Journal of Plant Physiology and Breeding

2015, 5(1): 47-54 ISSN: 2008-5168

# Effects of Foliar and Root Applications of Hydro-Alcoholic Solutions on Physiological and Biochemical Attributes and Fruit Yield and Weight of Strawberry

**Zeinab Yavarpanah, Mahdi Alizadeh* and Esmaeil Seifi**

Received: June 1, 2014 Accepted: December 21, 2014
Department of Horticulture, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, P.O. Box.386, Golestan, Gorgan, Iran
*Corresponding author: E-mail: alizadehpub@gmail.com

**Abstract**
An investigation was undertaken to ascertain the influence of hydro-alcoholic solutions on plant growth as well as quality attributes of strawberry fruits. The mother plants of strawberry (*Fragaria ananasa* cv. Gaviota) were subjected to various aqueous solutions of ethanol (15, 30%), methanol (15, 30%) and the mixture of ethanol + methanol (15 or 30%) plus the control (water only) as foliar spraying or via irrigation. Application of alcoholic solutions affected the majority of the characters under investigation. The highest amount of leaf chlorophylls, carotenoids, fruit sucrose and total yield were recorded on the plants treated with combination of 15% ethanol and methanol. The maximum fruit weight and the lowest acidity were found in plants treated with 15% methanol or in combination with 15% ethanol. The foliar spraying was superior in most of the traits over the irrigation method. Further experimentations are required to suggest the results of the present study in a commercial scale to the strawberry growers.

**Keywords:** Alcoholic compounds; Ethanol; Foliar spray; Irrigation; Methanol; Strawberry

## Introduction

Due to serious problems in the past decades as a result of excessive application of chemical inputs and plant growth regulators to enhance agricultural production, nowadays, the need for new technologies to produce safe food and to protect environment has been of great interest to the international community. In this regard, the application of alcohols, especially ethanol and methanol solutions, to improve the performance of plants in the agricultural systems are important. Alcohols are the most important compounds in organic chemistry and have wide frequency in nature and are easily produced in the industry and chemical laboratories (Haakana *et al.* 2001). The plant responses to alcoholic compounds were basically declared by Nonomura and Benson (1992) who reported significant growth of some C3 plants following application of methanol. The C3 cultivated plants, such as strawberry, may lose $CO_2$ during photorespiration. When such plants are subjected to a situation where photorespiration is reduced or prevented, their growth and photosynthesis performance could be significantly increased up to 20-30% (Feibert *et al.* 1995). Many experiments have been carried out to prevent or partially remove photorespiration; all these efforts have been ineffective. However, the process has some useful applications, too (Hans-Walter Heldt 2004).

Photorespiration is a process in plant metabolism by which RuBP (a sugar) has oxygen added to it by the enzyme (rubisco), instead of carbon dioxide, during normal photosynthesis in $C_3$ plants. During this metabolic process $CO_2$ and $NH_3$ are produced and ATP and reducing equivalents are consumed, thus making the photorespiration a wasteful process. Photorespiration loses 25% of the carbon it takes from the Calvin cycle (Krishna 2013). The enhancement of assimilation of methanol into plant photosynthetic organs and subsequent increases in biomass, plant growth and development were already reported in some C3 plants such as tomato, eggplant, cotton, cabbage, wheat, rose, palm and watermelon (Nonomura and Benson 1992; Yavarpanah 2013). The former citation also stated that application of methanol to strawberry plants during periods of elevated photorespiration resulted in the improved plant growth up to 50%. However, there is no report on changes in biochemical and quality characters of strawberry fruits. Therefore, the main objectives of the present study were to evaluate the physiological and biochemical changes of strawberry plants to methanol/ethanol application. Furthermore, the effects of alcoholic concentration and method of application (foliar or root application) were compared. Finally, the likely positive responses and improvement in fruit quality traits as well as yield were studied.

**Materials and Methods**

The present study was undertaken in a private greenhouse on strawberry (*Fragaria ananasa* cv. Gaviota) plantlets. The strawberry mother plants were transferred to the greenhouse and immediately planted in seven-liter plastic pots. The greenhouse was a tunnel shaped plastic structure, equipped with water cooler apparatus and exhaust fans. Air temperature and light intensity fluctuations on the pot surfaces were 25-30°C and 16,000-18,000 Lux during mid-day, respectively. Potting soil mixture consisted of leaf compost: sand: soil (2:1:1). The whole experiment was consisted of totally 14 treatments including ethanol (15, 30%), methanol (15, 30%), their mixtures [ethanol + methanol (15%); ethanol + methanol (30%)] and the control (water). Concentrations of 15 and 30% of ethanol and methanol solutions and their mixtures were prepared using normal tap water. These hydro-alcoholic solutions were applied to strawberry plants by both spraying and irrigation methods in four replications. The first spraying was performed just after releasing mother plants from chilling requirement (spring season). The second and third sprayings were done in a weekly interval apart from the first application. For the irrigation method, the amount of 100 ml of each solution was added to each pot, three times in weekly intervals. For the spraying method, 30 ml of each solution was sprayed on each plant. Two to three drops of tween-20 as surfactant was also added per liter for better efficiency of spraying treatments.The strawberry plants were then subjected to a standard maintenance procedure and their runners were eliminated immediately after emergence during whole experiment. Three approximately average size leaves from each pot were traced out on a graph paper. The leaf shape was cut out from the graph paper and its area was then measured by a digital area meter (DELTA-T, Co. Durham, UK). The mature, fully colored fruits were periodically harvested and stored in freezer ($0^{\circ C}$) following measurement of their physical characters (weight, volume, length). Then, the yield was calculated based on cumulative yield of each pot during the whole experimental period. The fruit biochemical characteristics were also measured using frozen samples in the laboratory. Leaf photosynthetic pigments (Barnes *et al*. 1992), fruit anthocyanin (Wagner 1979), total sugars (McCready *et al*.

1950), glucose (Miller *et al*. 1959), sucrose (Handel 1968) and total acidity (AOAC 1984) of fruits were also measured by standard procedures. The experiment was conducted as a completely randomized design and the data was analyzed using SAS software. The means were also compared using Duncan's multiple range test.

## Results and Discussion

The results of the analysis of variance of morpho-physiological and biochemical characters in strawberry fruits and leaves showed that the effects of hydro-alcoholic compounds on all of the measured traits were statistically significant (data not presented).

***Leaf area*:** The treatments were found to be highly effective on the leaf area expansion (Table 1). The highest leaf areas (230.75 and 218.05 $cm^2$) were recorded in plants sprayed with 15% ethanol and 30% methanol, respectively. It was equal to 90.74% and 80.25% increase, respectively, over the control plants. Similar results were also reported by Madhayan *et al*. (2006) who observed enhancement in plant height and leaf area in cotton due to methanol spraying. Methanol spraying on the plant leaf causes pectin methyl esterase gene activation which increases calcium ion in leaf cells and finally, results in leaf growth enhancement (Ramirez *et al.* 2006). The increasing in the leaf growth with methanol spraying can be attributed to cytokinin and auxin hormones. Methanol application makes the methylotrophic bacteria in leaves indirectly stimulated. These bacteria produce auxin and cytokinin and accelerate the growth of plants (Ivanova *et al*. 2001).

***Leaf pigments:*** The chlorophyll content (a, b and total) as well as carotenoids were considerably affected by the applied treatments (Table 1). The methanol either singly or in combination with ethanol when sprayed on strawberry plants had significant effect on chlorophyll content (Table 1). When the mixture of ethanol and methanol at the rate of 15% was sprayed, the amount of leaf total chlorophyll was increased by about 65.6% as compared to the control treatment. Leaf chlorophyll content is an important factor in determination of photosynthetic capacity of leaves. In different experiments, there were positive correlations between the rate of leaf photosynthesis and chlorophyll content per unit of leaf area (Hesketh *et al.* 1983).

Changes in leaf carotenoid content were almost similar to chlorophylls (Table 1). Combination of ethanol and methanol at the rate of 15% had the highest effect on the carotenoid content although it was not significantly different from some other treatments. The tetraterpenes or carotenoids are considered as supplemental photoreceptors. According to some researchers these pigments absorb wavelengths of light responsible for light oxidation of chlorophyll and therefore, they protect chlorophyll. The rate of photosynthesis is dependent on the leaf chlorophyll content (Krishna 2013). On the other hand, chlorophyll formation in the presence of light needs cytokinin hormone. Alcohols increase plant cytokinin content (Ivanova *et al*. 2001), so they will increase the chlorophyll pigments and carotenoids.

***Fruit weight:*** The strawberry fruits fresh weight was significantly increased following the application of alcoholic compounds. The highest fruit weight was recorded in the strawberry plants sprayed with 15% methanol, either alone (12.58 g) or mixed with 15% ethanol (12.39 g). These findings are in consistent with Lee *et al.* (2006) who also confirmed higher seed weight in soybean plants following methanol spray application. Increased fruit weight could be due to an increase in carbon dioxide fixation and also increased photosynthetic assimilate allocation (Ramirez *et al.* 2006).

***Fruit yield:*** The fruit yield was significantly increased in the treated plants as compared to the control treatments (Table 1). The yield improvement was so obvious even prior to any statistical analysis. The treated fruits were appeared even different in case of dimension (length, width, volume) as compared to the control (data are not shown, Figure 1). The highest yield (425.20 g/plant) was observed in plants sprayed with combination of 15% ethanol and methanol, while the minimum yield (103.28 g/plant) was related to plants irrigated with water only. The results of this research work are comparable with Nonomura and Benson (1992) who found that the methanol treatment increases yield. As 25% of the total fixed carbon is exploited during photorespiration, application of alcoholic compounds can minimize this process (Ramberg *et al.* 2002) and as a result increase fruit yield (Nonomura and Benson 1992). Moreover, Ramirez *et al.* (2006) stated that methanol may delay leaf senescence, enhance their photosynthetic activity and improve yield. In another study, application of methanol significantly increased yield of soybean (Li *et al.* 1995), however, the chlorophyll content or net photosynthetic rate of the treated plants were not significantly affected.

**Table 1. Means of physiological attributes, fruit yield and fruit weight recorded on the strawberry plantlets following application of different concentrations of hydro-alcoholic compounds by the foliar spraying and irrigation methods**

| Treatment | Yield per plant (g) | Fruit weight (g) | Chlorophyll a (mg/g) | Chlorophyll b (mg/g) | Total chlorophylls (mg/g) | Carotenoids (mg/g) | Leaf area ($Cm^2$) |
|---|---|---|---|---|---|---|---|
| Control (Spray) | $106.51^{g+}$ | $7.608^{c}$ | $8.064^{g}$ | $7.738^{g}$ | $6.906^{e}$ | $6.741^{f}$ | $120.97^{de}$ |
| Control (Irrigation) | $103.28^{g}$ | $8.818^{c}$ | $5.771^{h}$ | $7.347^{g}$ | $6.364^{e}$ | $6.267^{f}$ | $83.47^{e}$ |
| Spray 15% methanol | $276.85^{cd}$ | $12.584^{a}$ | $12.993^{ef}$ | $9.627^{ef}$ | $9.426^{cd}$ | $9.032^{bcd}$ | $122.04^{de}$ |
| Irrigation 15% methanol | $331.87^{bc}$ | $11.312^{ab}$ | $15.386^{a}$ | $10.188^{bcde}$ | $10.495^{abc}$ | $9.324^{abc}$ | $117.86^{de}$ |
| Spray 30% methanol | $193.30^{ef}$ | $9.205^{b}$ | $13.164^{ef}$ | $8.991^{fg}$ | $9.275^{de}$ | $8.538^{de}$ | $218.05^{ab}$ |
| Irrigation 30% methanol | $332.82^{bc}$ | $9.087^{b}$ | $12.619^{f}$ | $8.868^{fg}$ | $9.112^{de}$ | $8.147^{ef}$ | $162.53^{cde}$ |
| Spray 15% Ethanol | $227.82^{de}$ | $9.492^{b}$ | $14.187^{bcd}$ | $9.718^{def}$ | $9.666^{cd}$ | $8.784^{bcde}$ | $230.75^{a}$ |
| Irrigation 15% Ethanol | $279.75^{d}$ | $9.325^{b}$ | $14.379^{bcd}$ | $9.512^{ef}$ | $9.822^{cd}$ | $8.651^{cde}$ | $173.85^{cd}$ |
| Spray 30% Ethanol | $273.76^{d}$ | $8.181^{bc}$ | $14.805^{abc}$ | $10.784^{abc}$ | $11.029^{ab}$ | $9.374^{ab}$ | $186.03^{cd}$ |
| Irrigation 30% Ethanol | $156.27^{fg}$ | $9.192^{b}$ | $13.479^{def}$ | $9.842^{cdef}$ | $10.063^{bcd}$ | $8.835^{bcde}$ | $142.31^{de}$ |
| Spray 15% mix (Eth.+ Meth.) | $425.20^{a}$ | $12.385^{a}$ | $15.384^{a}$ | $11.179^{ab}$ | $11.437^{a}$ | $9.763^{a}$ | $135.11^{de}$ |
| Irrigation 15% mix (Eth.+ Meth.) | $383.99^{ab}$ | $9.495^{b}$ | $13.834^{cde}$ | $11.401^{a}$ | $11.039^{ab}$ | $9.632^{a}$ | $205.65^{bc}$ |
| Spray 30% mix (Eth.+ Meth.) | $339.06^{b}$ | $9.347^{b}$ | $13.601^{def}$ | $11.269^{a}$ | $11.147^{a}$ | $9.331^{abc}$ | $151.90^{de}$ |
| Irrigation 30% mix (Eth.+ Meth.) | $211.64^{e}$ | $8.381^{bc}$ | $14.892^{ab}$ | $10.671^{abcd}$ | $10.936^{ab}$ | $9.496^{ab}$ | $164.91^{cde}$ |

[+]The means in each column followed by similar letters are not significantly different.

## Biochemical attributes

***Fruit sugars*:** Total sugars were recorded to be in the highest level (3.97 mg/g) when the strawberry mother plants were irrigated with combination of 30% ethanol and methanol (Table 2). This sugar content was 12.81% higher than fruits of corresponding control plantlets. The utmost glucose level was related to plants sprayed with 30% methanol (2.736 mg/g) although it was not significantly different from the corresponding control. The highest sucrose content (0.704 mg/g FW) was obtained from fruits irrigated with combination of 15% ethanol and methanol. Madhayan *et al*. (2006) demonstrated that the application of 30% methanol can increase total sugars of sugarcane up to 8.7%. Furthermore, Nonomura (1997) reported analogous results on the increase in sugar content of the plants treated with methanol. Hemming *et al*. (1995) stated that sufficient amount of $CO_2$ generated following spraying methanol may alter the photorespiration from catabolism to an anabolic reaction. In fact, the nature of photorespiration is changed following foliar application of alcohols. It means, inside the mitochondria, two glycine molecules are converted to a serine molecule and one $CO_2$. Therefore, the glycine breakdown stage is considered as a source of $CO_2$ required in photorespiration. Since methanol treated plants produce 2 serine molecules in their mitochondria, such situation leads to increased glucose, fructose and the doubling of sucrose and as a result increased yield. The reason for inhibition of photorespiration in the methanol treated plants is due to rapid oxidation of methanol to $CO_2$ and combination with Ribulose-1, 5-bisphosphate carboxylase and decreasing competition with oxygen (Ramirez *et al.* 2006).

***Fruit anthocyanin content*:** Though all strawberry fruits obtained an acceptable red color during their maturation, but the amount of anthocyanin was not equal in alcohol treated and control plants. The pigment was more intense in the treated fruits as compared to control (Table 2). The maximum effect was imposed by 30% ethanol spray (0.301 μmol/g), however, it was not significantly different from those of 15% ethanol + methanol spray (0.288 μmol/g) and 15% methanol irrigation (0,286 μmol/g). According to Nonomura (1997), methanol enhances sugar content of plants. Sugars are preliminary compounds for the synthesis of anthocyanin. Furthermore, sugar and color development are closely correlated with each other (Drake and Fellman 1987). Hence, the increase in fruit anthocyanin content following the alcohol treatment is certainly due to high sugar content. Anthocyanin synthesis and accumulation in plant tissues are affected by many factors such as light intensity and quality and also the carbohydrate content in tissues (Taiz and Zeiger 2006).

***Total soluble solids (TSS) or Brix:*** A considerable part of the fruit TSS is constituted by sugars which are normally increased during ripening. The amount of TSS is also dependent on the species, type of plant and nutritional and environmental conditions (). In the present study the TSS was significantly affected by alcohols, their concentrations and method of application (Table 2). The fruits treated with 15% ethanol through irrigation method gained the highest brix (9.37%). Most of the other treatments also attained higher brix as compared to the control such as 15% ethanol + methanol irrigation (8.05%). The ethanol inside the plant is converted to acetaldehyde. Acetaldehyde also can be converted to acetate (acetic acid) by acetaldehyde dehydrogenase enzymes. Acetate is then converted to acetyl-coenzyme A and finally to water and $CO_2$ (Cossins and Beevers 1962). Increased $CO_2$ enhances plant photosynthesis and then carbohydrate and sugar synthesis in plant. Furthermore, since the methanol is a carbon-rich liquid, it can be a source of $CO_2$ production in higher plants. So, the rising in the levels of the sugars will be common following spraying either ethanol or methanol. It has been reported that methanol spray was effective to increase reducing sugars in tobacco plants (Moustakas and Ntzanis 2005). Therefore, it may be concluded that increasing Brix could be due to the final conversion of alcohols to carbon dioxide and increasing photosynthetic materials, especially sugars.

***Total acidity:*** The accumulation of free organic acids and its salts in fruits is considered as total acidity. This attribute was declined in all fruits that received alcoholic treatments (Table 2). The lowest amount of total acidity was recorded in plants irrigated with 15% methanol (0.032) and also sprayed with the mixture of 15% ethanol and methanol (0.038). According to Nonomura (1997), the sugar content can be increased due to methanol application on plants. So, the reduction in total acidity may be attributed to increased sugar content due to alcohols (ethanol or methanol) and also conversion of organic acids to sugars during fruit ripening.

***Alcohol Application technique:*** Most of the traits were found to be better affected by foliar spraying method as compared to irrigation. Furthermore, in a primary experiment (data are not shown) we even lost some plants due to root application of high concentration (30%) of methanol. In a greenhouse experiment on tomato, Rowe *et al.* (1994) applied 0% to 20% solutions of methanol or ethanol to the foliage and roots. A 5% solution of methanol applied to roots severely decreased shoot growth, and higher concentrations killed the plants. But, foliar applications increased the stem weight, fresh and dry leaf weight up to 19%. They also obtained somewhat better results with methanol as compared to ethanol.

**Conclusion**

The results of the present research work clearly confirmed the special physiological, agronomic and biochemical changes in the strawberry fruits following foliar application of alcoholic solutions. The highest amounts of chlorophylls, carotenoids, sucrose and fruit yield were recorded in plants treated mostly with the combination of 15% ethanol and methanol. While, the greatest fruit weight and the lowest acidity were found in plants treated either with 15% methanol or in combination with 15% ethanol. In general, considering the entire observed data it can be concluded that the combination of ethanol and methanol (mostly at the concentration of 15%) was superior to other treatments, followed by the 15% methanol alone. The foliar spraying was superior to irrigation method with regard to most of the characters under study. Since the present study was undertaken under greenhouse conditions, further research in a large scale is needed to commercialize these results.

**Table 2. Biochemical changes recorded in strawberry plantlets following application of different concentrations of hydro-alcoholic compounds as foliar spraying as well as via irrigation method**

| Treatments | Total sugars (mg/g) | Glucose (mg/g) | Sucrose (mg/g) | Brix (%) | Fruit total acidity | Fruit Anthocyanins (µmol/g) |
|---|---|---|---|---|---|---|
| Control (Spray) | $3.549^{bc}$ | $2.512^{abc}$ | $0.437^{def}$ | $5.275^{f}$ | $0.102^{a}$ | $0.184^{e}$ |
| Control (Irrigation) | $3.526^{bc}$ | $2.167^{cde}$ | $0.476^{bcde}$ | $6.025^{ef}$ | $0.088^{b}$ | $0.197^{e}$ |
| Spray 15% methanol | $2.678^{ef}$ | $2.062^{def}$ | $0.297^{gh}$ | $5.901^{e}$ | $0.049^{fg}$ | $0.212^{de}$ |
| Irrigation 15% methanol | $2.728^{ef}$ | $2.015^{ef}$ | $0.411^{ef}$ | $6.701^{cd}$ | $0.032^{h}$ | $0.286^{ab}$ |
| Spray 30% methanol | $3.336^{cd}$ | $2.736^{a}$ | $0.342^{fg}$ | $5.566^{ef}$ | $0.077^{cd}$ | $0.258^{bc}$ |
| Irrigation 30% methanol | $3.041^{de}$ | $2.119^{cde}$ | $0.433^{ef}$ | $7.525^{c}$ | $0.051^{fg}$ | $0.253^{bc}$ |
| Spray 15% Ethanol | $3.876^{ab}$ | $1.951^{efg}$ | $0.151^{h}$ | $5.975^{de}$ | $0.065^{de}$ | $0.272^{abc}$ |
| Irrigation 15% Ethanol | $2.378^{f}$ | $1.591^{g}$ | $0.546^{bc}$ | $9.375^{a}$ | $0.054^{ef}$ | $0.252^{bc}$ |
| Spray 30% Ethanol | $2.585^{f}$ | $2.328^{abcde}$ | $0.451^{ced}$ | $6.433^{cd}$ | $0.074^{d}$ | $0.301^{a}$ |
| Irrigation 30% Ethanol | $3.071^{de}$ | $2.308^{bcde}$ | $0.451^{cde}$ | $7.525^{c}$ | $0.066^{de}$ | $0.206^{de}$ |
| Spray 15% mix (Eth.+ Meth.) | $2.579^{f}$ | $1.642^{f}$ | $0.542^{bcd}$ | $7.816^{c}$ | $0.038^{gh}$ | $0.288^{ab}$ |
| Irrigation 15% mix (Eth.+ Meth.) | $2.499^{f}$ | $2.351^{abcd}$ | $0.704^{a}$ | $8.053^{b}$ | $0.075^{d}$ | $0.243^{cd}$ |
| Spray 30% mix (Eth.+ Meth.) | $2.698^{ef}$ | $2.435^{abcd}$ | $0.233^{gh}$ | $7.625^{c}$ | $0.067^{de}$ | $0.254^{bc}$ |
| | $3.978^{a}$ | $2.655^{ab}$ | $0.568^{b}$ | $8.201^{b}$ | $0.068^{de}$ | $0.255^{bc}$ |

[+]The means in each column followed by similar letters are not significantly different.

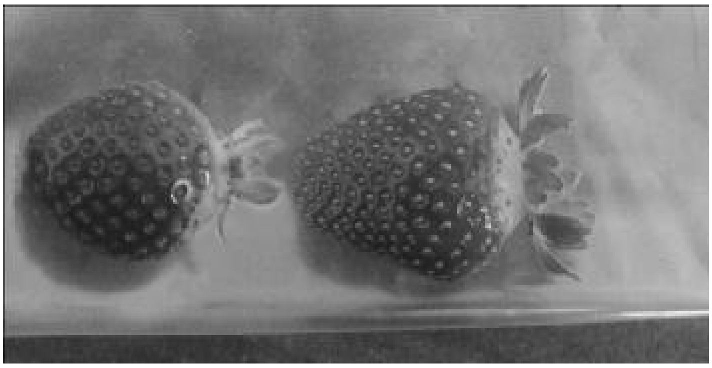

**Figure 1. Morphological changes in the strawberry fruits following application of hydro-alcoholic compounds (right) as compared to the control (left).**

## References


AOAC, 1984. Official Methods of Analysis, Association of Official Analytical Chemists, 14th ed., Benjamin Franklin Station Washington, DC.

Barnes JD, Balaguer L, Manrique E, Elvira S and Davison AW, 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. Environ Exp Bot 32(2): 85-90.

Cossins EA and Beevers H, 1962. Ethanol metabolism in plant tissue. Plant Physiol 48: 375-380.

Drake DR and Fellman JH, 1987. Indicator of maturity and storage quality of Rainier sweet cherry. HortSci 22(2): 283-285.

Feibert EBG, James SR, Rykbost KA, Mitchell AR and Shock C, 1995. Potato yield and quality not changed by foliar-applied methanol. HortSci 30(3): 494-495.

Haakana K, Sarkka L and Somersalo S, 2001. Gaseous ethanol penetration of plant tissues positively effects the growth and commercial quality of miniature roses and dill. Sci Hort 88(1): 71-84.

Handel EV, 1968. Direct microdetermination of sucrose. Anal Biochem 22: 280-283.

Hans-Walter H, 2004. Plant Biochemistry. Third edition. Elsevier, 630p.

Hemming DJB, Criddle RC and Hansen LD, 1995. Effects of methanol on plant respiration. J Plant Physiol 146: 193-198.

Hesketh JD, Orgen WL, Hageman ME and Peters DB, 1981. Correlation among leaf CO2-exchange rates, areas and enzyme activities among soybean cultivars. Photosynth Res 2: 21-30.

Ivanova EG, Dornina NV and Trotsenko YA, 2001. Aerobic methylobacteria are capable of synthesizing auxins. Microbiol 70: 392-397.

Krishna H, 2013. Physiology of Fruit Production. Studium Press, India, 296p.

Lee HS, Madhaiyan M, Kim CW, Choi SJ, Chung KY and Sa TM, 2006. Physiological enhancement of early growth of rice seedlings (*Oryza sativa L.*) by Production of phytohormone of $N_2$-fixing methylotrophic strains. Biol Ferti Soils 42: 402-408.

Li Y, Gupta G, Joshi JM and Siyumbano AK,1995. Effect of methanol on soybean photosynthesis and chlorophyll. J Plant Nutr 18: 1875–1880.

Madhayan, T, Poonguzhali S, Sundaram SP and Sa T, 2006. A new insight into foliar applied methanol influencing phylloplane ethylotrophic dynamics and growth promotion of cotton (*Gossypium hirsutum* L.) and sugarcane (*Saccharum officinarum* L.). Env Exp Bot 57: 168-176.

McCready RM, Guggolz J, Silviera V and Owens HS, 1950. Determination of starch and amylase in vegetables. Anal Chem 22: 1156-1158.

Miller GL, 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31: 426-428.

Moustakas NK and Ntzanis H, 2005. Dry matter accumulation and nutrient uptake in flue-cured tobacco (*Nicotiana tobacum* L.). Field Crops Res 94: 1-13.

Nonomura AM, 1997. Method and composition for enhancing carbon fixation in plants. Proc Natl Acad Sci, USA 89: 9794-9798.

Nonomura AM and Benson A, 1992. The path of carbon in photosynthesis: Improved crop yields with methanol. Proc Natl Acad Sci, USA 89: 9794-9798.

Ramberg HA, Bradley JSC, Olson JSC, Nishio JN, Markwell J and Osterman JC, 2002. The role of methanol in promoting plant growth: An update. Rev Plant Biochem Biotechnol 1: 113-126.

Ramirez IF, Dorta V, Espinoza E, Jimenez A and Mercado H, 2006. Effects of foliar and root applications of methanol on the growth of Arabidopsis, tobacco and tomato plants. J Plant Growth Regul 25: 30-44.

Rowe RN, Farr DJ and Richards BAJ, 1994. Effects of foliar and root applications of methanol and ethanol on the growth of tomato plants (*Lycopersicon esculentum* Mill). New Zeal J Crop and Hort Sci 22: 335–337.

Taiz L and Zeiger E, 2006. Plant physiology, (4th Edition). Sinauer Associates, Sunderland, Mass, 623p.

Wand SJE, Theron KI, Ackerman J and Marais SJS, 2006. Harvest and post-harvest apple fruit quality following applications of kaolin particle film in South African orchards. Sci Hort 107: 271–276.

Wagner GJ, 1979. Content and vacuole/extra vacuole distribution of neutral sugars, free amino acids and anthocyanins in protoplast. Plant Physiol 64: 88-93.