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STUDY THE EFFECT OF METHANOL FOLIAGE SPRAYING ON CHICKPEA CULTIVARS IN RAINFED CONDITION

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Abstract

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In order to study the effects of methanol foliage spraying on some characters of chickpea cultivars in rainfed condition, an experiment was carried out as split plot experiment based on a randomized complete block design with three replications at Research Field of Faculty of Agriculture and Natural Resources, Islamic Azad University-Sanandaj branch during 2012 growing season. The first factor was Methanol foliage spraying in concentrations as: 0 (control), 10, 20 and 30 volumetric percentages as the main factor and second factor was four chickpea cultivars in clouded as: pirozand kaka (desi type) and jam and ILC-482 (kabuli type) that considered as subplot. The results showed that methanol has a significant effect on yield and yield components. Methanol spraying with a concentration of 30% was more effective than other treatments on yield and yield components, as it increased the number of pods, number of seeds per pod and 100 seed weight. In this study, the most and lowest seed yields were recorded in 30% and 0% (control) methanol treatments as: 899.67 and 538.95 kg/ha respectively. The yield of desi type cultivars were more than Kabuli type, also. As the most and lowest seed yield were achieved in kaka and jam cultivars as: 963.97 and 462.01 kg/ha respectively. In this study other characters as: chlorophyll content and canopy temperature in different growth stages and seed protein were affected significantly by methanol and cultivars. Finally seed yield increased as: 28.52% - 66.93% by methanol spraying at different concentration from 10% - 30%.

Key words: methanol, chickpea, seed yield, chlorophyll, canopy temperature

Introduction

Due to water limitation and low rate of rainfall productivity of crops in rainfed area of Iran that is considered as dry and semi dry country is 42 percent of irrigated field (Sabaghpour, 2003). Also in Iran 49.78 percent of crops are planted in rainfed. Chickpea (*Cicer arietinum* L.) is planted on 700 000 ha in Iran and ranks fourth in the world after India, Turkey and Pakistan; of course Chickpea productivity in Iran is less than half of world average yield. Therefore, application suitable methods are very important for optimum usage of available water and increase productivity in growing season under rainfed condition. Liang et al. (1997) found that when stomata are closed due to drought or high temperature, the available CO_2 in intercellular space (Ci) would be reduced, leading to reduced electron transport capacity and restricted assimilation potential. In other words, Abdel-Latif et al. (1996) reported that 90% of plant dry weight is resulted from CO_2 assimilation during photosynthesis, and also numerous experiments have shown an increase of yield due to an increase of the CO_2 content in the atmosphere (Devlin et al., 1994). Furthermore, Zbiec et al. (1999) found that plants grown in CO_2-enriched atmosphere were less susceptible to drought due to a decrease of stomatal conductance and transpiration and an increase of net photosynthesis. Therefore looking for methods or compounds that can be used in field is interested in order to raise plant internal CO_2 concentration and so for increase their yield.

Nonomura and Beson (1992) found that using some compounds such as methanol, ethanol, bothanol, propanol and some amino acids like as glycine, aspartat and glutamate, on the foliage of numerous C3 cropscan improve yields of them,

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under the warm and arid growing conditions of Arizona, they also observed that application of methanol provides the potential to reduce water requirements and to improve crop yields. They suggested that this increased yield has resulted from a reduced photorespiration along with an increased cell turgor of plant's tissues and from an enhanced photosynthesis capacity during reproductive stage due to an increased Ci. They also observed that methanol can act as an alternative source of carbon especially for C3 plants, causing a substantial increase in their CO_2 fixation, growth and yield, primarily due to inhibiting their photorespiration that is resulting to lost 25% of total plant carbon gain.

Ramberg et al. (2002) concluded that rapid uptake of methanol by plants and its quick metabolizing to CO_2 in plant tissues, duo to smaller size of methanol molecules compared to CO_2. Metabolism of methanol and its conversion to sugars, change the osmotic potential of the leaves. In addition, it increases the turgor pressure and the pores as well. In fact, keeping the pores open causes assimilation and the growth rate of a plant to increase which in itself would lead to early maturity and less water requirement (Nonomura and Benson, 1992; Makhdum et al., 2002). In plants which suffer a shortage of water, methanol spraying of the aerial parts increases the chlorophyll concentration and prevent from increasing photorespiration that induced in stressed plant (Ramberg et al., 2002). Also, Nemecek-Marshall et al., 1995 found that methanol spraying results in increased production and reduces plants' water requirement in warm and dry conditions. Hemming et al. (1995) measured metabolic heat rate, carbon dioxide production and oxygen uptake rates of bell pepper (*Capsicum annuum* L.) after exposing leaf tissues to methanol. They reported a strict increase in metabolic heat rate resulting in increased carbon conversion efficiency which lasted several weeks.

Methanol enhanced the growth of oilseed rape, soybeans, small beans, cabbage and sugar beet (Zbieć et al., 2003). Furthermore, Makhdum et al. (2002) reported that foliar application of methanol caused increase in seed cotton yield and it had positive effect on physiological processes, water relation and plant structure. Also Bhattacharya et al. (1985) studied the effects of ethanol, methanol and acetone on mung bean and found that they increased the yield, accelerated the maturity and reduced the drought stress and the plant's water requirement. SafarzadeVishgahi et al. (2005) concluded that applying a 20% volumetric solution of methanol on peanut (*Arachis hypogaea*) plants increased LAI, CGR, RUE, protein content and grain yield.

Methanol application also can enhance plant photosynthetic capacity by delaying leaves senescence and therefore extending photosynthesis active course (Hemming and Criddle, 1995). Zbiec et al. (2003) found that loss of photorespiration in plants treated with methanol related to rapid oxidation of methanol to carbon dioxide and ribulose-l, 5-bisphosphate and less competitive of O_2. Li et al. (1995) found that grain yield, seeds weight, and number of pods per soybeans plant compared to control significantly increase by methanol spraying and applying a 25% volumetric solution of methanol prepare the greatest effect on soybean growth and yield. Andres et al. (1990) found that foliar application of methanol can enhance activity of FBPase, an important enzyme controlling photosynthesis. Aslani et al. (2011) concluded that the largest numbers of seeds per pod and harvest index were in 30% methanol, while the highest seed yield was that of the 20% methanol. Paknejad et al. (2009) suggested that methanol foliar application influenced significantly Chl, RWC, Grain Yield, they reported that applying methanol on water stressed soybean plants can reduce harmful effects of drought and improve plant potential to cope with stress. They also founded that moderate foliar applications of methanol can enhance chlorophyll and photosynthetic capacity for dry matter production, but higher methanol concentration can destroy chlorophyll content. Hossinzadeh et al. (2012) were studied different levels of methanol and moisture regimes on chickpea and found that spraying with 25% methanol had the best result.

Farajpour et al. (2012) showed that the fresh and dry yield of tobacco plant, in 30% methanol concentration and afternoon time treatments were significantly higher than the others. Nadali et al. (2010) demonstrated that 21% methanol spray poses the greatest impact on yield, and other physiological traits. Jafari-Paskiabi et al. (2011) indicated that concentration and time spraying methanol affected on pod and seed yield of cowpea also among methanol concentration treatments, maximum pod and seed were recorded for the 20% and 30% methanol treatments, respectively. Thus, until now do not any report about effect of methanol in rainfed condition, therefore, this study was conducted to determine the effects of methanol foliage spraying on chickpea cultivars under rainfed condition in Sanandaj city in the northwest of Iran.

Materials and Methods

In order to study the effect of methanol foliage spraying on chickpea cultivars (*Cicer arietinum* L.) in rainfed condition a split-plot experiment based on a randomized complete block design with tree replications was done at Research Field of Faculty of Agriculture and Natural Resources, Islamic Azad University - Sanandaj Branch, Iran, during 2012 in Sanandaj city located in the northwest of Iran (situated at 35°10′N and 46°59′E). The location has a semi-arid climate with 450 mm annual precipitation in average. Soil type was clay with pH of 7.8 at the depth of

0-30 and 30-60 cm. The experiment was conducted in 2.5×1.2 m plots, each of which having four cultivation rows. Distances between plots in each replication and between replications were 1 m and 1.5 m, respectively. Also the distance between rows was 30 cm; while the distance between plants on the rows was 10 cm. Seeds were sown in 23 March 2012.

In this study the first factor was methanol foliage spraying as the main factor in concentrations as: 0 (control: distilled water), 10, 20 and 30 volumetric percentage (v/v), based on Nanomora and Benson (1992) recommendation, foliar requirements for methanol differed widely, for example, in their study, 50% methanol was applied to palm and eucalyptus leaves, but eggplant was treated with 10% methanol. The second factor was four chickpea cultivars in clouded as: piroz and kaka (desi type) and jam and ILC-482 (kabuli type) that considered as subplot.

Methanol was sprayed three times during the growing season at intervals of 45, 60 and 75 days after planting on the foliage of chickpea plants. To avoid the toxicity in the presence of direct sunlight and chlorophyll degradation, 2 g lit-1 of glycine was added to prepared solution, addition of glycine eliminated methanol toxicity, actually allowing increased concentrations of methanol to be applied without injury (Nanomora and Benson, 1992).

Methanol spraying was carried out at 18:00 pm during bright sunny days with temperate temperature, by back handle sprayer with capacity of 12 L. The sprayer was held 30 cm above the plants and spraying was conducted on all above ground parts of chickpea and continued until the solution small drops was appeared on foliage surface. In the first spraying one liter of solution was applied in each main plot and while plant growth increasing in 60 and 75 day after planting, 2.5 and 3.5 liter of solution were used in second and third spraying, respectively. Also in another field next to this study, in order to possibility of achievement of more increasing yield, methanol solution with more concentrations as: 40%, 50% and 60% were applied, but in the first spraying all the plant was yellowish and than fully dried and finally destroyed, it seem that higher methanol concentration (above 30%) maybe imposes a toxic effect that can destroy chlorophyll content (Paknejad et al., 2009).

The studied characters in this study were: content of leaf chlorophyll (estimated by SPAD-502 device at flowering and poding stages) and canopy temperature (estimated by infrared thermometer at flowering, poding and ripening stages), number of pods per plant, number of seeds per pod, 100-seeds weight, seed yield in the unit of surface and protein percent. Finally data were statistically analyzed using the SAS software package (SAS Institute, 2001) and means of treatments were compared by Duncan's multiple range tests at the 0.05 probability level.

Results and Discussion

Chlorophyll content (SPAD)

The results of analysis variance demonstrated that chlorophyll content (SPAD) was significant at the probability level of 1% in both flowering and poding stages by methanol foliar spraying at different concentration (Table 1), as chlorophyll content increased when methanol concentration increased (Table 2). Methanol foliage spraying had the best effect at 30% concentration. In the various study shown that foliar application of methanol on some crops caused an increase of Chlorophyll concentration in their leaves. Paknejad et al. (2009) suggested that applying methanol on water stressed soybean plants can reduce harmful effects of drought and improve plant potential to cope with stress, they also founded that moderate foliar applications of methanol can enhance chlorophyll and photosynthetic capacity for dry matter production, but higher methanol concentration can destroy chlo-

Table 1

Anova analyses for study the effects of methanol foliage spraying on physiological characters of chickpea cultivars in rainfed condition, data are Mean of Squares (MS)

		chlorophyll content, SPAD		canopy temperature, ºc		
SOV	df	flowering	poding	flowering	poding	ripening
Replication	2	4.77 ns	2.98 ns	15.84 ns	6.75 ns	5.58 ns
Methanol spraying (A)	3	258.01 **	251.69 **	84.77**	51.8**	51.53**
Ea	6	1.15	1.57	3.94	2.12	2.05
Cultivar (B)	3	678.55**	692.85**	3.19 ns	3.58 *	36.5**
Methanol spraying × Cultivar (AB)	9	3.29**	3.94 **	1.88 ns	1.68 ns	9.68**
E	24	0.79	0.72	4.64	1.53	2.48
CV, %		1.78	1.66	8.51	5.25	4.04

**, Significant at 0.01 level *, Significant at 0.05 level, ns: non significant.

rophyll content. An increased SPAD value in moderate methanol applications (10-30 volumetric percent) and negative impacts of higher doses also has been reported for peanut.

Ramberg et al. (2002) and Ramirez et al. (2006) founded that spraying methanol on water-deficit plants can increase chlorophyll content of their leaves. Zheng et al. (2006) founded that foliar application of methanol on wheat plants increased leaf chlorophyll content and photochemical efficiency of photosystem II that led to increase photosynthesis and stomata conductance flag leaf plant and had a significant effect on grain yield. Methanol application also can enhance plant photosynthetic capacity by delaying leaves senescence and therefore extending photosynthesis active course (Hemming and Criddle, 1995). In this study methanol foliage spraying also was affected significantly chlorophyll content (SPAD) among cultivars, as ILC-482 (kabuli type) had the most SPAD in both flowering and poding stages. The interaction of methanol foliage spraying at with cultivars was significant also (1% probability).

Canopy temperature

Results showed a significant difference between different methanol concentrations at all growing season (flowering, poding and ripening stages) on canopy temperature. According Table 2 canopy temperature decreased significantly in all stages and with increasing methanol concentration spraying, canopy temperature more decreased. However canopy temperature is conjugated with environment temperatures, as, low canopy temperature in poding stage may due to environment condition, following that at ripening stage, canopy temperature increased. Of course it seems that evaporation of methanol from foliage plant can retard canopy temperature and increase cell turgor of plant tissues in growing season. Canopy temperature of cultivars and interaction of methanol foliar spraying with cultivars were affected in poding and ripening stage. Nonomura and Beson (1992) found that methanol increased cell turgor of plant's tissues and than reduce water requirements and lead to early maturity and also to improve crop yields. Makhdum et al. (2002) reported that foliar application of methanol caused increase in seed cotton yield and it had positive effect on physiological processes, water relation and plant structure.

Yield component

Number of pods in plant

This character was affected significantly by methanol spraying (Table 3) as the highest pod in plant was observed in 30% methanol (Table 4), probably increasing chlorophyll content and low canopy temperature induced by methanol spraying can help to produce more number of pods in plant. So this finding agreement with those obtained by Mirakhori et al. (2009) in their study on soybean, they noted that methanol spraying was done before soybean's pod formation stage. Li et al. (1995) found that number of pods per soybeans plant compared to control significantly increase by methanol spraying at 25% volumetric solution. Jafari-Paskiabiet al. (2011) indicated that maximum pod was recorded for the 20% methanol treatments. Also among chickpea cultivars studied, the most pods in plant were obtained in piroz (desi type). Number of pods in chickpea plant is a genetically characters that low affected by environmental condition, it seems that low canopy temperature in different growing stage of piroz (Table 2) resulting to better water relation and hence it can produce more pod in plant.

Table 2
Mean comparison effect of methanol foliage spraying on physiological characters of chickpea cultivars in rainfed condition

	chlorophyll content, SPAD		canopy temperature, °c		
Treatment	flowering	poding	flowering	poding	ripening
Methanol concentration spraying					
0% (control: distilled water)	43.97 d	45.23 d	28.45 a	25.08 a	41.77 a
10%	48.77 c	50.23 c	26.04 b	24.41 b	39.22 b
20%	51.99 b	52.87 b	24.72 b	22.91 c	37.68 c
30%	54.78 a	56.05 a	22.08 c	21 d	37.14 c
Cultivar					
piroz (desi type)	46.29 c	47.37 c	24.68 a	23.1 b	36.56 c
kaka (desi type)	41.03 d	42.22 d	25.54 a	24.31 a	39.04 b
jam (kabuli type)	55.28 b	56.55 b	25.18 a	23.56 ab	39.52 ab
ILC-482 (kabuli type)	56.9 a	58.23 a	25.89 a	23.2 b	40.7 a

Mean with the common letters in each column have not significant differences at 0.05 probability level by Duncan's multiple range test

Number of seeds per pod

In this study, number of seeds per pod was significant at the probability level of 1% and it increased following more methanol spraying, as the largest numbers of seeds per pod were in 30% methanol, this result was agreement with other researcher studies. Aslani et al. (2011) concluded that the largest numbers of seeds per pod was in 30% methanol. Jafari-Paskiabi et al. (2011) indicated that maximum seed was recorded for the 30% methanol treatments.

In this study kaka (desi type) were produce more seed in pod (1.26), however number of seed in pod is a genetically characters that low affected by treatments in various cultivars.

100-seeds weight

Methanol spraying had significant effect on the 100-seeds weight, as it increased with more foliar concentration (Tables 3 and 4).The same results found by Li et al. (1995) that found maximum seeds weight of soybean in 25% volumetric solution of methanol. Also Mirakhori et al. (2009) concluded that methanol spraying had a positive effect on the 100-seeds weight of soybean. In this research significant difference was observed among cultivars as the highest seed weight obtained in jam and ILC-482 (kabuli type), of course, the 100-seeds weight also is a genetically trait and is less affected in different experiments. Kabuli type chickpea cultivars have bigger seed size and so more weight genetically.

Seed yield

Applied methanol levels had significant and positive effect on increasing seed yield in compare with control (Table 3). The maximum grain yield (899.67 kg ha^{-1}) was achieved with 30% methanol spraying and the lowest grain yield (538.95 kg ha^{-1}) were found in 0% methanol concentration and without

Table 3
Anova analyses for study the effects of methanol foliage spraying on yield and yield component characters of chickpea cultivars in rainfed condition, data are Mean of Squares (MS)

SOV	df	number of pods	seeds per pod	100-seeds weight	seed yield	% protein
Replication	2	19.41 *	0.049 **	1.018 ^{ns}	10249.9 ^{ns}	0.0013 ^{ns}
Methanol spraying (A)	3	111.73 **	0.049 **	62.5 **	272562.57**	21.96**
Ea	6	1.32	0.001	5.27	12364.94	0.001
Cultivar (B)	3	319.52**	0.232**	507.38 **	601040.808 **	295.45**
Methanol spraying × Cultivar (AB)	9	2.34 ^{ns}	0.012 ns	1.56 ^{ns}	8713.37 ^{ns}	1.282**
E	24	4.84	0.01	3.68	7884.96	0.001
CV, %		12.28	9.55	8.87	12.24	0.1005

**, Significant at 0.01 level *, Significant at 0.05 level, ns: non significant.

Table 4
Mean comparison effect of methanol foliage spraying on yield and yield component of chickpea cultivars in rainfed condition

Treatment	number of pods	seeds per pod	100-seeds weight	seed yield	% protein
Methanol concentration spraying					
0% (control: distilled water)	14.58 ^{c}	1.00 ^{b}	19.35 ^{c}	538.95 ^{d}	34.42 ^{b}
10%	16.39 ^{c}	1.03 ^{b}	20.61 ^{bc}	692.7 ^{c}	36.16 ^{a}
20%	19.20 ^{b}	1.05 ^{b}	21.88 ^{b}	769.45 ^{b}	33.08 ^{d}
30%	21.5 ^{a}	1.15 ^{a}	24.69 ^{a}	899.67 ^{a}	33.6 ^{c}
Cultivar					
piroz (desi type)	23.06 ^{a}	1.02 ^{b}	18.95 ^{b}	846.21 ^{b}	37.76 ^{a}
kaka (desi type)	21.64^{a}	1.26 ^{a}	13.69 ^{c}	963.97 ^{a}	26.76 ^{d}
jam (kabuli type)	13.1 ^{b}	0.94 ^{b}	27.15 ^{a}	462.01 ^{d}	37.45 ^{b}
ILC-482 (kabuli type)	13.87^{b}	1.02 ^{b}	26.74 ^{a}	628.58 ^{c}	35.25 ^{a}

Mean with the common letters in each column have not significant differences at 0.05 probability level by Duncan's multiple range test methanol (control), respectively (Table 4), as in 30% methanol, obtained 28.52% - 66.93% more yield in compare with control. According to previous results, this is probably due to increasing in: chlorophyll content (SPAD), number of pod in plant, numbers of seeds in pod and seed weight and also decreasing canopy temperature in growing season that improve plant water relation.

Nemecek-Marshall et al. (1995) found that methanol spraying results in increased production and reduces plants' water requirement in warm and dry conditions. Methanol enhanced the growth of oilseed rape, soybeans, small beans, cabbage and sugar beet (Zbieć et al., 2003). Makhdum et al. (2002) reported that foliar application of methanol caused increase in seed cotton yield and it had positive effect on physiological processes, water relation and plant structure. Also Bhattacharya et al. (1985) studied the effects of ethanol, methanol and acetone on mung bean and found that they increased the yield, accelerated the maturity and reduced the drought stress and the plant's water requirement. Li et al. (1995) found that applying a 25% volumetric solution of methanol prepares the greatest effect on soybean growth and yield. Also, Aslani et al. (2011) concluded that the highest seed yield was that of the 20% methanol. Hossinzadeh et al. (2012) reported that Spraying with 25% methanol had the best result on chickpea in different moisture regimes. Also, Farajpour et al. (2012) showed the highest fresh and dry yield of tobacco plant, in 30% methanol concentration. Nadali et al. (2010) demonstrated that 21% (v/v) methanol spray poses the greatest impact on yield, and other physiological traits.

The positive effect of methanol application on seed yield may be due to abundant CO_2 supply from methanol as suggested by Hemming et al. (1995). This may have reduced photorespiration in favors of photosynthesis. Moreover, application of methanol play an important role in balancing the nutritional status of leaves by acting as a carbon source (Benson and Nonomura, 1992) or by enhancing the engendered root activity (Makhdum et al., 2002). Safarzadeh Vishekaei (2007) showed that spraying the aerial parts of peanut with a 20% methanol solution increased pod growth rate, radiation use efficiency, pod and seed yields, 100-seeds weight, number of mature pods and the protein content of a peanut seed. Makhdum et al. (2002) examined the effect of spraying methanol on cotton and found that consuming methanol increases the dry matter, photosynthesis, pore conductivity and seed yield. Furthermore, it reduces cotton's water requirement and increases the leaf surface.

Zbiec (2003) has found that the most important reason for the increase in leaf dry weight yield in methanol foliar application is increasing biomass production and carbon fixation, because with increasing methanol concentration entrance of carbon dioxide into the leaf and leaf stomata conductance are increased. According to the results, seed yield in kaka cultivar was higher than other cultivars (963.97 kg ha^{-1}) it seems that number of seed in pod was more increased by methanol as compare with other cultivars.

Seed protein percent

This character was affected by treatments significantly as the maximum seed protein was achieved in 10% methanol, this results was predictable due to negative relationship between seed yield and seed protein. Safarzade Vishgahi et al. (2005) concluded that applying a 20% volumetric solution of methanol on peanut (*Arachis hypogaea*) plants increased protein content and grain yield. In this study piroz had the greatest seed protein (37.76%) and also kaka had the lowest seed protein (26.76%).

Conclusions

In this study methanol foliage spraying had significant effect on all characters, as while methanol concentration was increased (up to 30%), more positive effect of methanol observed. The maximum grain yield (899.67 kg ha^{-1}) was achieved with 30% methanol spraying and the lowest grain yield (538.95 kg ha^{-1}) were found in 0% methanol concentration and without methanol (control), respectively.

It seems that increasing of chlorophyll content, numbers of pod in plant, numbers of seed in pod and seed weight and also decreasing canopy temperature that improve plant water relation, induced higher yield. Application of methanol increased chlorophyll content, hence photosynthesis capacity can increased as mentioned by Zheng et al. (2006), Ramirez et al. (2006), Paknejad et al. (2009), Nanomora and Benson (1992).

Among chickpea cultivars in this study kaka cultivar was achieved higher seed yield than other cultivars (963.97 kg ha^{-1}), it seems that number of seed in pod was more increased by methanol as compare with other cultivars. Finally in this study were used 194.4 liter ha^{-1} of methanol and seed yield increased by 360.72 kg ha^{-1} as it was`66.93% over control. In Nonomura and Benson (1992 b) studies, where foliar application of 32 liters of methanol improved fruit yield of watermelon crop by 12 t over controls.

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