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Effects of methanol on the yield and growth of soybean in different irrigation conditions

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Abstract

To investigate the effects of methanol on the yield and growth of soybean (*Glysine max* L.) in different conditions of irrigation a split plot experiment was conducted in the form of randomized complete block design during three replications at the Research field of Islamic Azad University, Karaj Branch in the year 2012. Aqueous solutions 0 (control), 7, 14, 21 and 28% (v/v) of methanol as well as three levels of irrigation 40, 65 and 70% soil water depletion were factors of this study. The results of the ANOVA indicated that the interaction effects of methanol and irrigation on harvest index, leaf area index, dry leaf weight, dry pods weight and phonological levels were significant. Generally, it can be stated that foliar application of methanol in moderate concentrations in different conditions of irrigation on soybean variety of Williams caused relative improvement in growth indexes. Due to the indeterminate growth of soybean, on the one hand increase in photosynthesis and dry matter production and the other hand relative accelerated flowering phase due to the use of methanol lead to decrease in remobilization time, therefore no significant difference was observed in grain yield.

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Introduction

Increasing the yield in the unit of surface is one of the important issues that have attracted many researchers' attention. Photosynthesis is the substantial process for the production of organic matter in plants. Usually, the amount of the production dry matter has a direct correlation with photosynthesis efficiency of the plant and also the way in which CO_2 fixation occurs in crops. Therefore, the acceleration of the photosynthesis rate could be useful for increasing the capacity of producing crops. Today, in order to achieve this goal, compounds such as methanol, ethanol, propanol, butanol are used. One of the main advantages of these compounds is their preventing and reducing the effects of stresses induced, this is due to their photorespiration which ultimately results in increasing the production of organic matter in plant along with increasing its growth and yield (Safarzadeh Vishekaei, 2007). Methanol is one of the simplest organic molecules and natural product of plant metabolism which is emitted from the leaves of most plants (Fall and Benson, 1996). The proportion of methanol produced that is recycled via metabolism in plants is not known, but it is clear that plant tissues can metabolize methanol (Gout *et al.*, 2000). In plants methanol can arise from a number of sources; for example, from pectin de-methylation in cell walls (Obendorf *et al.*, 1990), protein repair pathways (Mudgett and Clarke, 1993), and lignin degradation (Lewis and Yamamoto, 1990). A small proportion of this endogenous methanol reaches leaf surface, where it is volatilized or consumed by methylotrophic bacteria (Murrell and Dalton, 1992). Exposure to exogenous methanol from atmospheric pollution and deliberate application of methanol have been reported to increase growth and yield in a number of C_3, but not C_4, crops that have experienced drought stress (Nonomura and Benson, 1992). Foliar application of methanol causes an increase of fresh and dry weight in Arabidopsis and tobacco (Ramirez *et al.*, 2006). Radiotracer ^{14}C and ^{13}C NMR studies revealed that methanol is metabolized by alcohol oxidase to formaldehyde and formic acid, which are further converted to serine, methionine, purine and thymidylate (Gout *et al.*, 2000). The CO_2 produced from the oxidization of methanol is utilized within the Calvin-Benson cycle for glucose metabolism (Hanson and Roje, 2001).

Results of Zbiec *et al* (2003) showed that various crops such as tomato, bean, sugar beet, oil seed rape when treated with methanol solutions yielded 20-30% higher than the control. Results of field and pot trials on the effects of methanol on cotton and sugarcane conducted by Madhaiyan *et al.*, 2006 showed that application of 30% methanol as foliar spray significantly increased plant height, plant dry weight, leaf area, boll number and boll dry weight, leading to increase of cotton seed yield over control. As soybean has a low grain yield in Karaj region, the main aim of this study was recognition of the effects of methanol on the increase of soybean growth and yield in different irrigation conditions.

Matrials and methods

To evaluate effects of Methanol on the yield and growth of soybean in different conditions of irrigation, a field experiment has been conducted in Karaj Islamic Azad University Research farm- located (35 o 45' N, 51 o 6' E, 1313m) in the year 2012.The experiment was done in the form of a split plot in a randomized complete block design with three replications. The factors included three treatments of irrigation: 40%, 65% and 70% soil water depletion (calculated by gypsum block). The other treatments included five levels of Methanol foliar application: 0% (control, without application), 7, 14, 21 and 28% (v/v) of Methanol. These solutions applied over head three times in two week intervals on foliage parts of soybean. Each treatment planted at 6 lines (as furrow with type irrigation system) in 5 meters length and the distance between two rows was 50 cm and between two plants on each row was 7 cm. The distance among the plots and the replications were considered 1 meter and 2 meters respectively. Measurements for growth parameter calculated 10 days after last application of Methanol. Measurements for yield were done on two middle rows of each plot and 4 m² selected from that rows were measured for yield. 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.

Results

Plant height

The variance analysis showed that the irrigation treatment in the probability range of 1% had a significant effect on the plant height while the effect of Methanol and irrigation×Methanol on the height of the plant was not significant (Table 1). Among the irrigation regimes the most plant height was allocated to the Normal Irrigation 92.69 cm (at 40% water depletion) and the least height was 71.58 cm (at 70% water depletion). It can be deduced that having the maximum plant height in the irrigation treatment was thanks to two factors: 1. the indeterminate variety of Williams Soy bean. 2. The induction of the vegetative growth as a result an increase in the amount of water.

Table 1. Analysis of variance for seed yield and other traits under methanol and irrigation treatments.

S.O.V	df	MS										
		Plant height	Nods on main stem	Leaf Area Index	Dry leaf weight	Total dry matter	Pods weight	Phonological step	Biological yield	Harvest index	1000 seeds weight	Seed yield
Replication	2	290.76*	0.35^{ns}	3.28*	714828.40^{ns}	279185.8^{ns}	238423.58^{ns}	0.007^{ns}	3363147.4^{ns}	12.40^{ns}	246.14**	210431.007^{ns}
Rep*Irrigation	4	161.17^{ns}	17.18**	4.06*	1446107.71*	2329031.9^{ns}	793211.33^{ns}	0.29^{ns}	374819.9*	11.32^{ns}	90.17*	55052.03^{ns}
Irrigation (A)	2	1820.21**	9.60*	26.80**	6753033.98**	52976509.7**	6520695.15**	0.27^{ns}	64222406.1**	106.08**	651.39**	4099326.812**
Methanol (B)	4	23.48^{ns}	11.50**	6.63**	2426925.07**	8021650.00*	1971420.95*	2.88**	3155245.9*	14.52^{ns}	43.57^{ns}	28791.741^{ns}
(A)*(B)	8	23.93^{ns}	3.17^{ns}	4.29*	1143603.82*	8995636.5**	2244197.11**	1.04**	2356523.1*	30.89^{ns}	21.68^{ns}	24638.154^{ns}
Error	24	87.03	2.35	1.47	452226.49	1994900.9	531474.50	0.16	1078858.2	10.49	32.06	65122.30
C.V (%)		11.61	9.72	21.25	22.85	18.23	29.49	7.63	14.06	9.51	5.48	16.83

ns: Non-significant

* and **: significant at the 5% and 1% probability levels, respectively.

Number of nodes on the main stem

Considering the variance analysis, this trait had a significant difference in Irrigation and Methanol in the probability range of 5% and 1% respectively, but the interaction effect of Irrigation×Methanol was not significant (Table 1). The irrigation treatment had the furthest nodes on the main stem 16.58 (at 40% water depletion), and it did not have a significant statistical different by comparison to the irrigation treatment at 65% water depletion which was 15.80. Moreover, the irrigation treatment at 70% water depletion had the least number of nods on the main stem (14.98). Among the treatments, Methanol 14%(v/v) with 17.25 nods created a significant statistical difference with control having 14.11 nodes (Table 2). Although Methanol increased the number of nodes on the main stem, it did not cause a significant change in the plant height, which can be due to its neutral effect on the distances among the internodes.

Leaf Area Index

The effect of irrigation and Methanol on the leaf area index in probability range of 1% and the interaction effects of irrigation×Methanol in probability range of 5% were significant (Table 1). According to the results, among the irrigation treatments, the most LAI, which was 6.99 was observed in the irrigation treatment 40% water depletion the least one, which was 4.33 was shown by the irrigation treatment at 70% water depletion (Table 2). Lambert and Heartherly, 1995 in the study of the effect of irrigation on the genotypes of soybean asserted that the complete irrigation in comparison with the condition of drought stress can increase the LAI of soybean remarkably. Among the spray treatments; however, there wasn't any noticeable statistical difference among various concentrations of Methanol with the control, the Methanol of 14%(v/v) caused the most LAI with the number of 6.53.whereas the highest concentrations of Methanol created a significant reduction in the LAI, as far as the least LAI (4.55) was allocated to the treatment of Methanol 28%(v/v). The reduction may be brought about by the accumulation of Formaldehyde and the PH variations in the herbaceous cells as a result of the increase of Methanol concentration, and finally their effect on Methanol's assimilation. Also, the mean of interaction effects of irrigation×Methnol (Table 3) has demonstrated that the irrigation treatment at 40% water depletion with Methanol 14% has had the most Leaf area index (8.53), and the least Leaf area index has been observed in the treatment at 70% water depletion and Methanol 28% (2.67). It may be conducted that Methanol caused the most LAI in the irrigation condition after 40% of water depletion as compared with other irrigation treatments. According to the Ramirez *et al.*, 2006 Methanol spraying activates Pectinmethylesterase gene in the leaf cells which increases ca^{2+} and effects the transmission of nutrients to the leaf cells; Hence, it plays an important role boosting the leaf area index and the leaf area duration.

Table 2. Mean comparison for seed yield and other traits under methanol and irrigation treatments.

Treatment	Plant height (cm)	Nods on main stem	Leaf Area Index	Dry leaf weight (kg/ha)	Total dry matter (kg/ha)	Pods weight (kg/ha)	Phonological step	Biological yield (kg/ha)	Harvest index (%)	1000 seeds weight (g)	Seed yield (kg/ha)
40% water depletion	92.69a	16.58a	6.99a	3530.8a	9480.1a	2265.2a	5.30a	9560.0a	34.13b	108.73a	2055.8a
65% water depletion	76.66b	15.80ab	5.84b	3083.6a	8008.3b	2593.6a	5.53a	7157.0b	36.66a	104.93a	1480.79b
70%water depletion	71.58b	14.98b	4.33c	2211.5b	5749.1c	1323.4b	5.30a	5440.7c	31.34c	95.90b	1012.11c
LSD (5%)	7.74	1.15	0.91	506.8	1064.4	549.41	0.30	782.78	2.44	3.96	192.32
control	80.36a	14.11c	5.91ab	3140.0b	7146.9bc	1619.6c	4.72b	7413.6ab	33.38a	100.33b	1469.3a
Methanol 7%	80.85a	15.94ab	6.47a	3350.6a	8372.1ab	1877.4bc	4.83b	7914.8a	34.05a	104.27ab	1540.5a
Methanol 14%	82.57a	17.25a	6.53a	3418.9a	8954.4a	2741.3a	6.00a	7950.6a	36.24a	106.12a	1587.8a
Methanol 21%	79.58a	15.55bc	5.14bc	2570.2bc	7751.5abc	2335.4ab	5.66a	7120.2ab	33.21a	103.23ab	1534.8a
Methanol 28%	78.19a	16.08ab	4.55c	2230.1c	6543.2c	1729.9bc	5.66a	6530.1b	33.34a	102.00ab	1448.9a
LSD (5%)	10.00	1.49	1.18	654.7	1374.2	709.29	0.39	1010.6	3.15	5.11	248.28

Means, in each column per factor, followed by at least one letter in common are not significantly different at the 5% probability level-using LSD test.

Leaf dry weight

The results showed that the effects of irrigation and Methanol put and impact on the leaf dry weight in probability range of 1% and the interaction effects of irrigation×Methanol in probability range of 5% were effective too (Table 1). The maximum leaf dry weight was obtained in irrigation treatment at 40% water depletion (3530.8) and the minimum in irrigation treatment at 70% water depletion (2211.5) (Table 2). Since soybean are indeterminate, frequent watering can stimulate vegetative growth, and also it can increase the number, size and leaf dry weight. Among Methanol treatments, the treatment of 14%(v/v) showed the highest leaf dry weight (3418.9) and the treatment of 28% (v/v) Methanol indicated the lowest weight (2230.1) (Table 2). According to Mauney and Gerik, 1994 Methanol has increased the leaf area and the leaf diameter in the treated plants.

Methanol affects the metabolism of Pectin in cell-wall of the leaves and rises their size. Actually, the effect of Methanol on demethylation of Pectin, an essential reaction for the growth of cells, causes the leaf growth. Moreover, the influence of Methanol on the activities of Methylotrophic bacteria, present in the leaves of soybeans, stimulates the production of effective hormones for the growth of leaves such as Sytoknin and Oxine (Galbally and Kiristine, 2002).

Considering the interaction effects of irrigation×Methanol, the highest leaf dry weight of the treatments was gained by interaction effects of irrigation at 40% water depletion and Methanol 7% (4417.97) which was placed in one statistical group with Methanol 14%. The lowest leaf dry weight was observed in the irrigation treatment at 70% water depletion and Methanol 28% (1306.68). It should be taken into account that in all of the irrigation treatments the concentration of Methanol 28% decreased the leaf dry weight which can be due to the reaction of leaf area index as a result of the poisonous effect of Methanol at such concentration.

Table 3. Interaction between methanol×irrigation for soybean traits under methanol and irrigation treatments.

Treatment	Total dry matter (kg/ha)	Leaf Area Index	Dry leaf weight (kg/ha)	Pods weight (kg/ha)	Phonological step
40% water depletion					
control	7662.40c	7.25ab	3631.53b	1832.24c	4.00b
Methanol 7%	11030.40ab	7.90ab	4417.97a	2230.37b	5.50a
Methanol 14%	12197.40a	8.53a	4293.34a	3597.19a	6.00a
Methanol 21%	9439.40b	7.65ab	3485.87b	1816.82c	5.50a
Methanol 28%	7071.20cd	4.32c	1825.32d	18.49.53c	5.50a
65% water depletion					
control	7231.40c	5.92bc	2870.42c	1394.86cd	5.00b
Methanol 7%	7341.60c	6.16b	3262.57b	2426.63b	5.66a
Methanol 14%	9890.60b	6.31b	3658.96b	3841.59a	6.00a
Methanol 21%	9716.20b	5.09bc	2918.13bc	3485.04a	5.50a
Methanol 28%	5861.80de	5.71bc	2708.11c	1819.62c	5.50a
70%water depletion					
control	4659.80e	5.14bc	2888.11bc	600.00d	4.00b
Methanol 7%	6854.60cd	5.34bc	2576.27c	640.19d	4.50ab
Methanol 14%	6292.60d	4.17c	2129.45cd	2151.87bc	6.00a
Methanol 21%	6105.80d	3.64cd	2156.92cd	1704.20c	6.00a
Methanol 28%	4832.60e	2.67d	1306.68d	1520.56cd	6.00a

Means, in each column per factor, followed by at least one letter in common are not significantly different at the 5% probability level-using LSD test.

Total dry matter

A significant difference was observed in the effects of the treatment of irrigation and Methanol on total dry matter in probability range of 1% and 5% respectively and interaction effects of irrigation×Methanol in probability ranges of 1% (Table 1). The maximum produced dry matter belonged to the irrigation treatment at 40% water depletion (9480.1) and the minimum amount was allocated to the irrigation treatment at 70% water depletion (5749.1) (Table 2). It was thanks to the more height of the bush and also an increase in the leaf area index and the leaf dry weight in normal irrigation treatment (i.e. an irrigation treatment at 40% water depletion) (Table 2). Among Methanol treatments, the treatment of Methanol 14% (v/v) had the most produced dry matter (8954.4). And the one with Methanol 28% had the least produced dry matter (6543.2).

The interaction effects of irrigation×Methanol demonstrated that Methanol spraying had the most effect on the production of the dry matter in the condition of irrigation at 40% water depletion, as the highest amount of the produced dry matter was obtained in the treatment at 40% water depletion and Methanol 14% (12197.40). By intensifying water stress the dry matter per unit area decreased and in each irrigation treatment the effect of sprayed Methanol on boosting the production of the dry matter was less in comparison with the control as well (Table 3). Also, it can be asserted that average concentrations of Methanol as compared with the control caused a growth in the amount of the produced dry matter; whereas Methanol with the concentration of 28% brought about a drop in the amount of the produced dry matter per unit among all the irrigation treatments. The application of 21% Methanol treatments increased 15% its light absorption thanks to the rise in its leaf area index. The Methanol 27 and 35% treatments had the least light absorption (Mirakhori *et al.*, 2010). Therefore, it can be conducted that the average concentrations of Methanol have increased the production of dry matter because of the rise in photosynthesis, leaf area and assimilation of CO_2. On the contrary, high concentrations of Methanol have decreased photosynthesis activity and subsequently the production of the dry matter.

Pod weight

The results showed that the irrigation treatments and the Methanol treatment in the probability ranges of 1% and 5% respectively had a significant effect on the pod weight (Table 1). The irrigation treatment at 65% water depletion revealed the maximum pod weight (2593.6) that its difference with another treatment at 40% water depletion with the weight of 2265.2 was not significant.

The minimum pod weight was observed in the treatment at 70% water depletion (1323.4) (Table 2). This reduction in the pod weight might be due the fall in the number of pods and also a rise in number of seedless pods as a result of the drought stress imposed to the plant. As some researchers have reported that drought stress can decrease pod number in the plant noticeably and increase the number of seedless pods which will lead to a decline in the yield (Weaver *et al.*, 1991). Among Methanol treatments, the 14% treatment showed the maximum pod yield (2741.3) whereas the control treatment had the least amount of this trait (1619.6) (Table 2). The increase in the pod weight can be thanks to the rise in the leaf area index, leaf weight and the total dry matter. All these factors give a boost to the production and the accumulation of the dry matter in the green parts of the plant; moreover they increase the accumulation of photosynthetic matters in the pods (Li *et al.*, 1995). Considering the interaction effects of irrigation×Methanol, the most pod yield was observed in the irrigation treatment at 65% water depletion and Methanol 14% (3841.59). Although it was placed in one statistical group with the treatment of Methanol 14% and irrigation at 40% water depletion (3597.19), the noticeable point is the proportional increase in the pod weight as a result of a slight stress in the treatment of 65% water depletion which probably accelerated the transmission of photosynthetic matters from the source towards the pods. In addition, the results showed that the least pod weight was in the treatment of 70% water depletion without the application of Methanol (600) (Table 3).

Phonological levels

Variance analysis study of phonological levels indicated that irrigation had no effect on this trait. Moreover, the effect of Methanol and the interaction effects of irrigation×Methanol in the probability range of 1% were significant (Table 1). The results showed that foliar application of Methanol caused a relative acceleration in the phonology of soybeans, as ten days after the last Methanol spraying the treatment with 14% (v/v) completed the phonological levels faster than the other treatments. However, statistically there was no significant difference among the treatments 14, 21 and 28% (v/v) (Table 2).

The interaction effects of irrigation×Methanol on phonological levels of soybean demonstrated that the application of Methanol accelerated phonological levels in all the irrigation treatments (Table 3). The above mentioned results accord with the study of Nonomura and Benson, 1992.

Biological yield

The results of biological yield variance analysis showed that a significant difference exists in the irrigation treatment (in the probability range of 1%), Methanol treatment and the interaction effects of irrigation×Methanol (in probability range of 5%) (Table 1). Among the irrigation treatments, the one at 40% water depletion revealed the number 9560 as the maximum and the treatment at 70% water depletion showed the number 5440.7 as the minimum biological yield. On the other hand among Methanol spraying treatments, the treatment with 14% (v/v) had the highest biological yield (7950.6), although it didn't differ from the control, 7 and 21% (v/v); and 28% Methanol treatment showed the lowest biological yield (6530.1) (Table 2).

In the study of Makhdum *et al*., 2002., 30% Methanol treatment had most effect on the biological yield of cotton. Also, in other researches on canola and tomato, the use of Methanol caused an increase in the biological yield (Zebiec *et al*., 2008. Row *et al*., 1994) on the levels of the most of plants, there are symbiotic bacteria by the name of Methylotrophic; which receive Methanol and in turn make the precursor for the production of hormones such as Oxine and Sytokinin. These hormones play a great role in the growth of leaves and by delaying the senescence period can increase the production and the accumulation of dry matter and Biomass (Heins, 1980).

Harvest Index

According to the variance analysis of the harvest index, there was a significant difference in the irrigation treatment (in probability range of 1%) (Table 1). The maximum harvest index was allocated to the irrigation treatment (in probability range of 1%). (Table 1). The maximum harvest index was allocated to the irrigation treatment at 65% water depletion, 36.66 and the minimum amount of to the irrigation treatment at 70% water depletion, 31.34. (Table 2). Regarding the results of the study of Ehyaee *et al.*, 2011, the irrigation regimes did not affect the harvest index. Taherabadi's survey showed that irrigation had a significant effect on the harvest index as the most and the least harvest index was observed in the complete watering and water requirement of 50% respectively. (Taherabadi *et al.*, 2012).

1000 seed weight

The 1000 seed weight was significant in irrigation treatment in probability range of 1% (Table 1). The highest amounts of 1000 seed weight were recorded in irrigation treatment at 40% water depletion (108.73) and 65% water depletion (104.93), whereas the lowest amount was observed in irrigation treatment at 70% water depletion (95.90) (Table 2). Water stress at grain filling period leads to a decrease in the size of the seeds and affects seed weight severely (Kpoghomou *et al.*, 1990). In fact, the reduction of 1000 seed weight can be due to two factors:

1. The drop in the allocation of photosynthetic matters to the seeds.
2. The fall of the harvest index as a result of the decrease in the production of dry matter in such a wet condition.

Seed yield

The results of seed yield variance analysis indicated that irrigation had a significant effect on the yield. On the contrary, there wasn't a significant difference in Methanol effects and interaction effects of irrigation×Methanol on the seed yield (Table 1). According to the data mean comparison, the most seed yield, which was 2055.84 was allocated to the irrigation treatment at 40% water depletion and the least amount (1012.11) was observed in the treatment at 70% water depletion (Table 2). The seed yield difference with 103% in the treatment at 40% water depletion in proportion to the stress condition of the treatment at 70% water depletion may be thanks to the biological yield and the increase in leaves and the accumulation of dry matter in irrigation treatment at 40% water depletion, which brought about a rise in the allocation of photosynthetic matters to the seeds and therefore boosted the seed yield.

Among Methanol treatments, although there was not a significant statistical difference in the trait of seed yield, the treatment with 14% (v/v) showed the highest seed yield; as in comparison with the control, there was an increase of 8.06%. Considering the average seed yield of soybeans and the little cost of Methanol (comparing with other nutrients), It is worth to study the increase of this trait practically. Moreover, regarding the fact that soybean variety of Williams is indeterminate and considering the boost of vegetative growth in Methanol treatments, more photosynthetic matters can probably be directed to the seeds from other parts of the plants and as a result the seed yield can be improved. This is possible by a change in density, planting array and also source-sink.

Conclusion

The methanol can be used as a rich source of CO_2 in order to increase photosynthesis and soybean biomass. Regarding the fact that soybean variety of Williams is indeterminate and considering the boost of vegetative growth in Methanol treatments, more photosynthetic matters can probably be directed to the seeds from other parts of the plants and as a result the seed yield can be improved. This is possible by a change in density, planting array and also source-sink.

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