophyll b^1 were obtained from M20AsA1000, M20AsA2000, M20AsA3000, M30AsA2000 and M30AsA3000 treatments. The lowest chlorophyll b^1 were recorded from M0AsA0, M0AsA1000, M0AsA2000 and M0AsA3000 treatments.

Positive and significant correlations ($p<0.01$) were found among chlorophyll meter readings $(SPAD)^1$ ($r= +0.797^{**}$), chlorophyll meter readings $(SPAD)^2$ ($r= +0.454^{**}$), chlorophyll a^1 ($r= +0.990^{**}$), chlorophyll a^2 ($r= +0.892^{**}$), chlorophyll b^2 ($r= +0.856^{**}$), total $chlorophyll^1$ ($r= +0.991^{**}$), total $chlorophyll^2$ ($r= +0.889^{**}$), $carotenoid^1$ ($r= +0.671^{**}$), $carotenoid^2$ ($r= +0.612^{**}$), with chlorophyll b^1 according to the two-years results of the research, as seen in table 3. Positive and significant correlations ($p<0.01$) were found among chlorophyll meter readings $(SPAD)^1$ ($r= +0.493^{**}$), chlorophyll meter readings $(SPAD)^2$ ($r= +0.782^{**}$), chlorophyll a^1 ($r= +0.889^{**}$), chlorophyll a^2 ($r= +0.989^{**}$), chlorophyll b^1 ($r= +0.856^{**}$), total $chlorophyll^1$ ($r= +0.886^{**}$), total $chlorophyll^2$ ($r= +0.991^{**}$), $carotenoid^1$ ($r= +0.614^{**}$), $carotenoid^2$ ($r= +0.700^{**}$), with chlorophyll b^2 according to the two-years results of the research, as seen in table 3.

Total Chlorophyll

With attention to results of data variance analysis table (Table 1), the effect of methanol and ascorbic acid foliar application and theses interaction effect showed significant ($p\leq0.01$), on total $chlorophyll^1$. But effect of year and other interaction effect treatments were non significant (Table 1). With attention to results of data variance analysis table (Table 1), the effect of year, methanol and ascorbic acid foliar showed significant differences at 5%, 1% and 5% probability level respectively, on total $chlorophyll^2$. But interaction effect of methanol × ascorbic acid foliar application and other interaction effect treatments were non significant (Table 1). The highest total $chlorophyll^2$ of peanut obtained in the second year with 1.98 mg/g fw. Results showed that, with increasing concentration of methanol foliar application on plants the total $chlorophyll^1$ positively increased (Table 2). Between methanol foliar application levels, the highest amount of total $chlorophyll^1$ were obtained from M20 and M30 treatments (20-30 v/v) with 2.38 and 2.38 mg/g fw respectively. Also, the lowest total $chlorophyll^1$ with 1.50 mg/g fw was found from M0 treatment (control). Results showed that, with increasing concentration of methanol foliar application on plants the total $chlorophyll^2$ positively increased (Table 2). Between methanol foliar application levels, the highest amount of total $chlorophyll^2$ were obtained from M20 and M30 treatments (20-30 v/v) with 2.27 and 2.29 mg/g fw respectively. Also, the lowest total $chlorophyll^2$ with 1.44 mg/g fw was found from M0 treatment (control). Results showed that, with increasing concentration of ascorbic acid foliar application on plants the total $chlorophyll^1$ positively increased (Table 2). Between ascorbic acid foliar application levels, the highest amount of total $chlorophyll^1$ were obtained from AsA2000 and AsA3000 treatments (2000-3000 g/lit) with 2.07 and 2.11 mg/g fw respectively. Also, the lowest total $chlorophyll^1$ with 1.96 and 2.01 mg/g fw were found from AsA0 (control) and AsA1000 treatments respectively. Results showed that, with increasing concentration of ascorbic acid foliar application on plants the total $chlorophyll^2$ positively increased (Table 2). Between ascorbic acid foliar application levels, the highest amount of total $chlorophyll^2$ were obtained from AsA1000, AsA2000 and AsA3000 treatments (1000-3000 g/lit) with 1.96, 1.99 and 1.97 mg/g fw respectively. Also, the lowest total $chlorophyll^2$ with 1.91 mg/g fw was found from AsA0 treatment (control). With attention to interaction effect of methanol × ascorbic acid foliar application on total $chlorophyll^1$ (Figure 4), the highest amount of total $chlorophyll^1$ were obtained from M20AsA1000, M20AsA2000, M20AsA3000, M30AsA2000 and M30AsA3000 treatments. The lowest total $chlorophyll^1$ were recorded from M0AsA0, M0AsA1000, M0AsA2000 and M0AsA3000 treatments.

Positive and significant correlations ($p<0.01$) were found among chlorophyll meter readings (SPAD)[1] (r= +0.727[**]), chlorophyll meter readings (SPAD)[2] (r= +0.646[**]), chlorophyll a[1] (r= +0.995[**]), chlorophyll a[2] (r= +0.926[**]), chlorophyll b[1] (r= +0.991[**]), chlorophyll b[2] (r= +0.886[**]), total chlorophyll[2] (r= +0.923[**]), carotenoid[1] (r= +0.674[**]), carotenoid[2] (r= +0.632[**]), with total chlorophyll[1] according to the two-years results of the research, as seen in table 3. Positive and significant correlations ($p<0.01$) were found among chlorophyll meter readings (SPAD)[1] (r= +0.505[**]), chlorophyll meter readings (SPAD)[2] (r= +0.709[**]), chlorophyll a[1] (r= +0.926[**]), chlorophyll a[2] (r= +0.995[**]), chlorophyll b[1] (r= +0.889[**]), chlorophyll b[2] (r= +0.991[**]), total chlorophyll[1] (r= +0.923[**]), carotenoid[1] (r= +0.624[**]), carotenoid[2] (r= +0.690[**]), with total chlorophyll[2] according to the two-years results of the research, as seen in table 3.

Carotenoid

With attention to results of data variance analysis table (Table 1), the effect of methanol foliar application showed significant differences at 1% probability level on carotenoid[1].

Table 2: Comparison of mean effect of methanol and ascorbic acid foliar application on photosynthetic pigments of peanut under rainfed condition

Treatment	SPAD[1]	SPAD[2]	Car[1]	Car[2]	T Chl[1]	T Chl[2]	Chl b[1]	Chl b[2]	Chl a[1]	Chl a[2]
	-		(mg/ g FW)		(mg/ g FW)		(mg/ g FW)		(mg/ g FW)	
Year										
Y1	42.69 a	42.35 a	0.293 a	0.305 a	2.02 a	1.94 b	0.307 a	0.302 a	1.72 a	1.64 b
Y2	43.32 a	42.85 a	0.297 a	0.310 a	2.06 a	1.98 a	0.312 a	0.308 a	1.74 a	1.67 a
LSD	0.871	0.650	0.0084	0.0083	0.036	0.041	0.0055	0.0063	0.031	0.035
Methanol (v/v)										
M0	41.07 c	40.87 c	0.222 c	0.231 c	1.50 c	1.44 c	0.253 c	0.248 c	1.25 c	1.19 c
M10	42.73 b	42.03 b	0.277 b	0.288 b	1.90 b	1.83 b	0.297 b	0.294 b	1.60 b	1.54 b
M20	44.01 a	43.83 a	0.339 a	0.356 a	2.38 a	2.27 a	0.344 a	0.338 a	2.04 a	1.94 a
M30	44.22 a	43.67 a	0.341 a	0.356 a	2.38 a	2.29 a	0.343 a	0.339 a	2.03 a	1.95 a
LSD	1.23	0.919	0.0119	0.0117	0.051	0.058	0.009	0.0078	0.044	0.049
Ascorbic acid (mg/lit)										
AsA0	42.09 b	40.90 c	0.288 a	0.297 c	1.96 b	1.91 b	0.298 b	0.298 b	1.66 b	1.62 b
AsA1000	43.10 ab	42.18 b	0.296 a	0.303 bc	2.01 b	1.96 ab	0.305 b	0.305 ab	1.71 b	1.66 ab
AsA2000	43.60 a	43.27 a	0.298 a	0.313 ab	2.07 a	1.99 a	0.314 a	0.309 a	1.76 a	1.68 a
AsA3000	43.23 ab	44.05 a	0.298 a	0.318 a	2.11 a	1.97 ab	0.320 a	0.306 ab	1.79 a	1.67 ab
LSD	1.23	0.919	0.0119	0.0117	0.051	0.058	0.009	0.0078	0.044	0.049

Means, in each column, with similar letters are not significantly different at the 5% probability level. (1: First foliar application, 2: Second foliar application).

But effect of year, ascorbic acid foliar application, interaction effect of methanol × ascorbic acid foliar application and other interaction effect treatments were non significant (Table 1). With attention to results of data variance analysis table (Table 1), the effect of methanol and ascorbic acid foliar application showed significant differences at 1% probability level, on carotenoid[2]. But effect of year, interaction effect of methanol × ascorbic acid foliar application and other interaction effect treatments were non significant (Table 1). Results showed that, with increasing concentration of methanol foliar application on plants the carotenoid[1] positively increased (Table 2).

Between methanol foliar application levels, the highest amount of carotenoid[1] were obtained from M20 and M30 treatments (20-30 v/v) with 0.339 and 0.341 mg/g fw respectively. Also, the lowest carotenoid[1] with 0.222 mg/g fw was found from M0 treatment (control). Results showed that, with increasing concentration of methanol foliar application on plants the carotenoid[2] positively increased (Table 2).

Between methanol foliar application levels, the highest amount of carotenoid[2] were obtained from M20 and M30 treatments (20-30 v/v) with 0.356 and 0.356 mg/g fw respectively.

Table 3: Simple correlation between photosynthetic pigments in peanut leaves

Parameter	Spad[1]	Spad[2]	Chl a[1]	Chl a[2]	Chl b[1]	Chl b[2]	T Chl[1]	T Chl[1]	Car[1]	Car[2]
Spad[1]	1									
Spad[2]	0.285**	1								
Chl a[1]	0.718**	0.468**	1							
Chl a[2]	0.205**	0.699**	0.929**	1						
Chl b[1]	0.797**	0.454**	0.990**	0.892**	1					
Chl b[2]	0.493**	0.782**	0.889**	0.989**	0.856**	1				
T Chl[1]	0.728**	0.468**	0.995**	0.926**	0.991**	0.886**	1			
T Chl[2]	0.505**	0.709**	0.926**	0.995**	0.889**	0.991**	0.923**	1		
Car[1]	0.512**	0.353**	0.672**	0.625**	0.671**	0.614**	0.674**	0.624**	1	
Car[2]	0.385**	0.528**	0.633**	0.689**	0.612**	0.7000**	0.632**	0.690**	0.958**	1

** and ** significant at level of 5 and 1%, respectively. (1: First foliar application, 2: Second foliar application)*

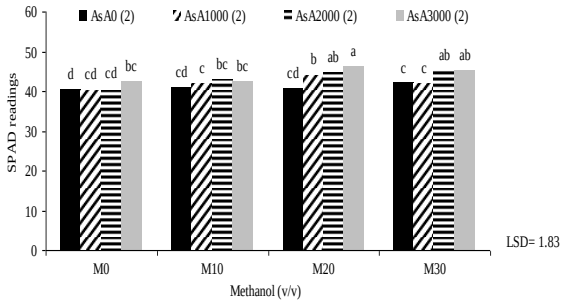

Figure 1: Interaction effect of methanol × ascorbic acid foliar application on SPAD

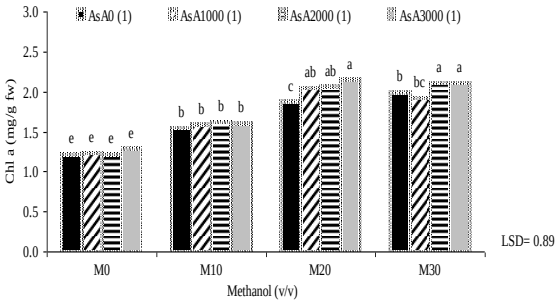

Figure 2: Interaction effect of methanol × ascorbic acid foliar application on chlorophyll a

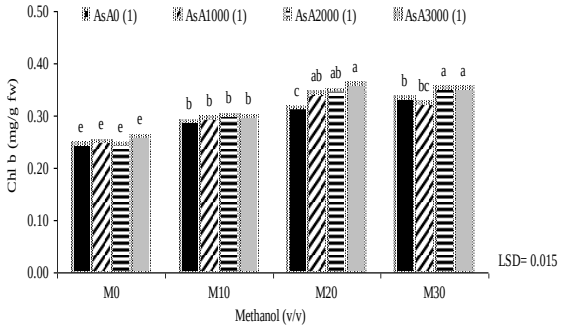

Figure 3: Interaction effect of methanol × ascorbic acid foliar application on chlorophyll b

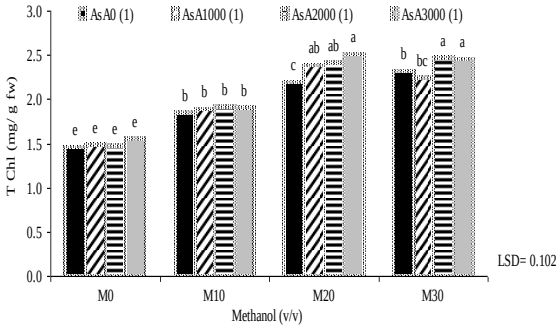

Figure 4: Interaction effect of methanol × ascorbic acid foliar application on total chlorophyll

Also, the lowest carotenoid[2] with 0.231 mg/g fw was found from M0 treatment (control). Results showed that, with increasing concentration of ascorbic acid foliar application on plants the carotenoid[2] positively increased (Table 2). Between ascorbic acid foliar application levels, the highest amount of carotenoid[2] were obtained from AsA2000 and AsA3000 treatments (2000-3000 g/lit) with 0.313 and 0.318 mg/g fw respectively. Also, the lowest carotenoid[2] with 0.297 mg/g fw was found from AsA0 treatment (control).
Positive and significant correlations ($p<0.01$) were found among chlorophyll meter readings (SPAD)[1] (r= +0.512**), chlorophyll meter readings (SPAD)[2] (r= +0.353**), chlorophyll a[1] (r= +0.672**), chlorophyll a[2] (r= +0.625**), chlorophyll b[1] (r= +0.671**), chlorophyll b[2] (r= +0.614**), total chlorophyll[1] (r= +0.674**), total chlorophyll[2] (r= +0.626**), carotenoid[2] (r= +0.958**), with carotenoid[1] according to the two-years results of the research, as seen in table 3. Positive and significant correlations ($p<0.01$) were found among chlorophyll meter readings (SPAD)[1] (r= +0.385**), chlorophyll meter readings (SPAD)[2] (r= +0.528**), chlorophyll a[1] (r= +0.633**), chlorophyll a[2] (r= +0.689**), chlorophyll b[1] (r= +0.612**), chlorophyll b[2] (r= +0.700**), total chlorophyll[1] (r= +0.632**), total chlorophyll[2] (r= +0.690**), carotenoid[1] (r= +0.958**), with carotenoid[2] according to the two-years results of the research, as seen in table 3.

Relationship between Chlorophyll Meter Readings (SPAD) and Chlorophyll Contents

Correlation coefficients (r) and regression equations between chlorophyll meter readings (SPAD) and chlorophyll contents were presented in Table 4. Correlation coefficients between chlorophyll meter readings (SPAD) and chlorophyll contents were highly significant at all leaves sampled. In terms of fresh weight basis, the Correlation coefficients (r) values between chlorophyll meter readings (SPAD) and chlorophyll a (0.693**), chlorophyll b (0.782**), and total chlorophyll (0.704**) were high, positive and significant. The Coefficients of determination (R^2) values for chlorophyll a, b and total chlorophyll on a fresh weight basis were 0.481**, 0.612** and 0.495**, respectively. Figures 5-7 showed that the relationships between chlorophyll meter readings (SPAD) and extracted chlorophyll in peanut leaves.

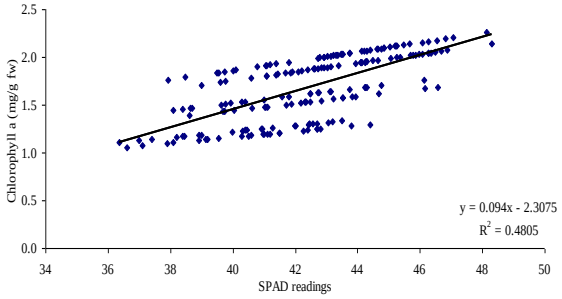

Figure 5: Relationship between SPAD reading and extracted Chl a in peanut leaves

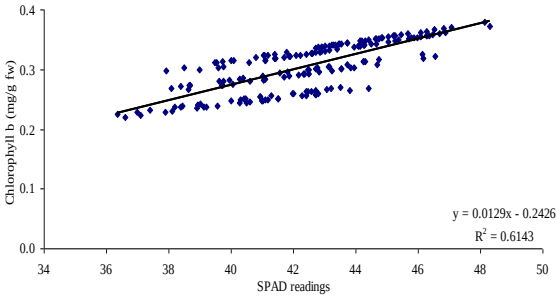

Figure 6: Relationship between SPAD reading and extracted Chl b in peanut leaves

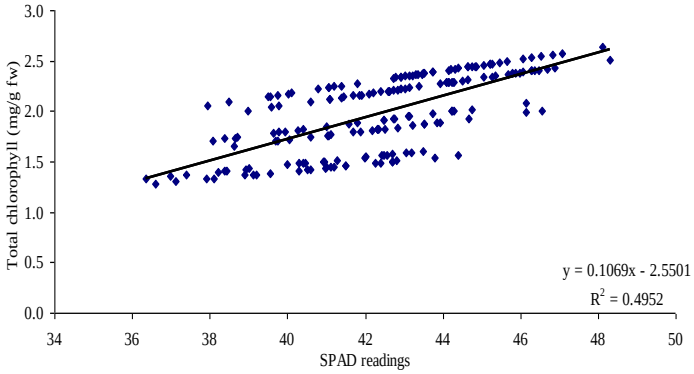

Figure 7: Relationship between SPAD reading and extracted T Chl in peanut leaves

The higher the chlorophyll meter readings (SPAD), the higher the chlorophyll pigments will be and vice versa. The regression lines (Figures 5-7) showed that these variables are linearly related with each other. The R^2 values for chlorophyll a, b and total chlorophyll on a fresh weight basis were 0.4805^{**}, 0.6143^{**}, and 0.4952^{**}, respectively.

Table 4: Relationship between extracted leaf chlorophyll (Y) and SPAD readings (X) in peanut leaves

Components	Simple regression	Correlation coefficients (r)	Coefficients of determination (R^2)
Extracted chlorophyll in fresh weight basis (mg/g fw)			
Chlorophyll a	Y = 33.831 + 5.110 X	0.693^{**}	0.481^{**}
Chlorophyll b	Y = 27.941 + 47.505 X	0.782^{**}	0.612^{**}
Total Chlorophyll	Y = 33.221 + 4.633 X	0.704^{**}	0.495^{**}

Discussion

Photosynthesis is the most important biochemical process occurring in plants and chlorophyll is the key pigment involved in it (Samdur *et al*., 2000). In photosynthesis, antenna pigments in leaf chloroplasts absorb solar radiation, and through resonance transfer the resulting excitation is channeled to the reaction centre pigments, which release electrons and set in motion the photochemical process (Richardson *et al*., 2002). The chlorophylls, Chl a and Chl b, are virtually essential pigments for the conversion of light energy to stored chemical energy (Gitelson *et al*., 2003). The amount of solar radiation absorbed by a leaf is a function of the photosynthetic pigment content; thus, chlorophyll content can directly determine photosynthetic potential and primary production (Curran *et al*., 1990; Filella *et al*., 1995; Ma *et al*., 1995). In General, drought is one of the most important limiting factors of crop yields in arid zones. The reduction of photosynthesis under drought stress is appeared to be associated with disturbance in biochemical reactions (Graan and Boyer, 1990).

Photosystem II (PSII) is highly sensitive to environmental inhibiting factors and water stress will damage its reaction centers severely. The chemical reaction of PSII is also affected strictly by water stress (Masojidek *et al*., 1991). When stomata are closed due to drought or high temperature, the available CO2 in intercellular space (Ci) would be reduced, leading to reduced electron transport capacity and restricted assimilation potential (Liang *et al*., 1997). On the other hand, stomata closure will result in evaluated temperatures of leaf and plant, limiting light reaction of photosynthesis. The study of chlorophyll fluorescence parameters is a simple, non-destructive method, rapidly lead to valuable results. One can detect the imbalance between two metabolic and anabolic processes, which are affected by heat and drought stress, by using chlorophyll fluorescence technique (Havaux *et al*., 1998). The chlorophyll

fluorescent measurements in field can reflect the exact response of photosynthetic apparatus which is more restricted under natural conditions (Araus *et al*., 1998).

In fact, results showed that deficit water stress did have an effect on photosynthesis, directly or indirectly, by decreasing CO2 availability caused by diffusion limitations (Flexas *et al*., 2007). The amount of photosynthetic pigment was reduced under drought conditions and this can be attributed to chloroplast destruction photosynthetic apparatus, photo oxidation of chlorophyll, interaction of chlorophyll with single oxygen, degradation of chlorophyll substrates, biosynthesis inhibition of new chlorophyll, and an increase in chlorophylase enzyme (El-Tayeb, 2005). In additional, Rubisco activase is susceptible to high temperatures (Craft–Brandner and Salvucci, 2000). This may be associated with drought stress. Severe drought is known to decrease amounts of Rubisco protein in plants (Majumdar *et al*., 1991). It is important that the mechanism that induces this decrease is linked to Rubisco activity (Salvucci, 1992). However, it has yet to be determined precisely how drought stress affects the expression and activity of Rubisco activase in plants (Law and Crafts-Brandner, 2001). So it is important to note that the amount and properties of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) affect leaf photosynthetic capacity. This enzyme catalyses competing reactions, the carboxylation and the oxygenation of ribulose-1,5-bisphosphate (RuBP), initiating the photosynthetic carbon assimilation and photorespiration, respectively (Bota *et al*., 2002). Given that we know that oxygen competes with the dioxide carbon for combination with Rubisco, as we know, under conditions of under rainfed condition and water stress, intracellular levels of carbon dioxide decrease. So, spray application of methanol serves to increase the intracellular CO2, and leads to an increase in the rate of photosynthesis and chlorophyll and carotenoid content. This increase is more tangible under conditions of drought stress, because the plant does not need to increase levels of carbon dioxide under normal irrigation (no water stress), since, in this condition there is sufficient CO2 for the production of chlorophyll. And, while even the use of a high 45% (v/v) concentration of methanol, may cause toxicity. So that production is much less than that in the control treatment. Much research has shown that methanol has a significantly positive effect on photosynthesis and chlorophyll and carotenoid content. An increased rate of photosynthesis induced by methanol treatment was also reported in David *et al*., (2003). Foliar application of methanol increases chlorophyll a, chlorophyll b, total chlorophyll, carotenoid and chlorophyll meter readings (SPAD) which were corresponded with our results (Akbari *et al*., 2014; Bagheri *et al*., 2014; Dawood *et al*., 2013; Ramadan and Omran, 2005).

Ascorbic acid (vitamin C) is synthesized from hexose sugars. Ascorbic acid is an important antioxidant and redox buffer in plants, playing important roles in metabolism and plant responses to abiotic stresses and pathogens. It also works as an enzyme cofactor, so it has multiple roles in various plant physiological processes. Humans have lost the ability to synthesize ascorbate and have to absorb ascorbic acid from the diet including fresh fruits and vegetables, as they are the major sources of ascorbate. Several pathways for ascorbic acid biosynthesis and metabolism have been identified in plants since 1998 (Zhang, 2013). Ascorbate has proposed functions in photosynthesis as an enzyme cofactor (including synthesis of ethylene, gibberellins and anthocyanins). It has a major role in photosynthesis, acting in the Mehler peroxidase reaction with APX to regulate the redox state of photosynthetic electron carriers and as a cofactor for violaxanthin deepoxidase, an enzyme involved in xanthophyll cycle-mediated photoprotection (Smirnoff and Wheeler, 2000). Ascorbate accumulation in Arabidopsis leaves is increased by high light along with expression and activity of GDP-L-galactose phosphorylase (GGP, also VTC2), the enzyme responsible for ascorbate synthesis. That indicates the multiple roles of ascorbate during photosynthesis. These roles may include modulation of hydrogen peroxide and singlet oxygen, enzyme cofactor in the xanthophyll cycle and, speculatively, a photosystem II electron donor during photoinhibition (Smirnoff, 2011). Role of ascorbate in photosynthesis is also supported by the transgenic plants regulating the enzyme for ascorbate synthesis. Suppressed expression of L-galactono-1,4-lactone dehydrogenase gene (GLDH), the gene encoding last step enzyme for ascorbate synthesis, in rice resulted in a loss of chlorophyll, a lower Ribulose 1,5-bisphosphate carboxylase/oxygenase protein content, and a lower rate of CO2 assimilation. As a consequence, a slower rate of plant growth and lower seed set were

observed. Conversely, increasing GLDH expression maintained high levels of chlorophyll, Rubisco protein, and a higher rate of net photosynthesis, resulting in higher seed set (Liu *et al*., 2011). These data at least indicate that the ascorbate level and/or GLDH enzyme is closely associated with plant photosynthesis and growth. Foliar application of ascorbic acid increases chlorophyll a, chlorophyll b, total chlorophyll, carotenoid and chlorophyll meter readings (SPAD) which were corresponded with our results (Abdul, 2014; Ebrahimian and Bybordi, 2012; Dewdar and Rady, 2013; Rahmawati *et al*., 2014; Zonouri *et al*., 2014).

Samdur *et al.,* (2000) in Arachis hypogaea found a positive and highly significant correlation between SPAD readings and chlorophyll content with r value of 0.94^{**} for chlorophyll a, 0.90^{**} for chlorophyll b, and 0.93^{**} for total chlorophyll. Ruiz-Espinoza *et al.,* (2010) in basil found a linear relationship between SPAD readings and the extractable leaf chlorophyll a, chlorophyll a and total chlorophyll with R^2 value 0.51**, 0.22** and 45**, respectively. Wang *et al.,* (2005) in tropical ornamental foliage plants, highly significantly linear relationships ($R^2 \geq 0.87$) found between SPAD readings and chlorophyll a, b, or total chlorophyll content. Finally, we conclude that the r and R2 values between chlorophyll meter readings (SPAD) and chlorophyll (a, b, and total) obtained in peanut leaves were high, positive and significant, indicating closer relationship of these traits with the chlorophyll meter readings (SPAD), i.e., higher the chlorophyll meter readings (SPAD) higher will be the chlorophyll pigments and vice versa. In general terms, the chlorophyll meter readings (SPAD) were strongly correlated with chlorophyll content (a, b, and total) determined by absorbance of extracted pigments, indicating that this instrument can be used in the field to monitor chlorophyll content in peanut leaves, and the data confirms too that chlorophyll meter (SPAD) is an effective tool for rapid and nondestructive estimation of relative chlorophyll content in peanut leaves during the growing season.

Conclusion

Our results showed that methanol and ascorbic acid foliar application were involved in the peanut response under rainfed condition, by changes in pigment contents. Methanol and ascorbic acid foliar application increases chlorophyll a, chlorophyll b, total chlorophyll, carotenoid and chlorophyll meter readings (SPAD) in peanut leaves. Findings suggest that chlorophyll meter readings (SPAD) can be used as a tool to improve peanut quality and for assessing the relative chlorophyll content during the growing season.

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