



Effect of Methanol Foliar Application on Seed Yield and it's Quality of Soybean (*Glycine max* L.) under Water Deficit Conditions

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ABSTRACT

To determine effects of methanol foliar application on soybean grain yield under, a factorial split-plot experiment based on a randomized complete block design with four replications was done at Research Field of Faculty of Agriculture and Natural Resources, Islamic Azad University-Karaj Branch, Karaj, Iran, during 2010. The first factor was drought stress in two levels (based on depletion of $a_1=40\%$ and $a_2=70\%$ of available soil moisture). The second factor was spraying times of methanol in two levels (in the morning at $b_1=8-10$ AM and in the evening at $b_2=19-21$ PM). Third factor was foliar application number of methanol with three levels (each $c_1=7$, $c_2=14$ and $c_3=21$ days, Methanol spray was applied 5, 3 and 2 times during growth season of soybean, respectively). All treatments were sprayed with 21% (v/v) methanol concentration. 2 g lit^{-1} Glycine was added to prepared solutions. Grain yield, biomass, harvest index, 1000-grain weight, protein and oil percentage and yields were measured in this study. The results showed that there was significant ($p>0.05$) differences between effects of drought stress level on measured parameters. Under normal irrigation, the highest (3187 kg ha^{-1}) and lowest (1526 kg ha^{-1}) soybean grain yield was obtained in a_1 and a_2 , respectively. Results of oil yield indicated that a_1 and a_2 were produced the most (731 kg ha^{-1}) and least (484 kg ha^{-1}), respectively. Besides, results showed that significant differences exists ($p>0.05$) between interaction effects $a*b$, $a*c$, $b*c$ and $a*b*c$ in some traits, as under normal and deficit irrigation maximum grain yield were observed by methanol spraying every other week in the evening and every 7 days in the morning, respectively. It seems applying aqueous solutions 21 % (v/v) methanol on water deficit condition on different periods on soybean plants and time application can reduce harmful effects of drought and improve plant potential to cope with stress.

Keywords: methanol, soybean, yield, biomass, harvest index.

INTRODUCTION

The amount of water needed for plant growth and development in soybean is an important factor and can have significant influence on yield and yield. Drought is one of the most important limiting factors of soybean growth [1]. Short maturation varieties compared to long duration varieties of soybean less respond to water stress, then in arid zones or areas where water is limited, Short maturation varieties should be planted [2]. Daneshian *et al.*, (2002) Showed that stress at pod formation stage increased flower and pod shedding and reduce the number of seeds in soybean [3]. It is reported that water stress during early reproductive stages increases the loss of flowers and pods [4]. Therefore, lack of available water is the most critical factor affecting flower and pod shedding, as adequate water supply prevents destructive changes in the area of pedicles falling. Egli and Zhemwen (2001) concluded that the drought in the early flowering caused a slight decrease in the number of pods per plant, because flowering in indeterminate cultivars of soybean occurs over a long period and the plant can compensate water deficit in late flowering by producing more flowers [5]. Vieira *et al.* (1992) claimed that water stress during grain filling considerable decreased soybean yield (32-42%) [6]. They found that drought stress during seed development

decreased yield, shortens the grain-filling stage and lowers final seed size and in severe stress, the seeds are very wrinkled and ill-formed. Most agricultural regions of Iran are located on a dry climate. And drought is one of the factors limiting photosynthesis and yield. Therefore, application of methanol by foliar spraying can significantly reduce the damage caused by water deficiency. Nonomura and Benson (1992) foliar application of methanol increase the growth and yield of C₃ species and methanol is considered as a source of carbon for plants [7]. Methanol molecules are smaller than the carbon dioxide and absorbed sooner by plant, moreover, foliar application of Methanol delayed senescence of leaves through ethylene production in plant, this increases photosynthetic active period and leaf area duration (LAD) [8]. Several studies have been shown that foliar application of methanol can prevent of biomass reduction [9-10]. Li *et al.* (1995) revealed that Grain yield, 1000-seed weight and number of pods per plant of soybean treated by in Methanol significantly increased compared to control [11]. Methanol spray is a method which increases crop CO₂ fixation unit area. Recent investigation showed that C₃ crops yield and growth increased via methanol spray and methanol may act as C source for these crops [12]. Foliar application with 5-10% methanol increase plant growth and yield [7]. In order to better absorption of methanol by the leaves, after foliar application, hours of darkness is necessary [13]. It has been reported that foliar application of methanol could enhance activity of FBPase, an important enzyme controlling photosynthesis [14]. Hemming and Criddle (1995) reported that foliar application of methanol cause to rise in Carbon conversion efficiency [15]. Experiments have shown that foliar application of 20% methanol to peanut could increase leaf area index, crop growth rate, pod growth rate, radiation use efficiency, pod and grain yield, 1000- grain weight, number of ripened pod and grain protein of peanut [16]. Mirakhori *et al.* (2009) [10] and Nadali *et al.* (2010) [17] stated that 21% (v/v) methanol spray poses the greatest impact on yield, and other physiological traits. Positive effects of foliar application of methanol on growth and yield of soybean have been confirmed in previous studies. Thus, the objectives of this study were to investigate the effects of time and number of foliar application of methanol on soybean quality yield under deficit irrigation.

MATERIALS AND METHODS

The experiment was carried out in 2010 at Karaj, Iran (35°41' 15"N, 50°56'51"E, 1190 m). Soil type was clay loam with pH of 7.6 and its salinity in 0-30 cm of soil profile was 2.7 dS m⁻¹. The experimental design was a randomized complete block in a factorial split-plot arrangement with four replications. In these experiments, the first factor was consisted of water stress in two levels (based on 40 and 70% depletion of available soil moisture a₁ and a₂, respectively), second factor was time of foliar application of methanol in two levels (8-10 AM=b₁ and 19-21 PM=b₂) and the third factor was in three levels (foliar application of methanol weekly=c₁, bi weekly=c₂ and three weekly=c₃ which was performed spraying 5, 3 and 2 times, respectively). To prevent of methanol poisoning at light presence and chlorophyll destruction, 2 gr lit⁻¹ of glycine was added to prepared solution [7, 18]. Moreover, plots related to other treatments were sprayed by water and glycine at time of spray solution. The first Methanol spray was performed 60 days after planting on July 16. Spray application was continued until solution drops flow from plant surface. Time of irrigation was determined using chalk block in term of Soil Moisture Depletion (SMD). Chalk blocks already calibrated and applied available moisture depletion curve provided by Paknejad *et al.* (2007) [19] from in Research Field of Islamic Azad University-Karaj Branch. Irrigation was done when the show 20 and 80. Plots were furrow irrigated soon after planting all treatments irrigated until the fifth stage (V₅). To avoid interference among watering treatments, 250 cm distance between drought treatments was considered. The soybean was planted at an average density of 40 plant m⁻², 0.5 m row spacing and 5 cm distance between seeds within rows. Each plot involved six 5m rows. Soybean seeds were disinfected, then inoculated by soybean inoculums and were manually sown in 4 May 2010. Soybean were seeded at high density and then thinned to the target densities (40 plant m⁻²) after their establishment. Plants were harvested at 125 days after planting and Grain yield, biomass, harvest index, 1000 grain weight, protein and oil percentage and yields were measured. In order to determination of Grain yield, harvest index, and 1000 grain weight, 4m⁻² of each plot were harvested. Kejeldal method was applied for protein content determination and for oil extraction socsole device was used. All data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 2002). Treatment means were separated using Duncan test at P < 0.05. The graphs were fitted using Excel 2003 statistical software.

RESULTS AND DISCUSSION

The results of ANOVA (Table 1) demonstrated that the effects of drought stress level on grain yield, biomass, harvest index, 1000-seed weight, protein and oil percentage and yields were significant, but time of spraying had no significant differences on all measured traits at P < 0.05 probability level. Moreover, there was a significant difference between numbers of methanol application on all evaluated features. Grain yield, besides, was further affected by the interactions of water stress and number of spraying, water stress and time of spraying, time and number of spraying. Based on the results, there was significant (p>0.05) differences between interaction effects of drought, time and number of methanol spraying was detected on all traits. The highest (3187 kg ha⁻¹) and lowest

(1526 kg ha⁻¹) Grain yield was obtained in under normal and deficit irrigation, respectively. Here, yield reduction equal to 52.1% was detected in deficit irrigation compared to normal irrigation (Table 2). Yield loss was anticipatable due to water stress was applied from primary growth stages. This result is supported by previously published works [9-10] in which the maximum soybean yield was produced under normal irrigation condition. Pandey (1984) found that soybean grain yield was linear affected by water [20]. Based on result, in normal irrigation condition the highest grain yield (3403 kg ha⁻¹) related to methanol spraying per 14 days in the evening. However, foliar application of methanol per 14 days in the evening did not differ significantly in comparison with foliar application of methanol per 7 days in the morning and evening. In other hand, the lowest grain yield (2928 kg ha⁻¹) was obtained in methanol spraying per 14 days in the morning and had no statistically significant with methanol spraying per 21 days in the morning. Maybe, methanol spraying per 21 days produced a less carbon assimilation. Under normal irrigation condition, there was no difference between spraying in the morning and evening and methanol spraying per 14 days was superior due to usage reduction of methanol and higher yield. For deficit irrigation, the highest (2025 kg ha⁻¹) and lowest (1090 kg ha⁻¹) grain yield were produced in methanol spraying per 7 days in the morning and methanol spraying per 21 days in the evening, respectively (figure 1). Under deficit irrigation, spraying in the morning is better. This is probably due to closing of stomata in the evening induced by decreasing turgor and then more foliar application of methanol compensated to some extent damage from deficit irrigation and the changes trend of yield under deficit irrigation conditions affected by the number of methanol application which these result is in correspond to that of Khashaman (2010) [21]. Drought stress cause reduces oil percentage as the most (27.34 %) and least (22.80 %) oil percentage were generated in a₁ and a₂, respectively. A₂ showed a reduction as much as 16.61 % related to normal conditions (a₁) (Table 2). Daneshian et al. (2002) reported that drought stress reduced soybean yield due to reduction of grain number per plant and 1000 grain weight [3]. They, also, found that as water stress increased, further seed oil and less protein rate is induced, however, eventually drought stress a marked negative effect on the oil and protein yield because of yield loss. Increasing effects of methanol spraying on the oil and protein percent have been confirmed in soybean [14]. In normal irrigation, spraying of methanol every other week in the morning produced the greatest oil percent (28.27 %) which had no significant different with spraying in the evening. on the contrary, spraying of methanol every 21 days in the morning induced the smallest oil percent (22.30 %) which placed statistically in a same group with spraying weekly in the evening (Figure 3). Thus, it may be concluded that, under normal irrigation more time methanol application can create destruction of chlorophyll and less applications of methanol can decrease plant assimilation. In deficit irrigation foliar application of methanol every other week in the evening made the utmost oil percent (24.21 %) and spraying of methanol every 21 days in the evening generated the minimum oil percent (20.70 %), however, it had no significant difference with methanol application in the morning every 7 days. Finally, methanol spraying suggested in the evening every other week. The highest (890.93 kg ha⁻¹) and lowest (429.90 kg ha⁻¹) oil yield were observed in a₁ and a₂, respectively (Table 2). In normal irrigation spraying of methanol every 7 days in the evening generated the peak oil yield (952.53 kg ha⁻¹), besides, methanol application every 21 days in the evening breed the lowest yield oil (554.90 kg ha⁻¹). As oil yield affected by grain yield, more application time of methanol has favorable effects on oil yield. Under deficit irrigation methanol spray every 7 days in the morning and foliar application of methanol every 21 days presented the uppermost (573.29 kg ha⁻¹) and least (364.30 kg ha⁻¹) oil yield, respectively. In deficit irrigation because of more application time of methanol, greatly reduce damage from deficit irrigation, and changes trend of yield affected by more application time of methanol (Figure 4). Thus, it can be deductible more application times of methanol impose positive influences on oil yield, because oil yield and grain yield have direct relation and consequence for both normal and drought stress recommended methanol spray every 7 days in the morning. According to Eduardo *et al.* (1993) drought stress reduced seed weight due to reduction of current photosynthesis and transported products rate [22]. Mean 1000- grain weight comparison revealed that the highest (165.52 g) and lowest (143.21 g) mean were concerned to a₁ and a₂, respectively (Table 2). In normal conditions of irrigation the most 1000 grain weight (175.42 g) was detected in treatments which received methanol spraying every 14 days in the morning and placed in a same group with spraying of methanol every 14 days in the evening. In contrast, application of methanol every 21 days in the morning produced the least 1000- grain weight (160.42 g). Under deficit irrigation, methanol application every 21 days in the morning created the greatest (152.68 g) and application of methanol every 14 days in the morning caused the smallest (136.63 g) 1000- grain weight (Figure 5). Since transportation of nutrition to grain was done through leave and according to Mitchell *et al.* (1994) that reported constantly methanol spray damage pepper leaves [23]. According to Mirakhori *et al.* (2009) [10] and Paknejad *et al.* (2009) [9] who declared that methanol spray increase 1000- grain weight, it can be found the reason for increasing of 1000- grain weight. Negative correlation between grain oil and protein percent has been reported [24]. The most (36.60%) and least (32.38%) protein percent was reported in a₁ and a₂, respectively. Under normal irrigation conditions, the maximum (39.23%) content of grain protein was discovered with methanol spray fortnight in the evening, however, showed no statistically significant ($P < 0.05$) difference relative to methanol application weekly in the evening and put in a top group, on the other hands, the minimum (34.28%) content of grain protein was observed by methanol spray every 21 days in the evening and did not significant different compared to foliar application of methanol in the morning every 21 days. In deficit irrigation, the highest (34.89%) and lowest

(32.28%) grain protein rate were found in treatments which received methanol every other week in the morning and every 7 days in the morning, respectively. nonetheless, foliar application of methanol every 14 days in the morning and evening were statistically similar. Accordingly, in normal and deficit irrigation foliar application of methanol fortnight in the evening and morning were appropriate (figure 6). The maximum protein yield ($1243.93 \text{ kg ha}^{-1}$) was observed in a_1 and minimum protein yield (698 kg ha^{-1}) was produced in a_2 which showed a yield loss as much as 44 % compared to a_1 (Table 2). Under normal irrigation, methanol spray weekly, in the morning gave the greatest protein yield ($1529.56 \text{ kg ha}^{-1}$) and was statistically similar to foliar application of methanol in the evening. Conversely, foliar application of methanol every other week in the morning presented the lowest protein yield ($1019.37 \text{ kg ha}^{-1}$), although, methanol spray in the morning and evening were not significantly different and put in a same group. In deficit conditions, methanol spray weekly in the morning and foliar application of methanol fortnight in the evening led to the utmost ($1077.29 \text{ kg ha}^{-1}$) and least ($921.56 \text{ kg ha}^{-1}$) protein yield (figure 7). Overall, foliar application every 7 days in the morning and evening in both normal and water stress is better. More time spraying of methanol can reduce damage from water stress and the changes trend of yield affected by time of methanol application which this finding is in agreement with Khashaman (2010) observations [21]. Highest (10550 kg ha^{-1}) and lowest (7437 kg ha^{-1}) value of soybean biomass was detected in a_1 and a_2 , respectively (Table 2). Maximum biomass (11777 kg ha^{-1}) in normal irrigation was related to methanol application in the morning every other week, nevertheless, there was no significant difference with methanol application spray in the evening every other week. Whereas, minimum biomass (9110 kg ha^{-1}) was recognized with methanol spray in the morning every 21 days. Moreover, in deficit irrigation the most soybean biomass (8265 kg ha^{-1}) was found when methanol spray was conducted every other week in the morning, and was statistically in the same group with methanol spray in the evening, in opposition, the least soybean biomass (6302 kg ha^{-1}) was distinguished in methanol spray every 21 days in the evening (Figure 2). Mitchell *et al.* (1994) found that foliar application of methanol continuously harmful influences on pepper leave, so, pepper biomass decreased in treatments receiving methanol spray weekly [23]. Indeed, main reason for yield and 1000-seed weight reduction of soybean is the result of biomass loss. Spaeth *et al.* (1984) confirmed that in soybean indeterminate cultivars harvest index has a more firmness [25]. The highest (31.01 %) and lowest (25.52%) harvest index were scrutinized in a_1 and a_2 , respectively. reduction in harvest index in a_2 compared to a_1 was 18%. Under normal irrigation, foliar application of methanol in the evening every 21 days provided the highest harvest index (33.24 %), however, there was no significant difference between methanol spray both in the evening and morning every 21 days. On the other hand, the minimum harvest index (29.09 %) obtained by methanol spray in the evening each 14 days; nonetheless, it put in the same group along with methanol spray in the morning weekly and methanol spray both in the morning and evening every other week. In deficit irrigation, the most soybean harvest index (27%) was related to foliar application of methanol in the morning weekly, although, it showed significant difference compared to methanol application in the evening weekly and no significant difference with every other week in the morning, and 21 days both in the morning and evening. Besides, methanol spray every week in the evening produced the least soybean harvest index (23.35 %), however, it had significant difference with spraying in the evening weekly (Figure 8). Harvest index affected by biomass and grain yield.

Table1: Analysis of variation for effects of timing and number of methanol foliar application and its period under water deficit conditions

Source of variation	df	Grain Yield	Biomass	Harvest Index	Grain Weight	Oil%	Oil yield	Protein%	Protein yield
Replication	3	0.00 ^{ns}	0.16 ^{ns}	0.42 ^{ns}	15.66 ^{ns}	2.07 ^{ns}	154.26 ^{ns}	0.44 ^{ns}	614.02 ^{ns}
Soil moisture (A)	1	33.1**	122.33**	360.96**	5970.15**	101.50**	728464.35**	166.35**	786388.48**
Time of foliar application (B)	1	0.03 ^{ns}	0.04 ^{ns}	4.00 ^{ns}	0.48 ^{ns}	0.20 ^{ns}	12.01 ^{ns}	2.97 ^{ns}	2.38 ^{ns}
A*B	2	0.01**	0.66*	5.02 ^{ns}	0.88 ^{ns}	0.47 ^{ns}	12506.24**	12.42*	10.40 ^{ns}
Error (AB)	9	0.10	0.33	1.24	5.95	1.61	21.33	1.28	19.76
Number of foliar application (C)	2	0.65**	16.03**	21.85**	173.98*	61.74**	274340.24**	48.79**	400224.74**
A*C	2	0.21**	0.76**	7.34*	164.89*	11.51*	46870.43**	9.35**	137407.97**
B*C	2	0.22**	0.01 ^{ns}	4.54 ^{ns}	83.22 ^{ns}	1.84 ^{ns}	159.24 ^{ns}	1.25 ^{ns}	1701.29*
A*B*C	2	0.06*	0.41 ^{ns}	6.57*	363.16**	11.17*	19627.36**	1.36 ^{ns}	1515.72*
Error	24	0.01	0.07	1.90	24.38	1.23	398.29	2.45	571.93
C.V	-	4.62	3.70	4.40	3.85	6.91	3.50	3.75	1.75

**, Significant at 0.01 level *, Significant at 0.05 level n.s, non significant

Table 2: Means comparison time and number of methanol foliar application under water deficit conditions (MS)

Treatment	Levels	Grain yield (kg.ha ⁻¹)	Biomass kg.ha ⁻¹	Harvest Index (%)	Grain weight (g)	Oil (%)	Oil yield (kg.ha ⁻¹)	Protein (%)	Protein yield (kg.ha ⁻¹)
Soil moisture (A)	a1=40%	3187 a	10550 a	31.01 a	165.52 a	27.34 a	890.93 a	36.60 a	1243.93 a
	a2=70%	1526 b	7437 b	25.52 b	143.21 b	22.80 b	429.90 b	32.38 b	698 b
Time of foliar application (B)	b1=8-10AM	2384 a	9075 a	28.55 a	154.64 a	23.31 a	608 a	34.11 a	1127 a
	b2=19-21PM	2330 a	9016 b	27.98 a	154.26 a	23.44 a	607 a	34.61 a	1128 b
Number of foliar application (C)	c1=each 7 days	2589 a	9134 b	27.84 b	151.30 b	22.13 b	757 a	34.19 b	1293 a
	c2=each 14 days	2213 b	9999 a	27.37 b	157.86 a	25.64 a	549 b	36.19 a	978 c
	c3=each 21 days	2268 b	8003 c	29.59 a	153.93 ab	22.36 b	516 c	32.71 c	1110 b

Mean with the same letters in each column does not have significant difference at the 5% level of probability

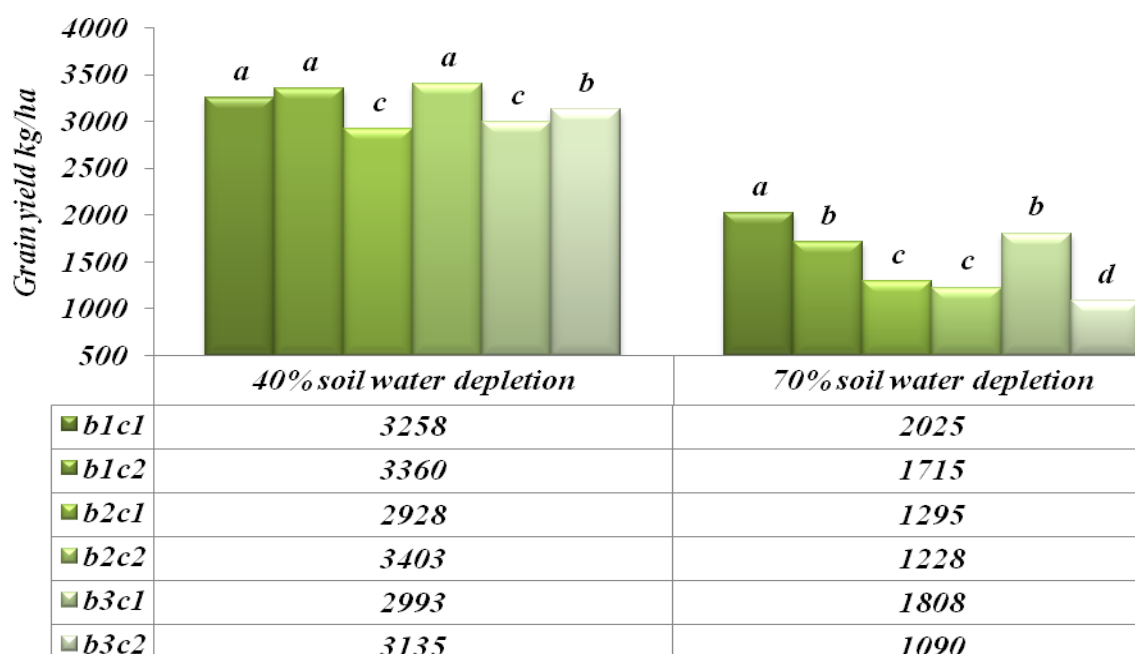


Fig 1: Effect timing (b1=8-10 AM and b2=19-21 PM) and number (c1=each 7 days ,c2=each 14 days ,c3=each 21 days) Foliar application of methanol on grain yield under water deficit conditions.

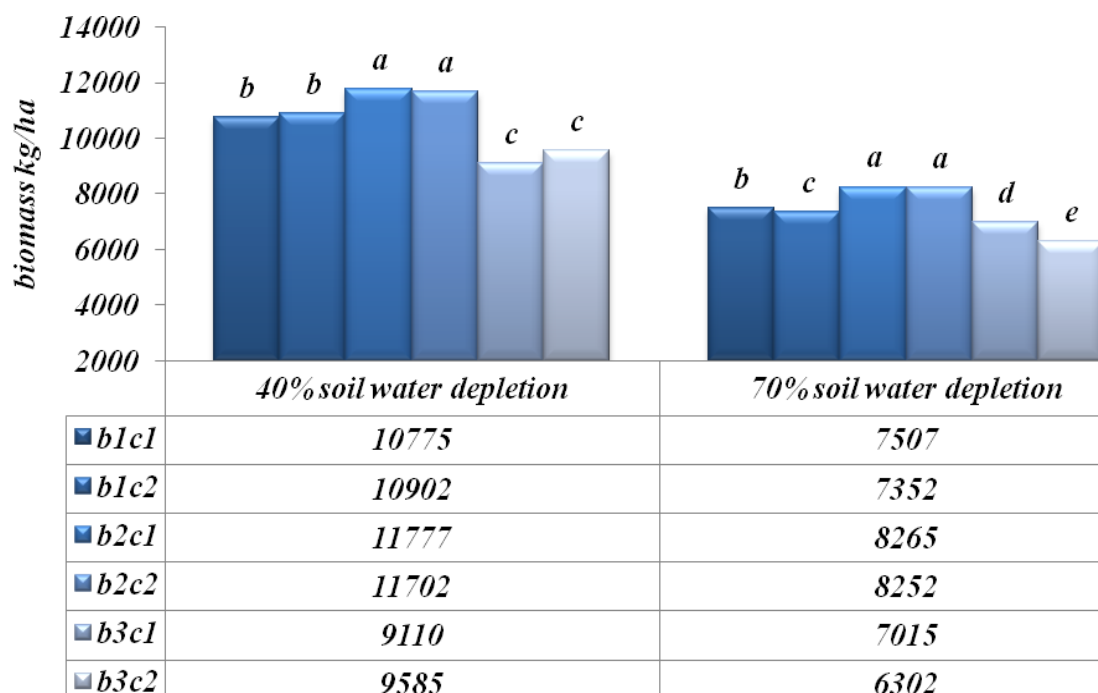


Fig 2: Effect timing (b1=8-10 AM and b2=19-21 PM) and number (c1=each 7 days ,c2=each 14 days ,c3=each 21 days) Foliar application of methanol on biomass under water deficit conditions.

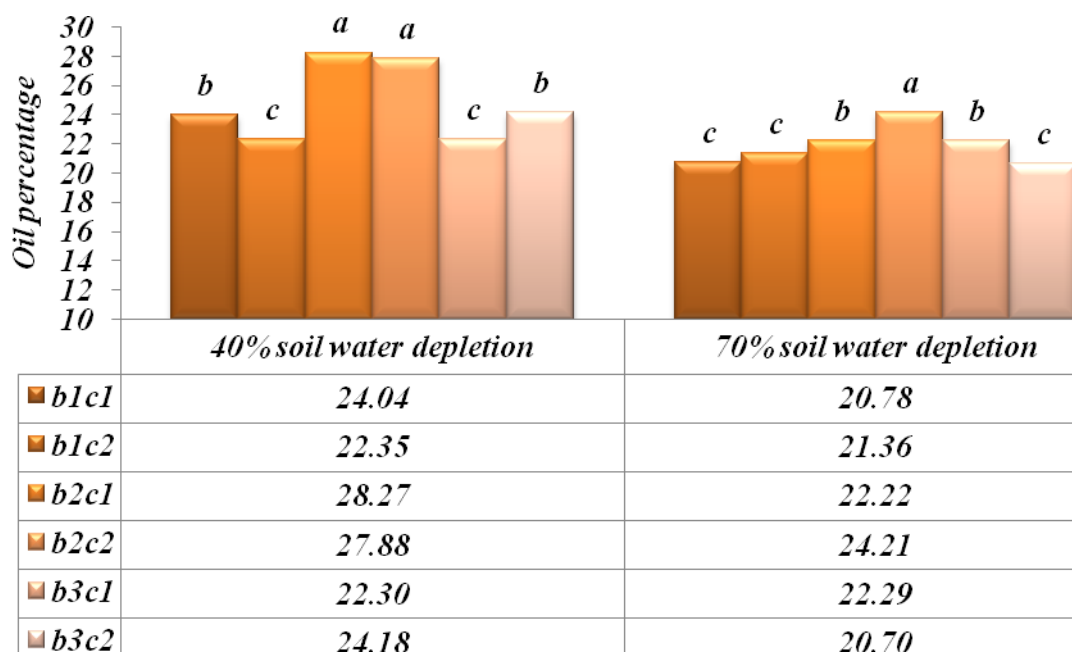


Fig 3: Effect timing (b1=8-10 AM and b2=19-21 PM) and number(c1=each 7 days ,c2=each 14 days ,c3=each 21 days) Foliar application of methanol on oil percentage under water deficit conditions.

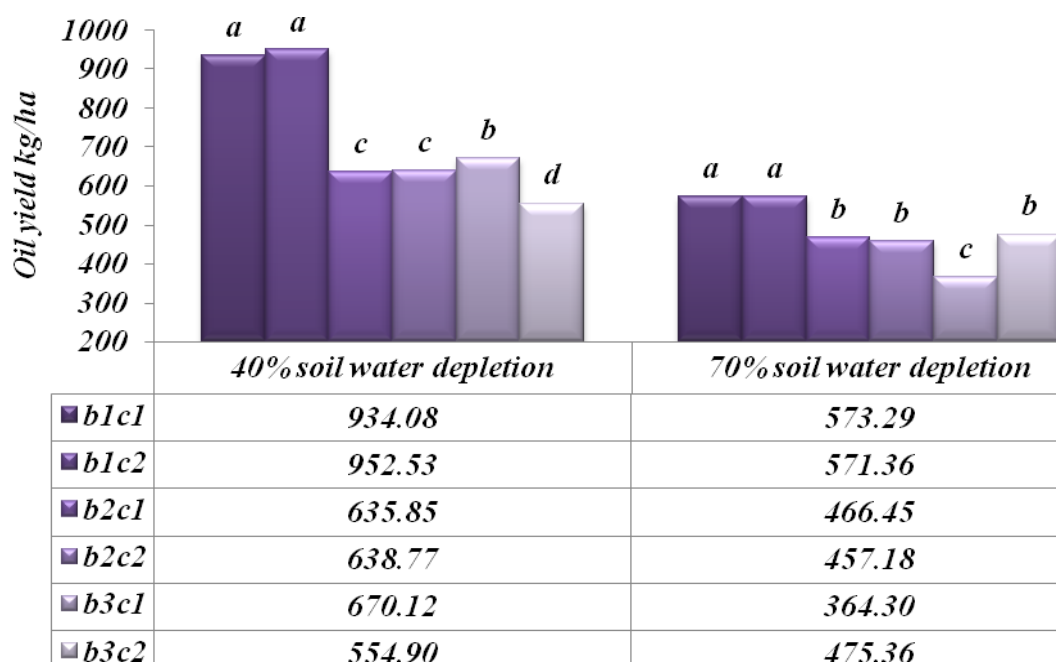


Fig 4: Effect timing (b1=8-10 AM and b2=19-21 PM) and number (c1=each 7 days, c2=each 14 days, c3=each 21 days) Foliar application of methanol on oil yield under water deficit conditions.

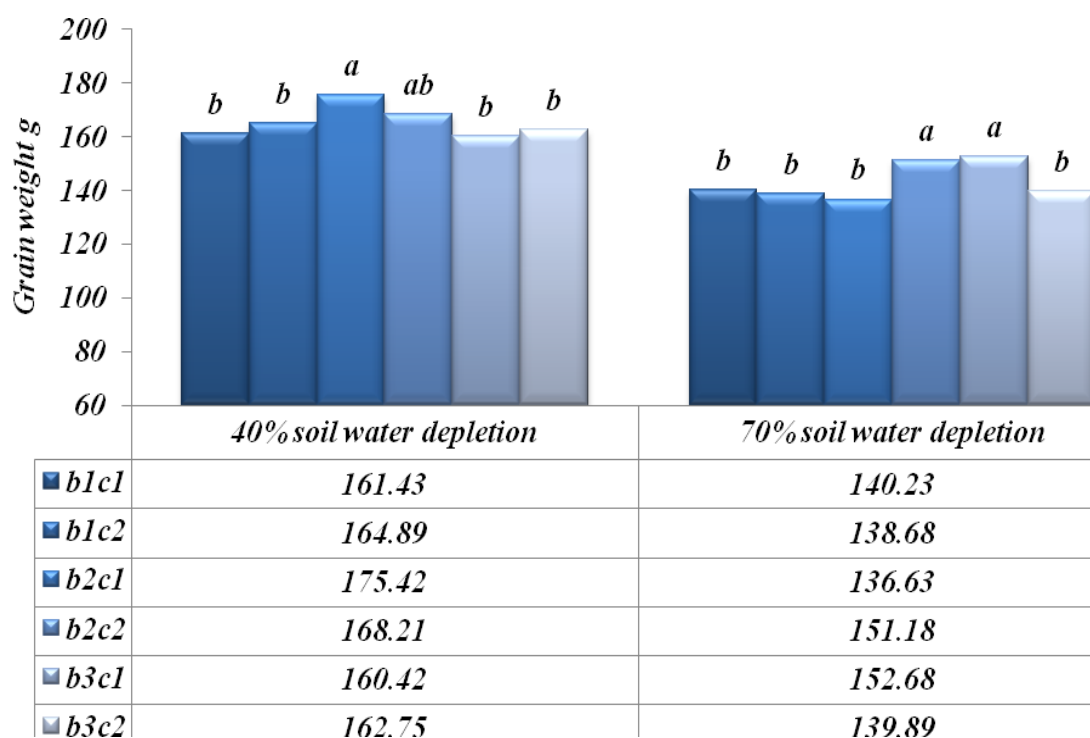


Fig 5: Effect timing (b1=8-10 AM and b2=19-21 PM) and number (c1=each 7 days ,c2=each 14 days ,c3=each 21 days) Foliar application of methanol on Grain weight under water deficit conditions.

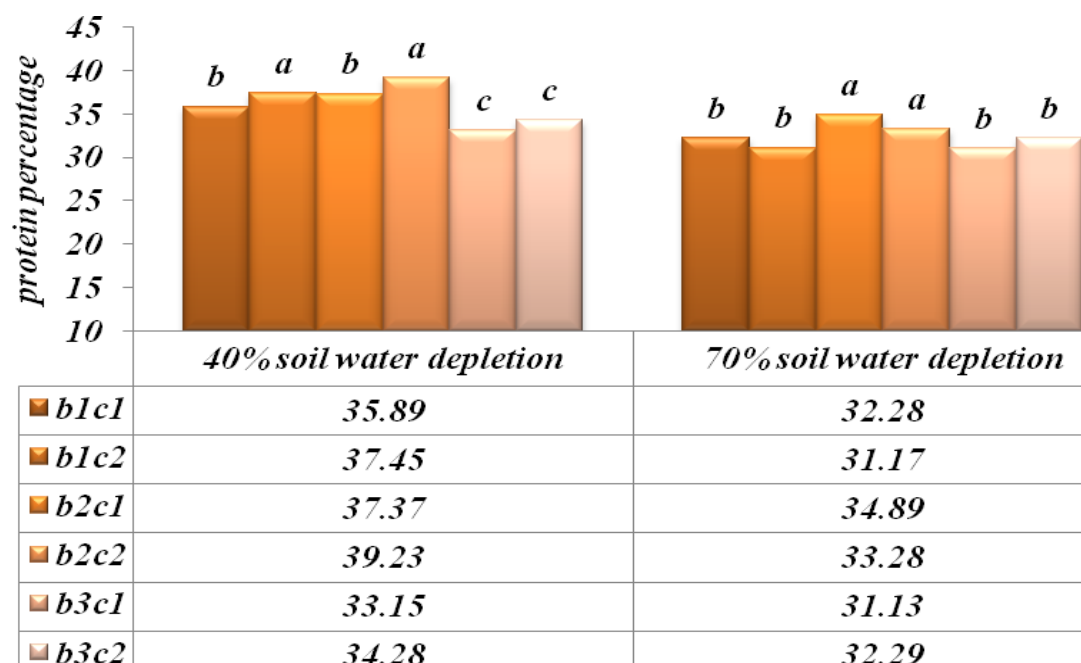


Fig 6: Effect timing (b1=8-10 AM and b2=19-21 PM) and number (c1=each 7 days ,c2=each 14 days ,c3=each 21 days) Foliar application of methanol on Protein percentage under water deficit conditions.

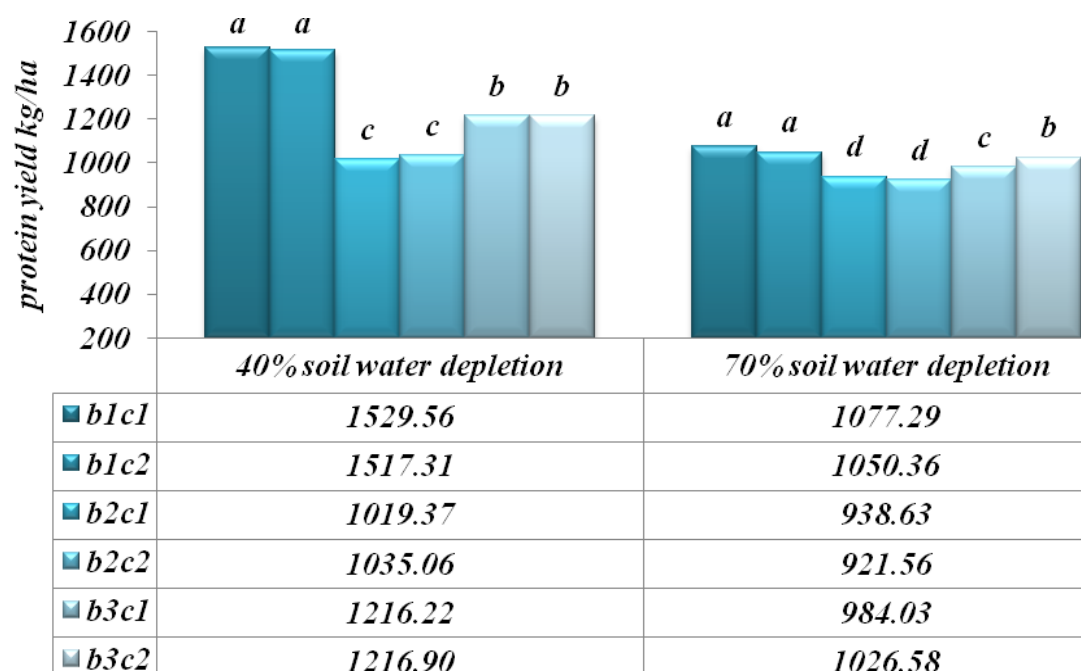


Fig 7: Effect timing (b1=8-10 AM and b2=19-21 PM) and number (c1=each 7 days ,c2=each 14 days ,c3=each 21 days) Foliar application of methanol on Protein yield under water deficit conditions.

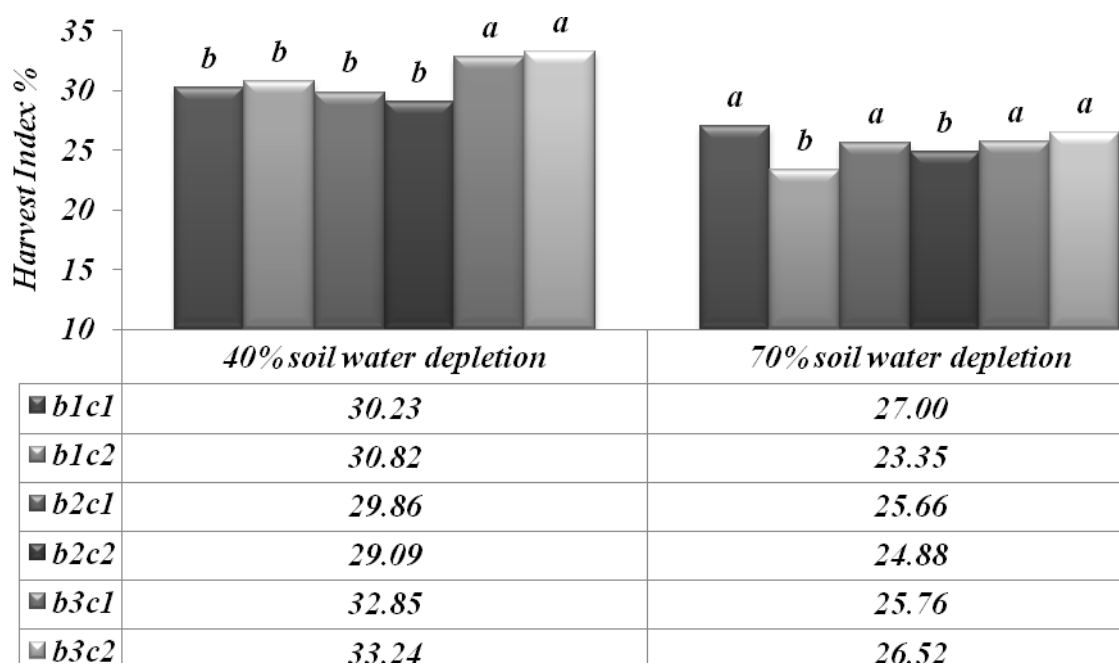


Fig8: Effect timing (b1=8-10 AM and b2=19-21 PM) and number (c1=each 7 days, c2=each 14 days, c3=each 21 days) Foliar application of methanol on Harvest Index under water deficit conditions.

CONCLUSION

Soybean yield is affected by methanol spraying and under water stress higher frequency of spraying somewhat can reduce destructive effects of drought and prevent of yield loss. Based on the results, the highest impacts under normal irrigation and drought conditions were found by methanol spraying every 14 and 7 days, respectively. Under normal irrigation condition foliar application of methanol in the morning and evening provided the maximum yield but in deficit irrigation condition the maximum yield obtained when methanol applied in the morning. It seems by increasing applications, methanol can compensate drought severe, prevents light respiration like an anti- stress substance and can be used by plant as a carbon source.

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