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Response of Qualitative Characteristics of Cowpea to Foliar Application of Methanol and Zinc Chelate

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ABSTRACT

BACKGROUND: Foliar fertilization has the advantage of low application rates, uniform distribution of fertilizer materials and quick responses to applied nutrients. Zinc Playing an important role in many biological processes and as an essential element for plant growth and development.

OBJECTIVES: This study was done to evaluate the effect of different level of Methanol and Zinc fertilizer on chlorophyll index, protein content, leaf area index and crop production.

METHODS: This study was carried out according Factorial experiment based on randomized complete blocks design with three replications along 2018 year. The test factors consisted of different levels of Methanol in distilled water at three levels (a_1: the absence of methanol or control, a_2: 10% Vol., a_3: 20% Vol.) and zinc fertilizer (b_1: the lack of zinc or control, b_2: 2 per thousand, b_3: 4 per thousand).

RESULT: Data of analysis of variance indicated effect of different level of Methanol and zinc fertilizer on all studied traits were significant but interaction effect of treatments (instead seed yield) was not significant. Mean comparison result of different level of Methanol indicated that maximum amount of plant height (153.49 cm), pod length (13.12 cm), leaf area index (4.43), chlorophyll index (58.33), protein content (31.51%), protein yield (68.71 $gr.m^{-2}$) and seed yield (218.08 $gr.m^{-2}$) was noted for 20%vol. and the minimum of mentioned traits were for control treatment. As for Duncan classification made with respect to different level of Zinc Chelate maximum and minimum amount of studied traits belonged to 4 per thousand concentration (also that doesn’t have significant differences with 2 per thousand) and control treatment.

CONCLUSION: Generally based on result of this study use 20%vol. Methanol and 2 per thousand concentration Zinc Chelate produce the maximum amount of mentioned characteristics and can be advice to farmers.

KEYWORDS: *Chlorophyll, Leaf area index, Plant height, Pod length, Protein.*

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1. BACKGROUND

Snap bean or 'French bean' (also referred to as green beans or string beans) is a strain of common bean, (*Phaseolus vulgaris* L), which is grown as a cash crop at large scale and smallholder farmers. So, snap bean is an important vegetable crop for local consumption and export (El-Awadi *et al*., 2011). Green bean is an important vegetable crop widely used as a protein source and for other nutrients in many developing countries. The total worldwide cultivated area of green beans is 1,527,613 hectares, producing 21,720,588 tons, as reported by FAO (2017). Application of micronutrients can increase water and nutrient absorption, and greater foliage expansion allows plants to absorb more radiation. These factors increase biomass yield and grain yield (Arnold Bruns and Abbas, 2005). A high pH level and presence of carbonates and bicarbonates in soils reduce absorption of many nutrients, especially micronutrients like iron and zinc (Sabet and Mortazaeinezhad, 2018). Deficiency of microelements causes health problems for people (Oliver and Gregory, 2015). More than two billion people in Asia, Africa, and Latin America suffer from severe malnutrition and iron (Fe) and zinc (Zn) deficiencies (Grujcic *et al*., 2018; Gupta *et al*., 2008). Zinc Playing an important role in many biological processes and as an essential element for plant growth and development as well as human and animal health, zinc enhances root system development, improves nutrient and water absorption, and activates enzymes (Cakmak, 2008; Noulas *et al*., 2018). Due to the low levels of zinc in almost half of the soils worldwide available for plants, the crop yields and quality have declined in cereal cultivation (Noulas *et al*., 2018). Presence of sufficient amounts of nutrients in plant organs results in better grain filling and increased grain weight. Zinc is necessary in the biosynthesis of growth regulators like indoleacetic acid and carbohydrates that improve yield and yield components. It may be due to their importance in accumulation of assimilates in grains in the final stages of plant growth, and as a result, production of larger and heavier grains. Reduced number of grains per plant under nutrient deficiency indicates the negative effects of absence of the aforementioned micronutrients, consequently preventing the reproductive organs from preparing for grain production (Bybordi and Mamedov, 2010; Xue *et al*., 2003). Increased production of assimilates improves storage of materials and hence enhances physiological performance. Reduction in the rate of plant aging, and hence possibility of more photosynthetic activity by plants, are among other reasons for the greater number of grains obtained by applying micronutrients (Bakhtavar *et al*., 2015). Among different commercial crops, green beans have the highest level of protein contents. There are favorable light and moisture conditions in Iran to plant such crops; sunny summers and cool nights as well as high-quality soil of Iran have prepared an ideal condition for planting and producing green beans, as the yield of this crop in Iran is 2.5 times more than the world average; in other words, the average yield of green beans in the world is 810 $kg.ha^{-1}$, while in Iran it is 1800 $kg.ha^{-1}$ (Ghasempour and Ashori, 2014). Zinc is an essential micronutrient in biological systems, which is required in small quantities. It is involved in the formation and activation of enzymes that impact on the growth, development and production of plants (Poblaciones and Rengel, 2016). Thalooth *et al.* (2005) indicated that foliar spraying with Zn had a positive effect on yield and yield attributes of sunflower plants. Several research reports have established the essentiality and role of micronutrient-zinc on plant growth, development and yield (Grzebisz *et al.*, 1999). Zinc deficiency is a global nutritional constraint for plant growth, particularly in calcareous soils of arid and semi arid regions. One of the most important micronutrient deficiencies is attributed to zinc deficiency that is a worldwide problem in human nutrition. More than 2 billion people suffer from micronutrient deficiency including zinc deficiency. The major reasons for the widespread occurrence of Zn deficiency in humans, especially in developing countries is a high proportion of cereal-based foods in the human being daily diet. Zinc deficiency causes a number of health problems like impairment in linear growth, sexual maturation, learning ability, immune functions and the central nervous system. Selection and breeding of plant genotypes for higher resistance against Zn deficiency is a realistic and long-term solution to overcome Zn deficiency in soils. Breeding genotypes for resistance to Zn deficiency may, however, take considerable time. Therefore, Zn fertilization is still a widely used agronomic practice for farmers to correct Zn deficiency (Ranjbar and Bahmaniar, 2007).

2. OBJECTIVES

This study was done to evaluate the effect of different level of Methanol and Zinc fertilizer on chlorophyll index, protein content, leaf area index and crop production.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

Current research was carried out to response of crop production of Cow Pea to apply different level of Methanol and Zinc fertilizer via Factorial experiment based on randomized complete blocks design with three replications along 2018 year. Place of research was located in Ahvaz City at longitude 48°40'E and latitude 31°20'N in Khuzestan province (Southwest of Iran). The test factors consisted of different levels of Methanol in distilled water at three levels (a_1: the absence of methanol or control, a_2: 10% Vol., a_3: 20% Vol.) and zinc fertilizer in three levels (b_1: the lack of zinc or control, b_2: 2 per thousand, b_3: 4 per thousand).

3.2. Farm Management

Base fertilizers (50 $kg.ha^{-1}$ Nitrogen from urea, 80 $kg.ha^{-1}$ phosphorus from ammonium phosphate and 80 $kg.ha^{-1}$ potassium from potassium sulfate) were added to the soil based on soil tests and the recommendations of the Iranian Soil and Water Research Institute at the planting stage. The light-disk harrow was used to mix soil and the fertilizer after soil fertilization. The furrower was used to make furrows at a distance of 50 cm. The zinc and manganese Nano-chelate were used in the furrows (with 4cm depth) before planting. The furrows were covered with soil. The seeds were planted 2 cm above the fertilizer. Physical and chemical properties of the soil are mentioned in table 1.

Table 1. Physical and chemical properties of studied field

Depth of soil (cm)	P (ppm)	K (ppm)	N (%)	pH	EC ($ds.m^{-1}$)	O.C (%)	Soil texture
0-15	5	224	5.3	7.2	4	0.64	Clayloam
15-30	4.41	219	5.1	7	3.94	0.57	Clayloam

3.3. Measured Traits

In order to determine the yield two planting lines from each plot harvested and after the removal of marginal effect were carried to the laboratory and were placed in the oven at 75°C for 48 hours and after ensuring that the samples were completely dry, they were weighed and finally the total yield was measured. Chlorophyll content of five ear leaves in each plot was measured at flowering stage by SPAD 502 device, accurately three points of leaf measured and the average of three numbers was considered. (SPAD 502, Minolta Company, Japan). To determine the leaf area of the linear relationship S= K. L.W was used in which S, L and W were the leaf area, L and W respectively, the maximum length and width of each leaf and K= 0.75 correction coefficient. The leaf area index was calculated from leaf area ratio to ground level. To determine the percentage of grain protein, the percentage of grain nitrogen was first measured by Kjeldahl method, which includes digestion, distillation and titration. To measure the amount of seed protein by multiplying the percentage of seed nitrogen by a factor of 6.25, the amount of protein in the seed was obtained. Then, by multiplying the percentage of protein in each treatment by its seed yield, the protein yield for each treatment was calculated (Keeney and Nelson, 1982).

3.4. Statistical Analysis

Analysis of variance and mean comparisons were done via SAS (Ver.8) software and Duncan multiple range test at 5% probability level.

4. RESULT AND DISCUSSION

4.1. Plant height

According result of analysis of variance effect of Methanol and Zinc Chelate on plant height was significant at 5% probability level but interaction effect of treatments was not significant (Table 2). Result of mean comparison revealed maximum of plant height (153.49 cm) was obtained for 20%vol. Methanol and minimum of that (105.59 cm) was for control treatment (Table 3). Evaluation mean comparison result indicated in different level of Zinc Chelate the maximum plant height (147.34 cm) was noted for 4 per thousand concentrations also it doesn't have significant differences with 2 per thousand (143.25 cm) and minimum of that (104.7 cm) belonged to control treatment (Table 4). Another researcher such as Harssine *et al.* (2014); Moallim and Eshqizade (2007) reported same result.

Table 2. Result analysis of variance of measured traits

S.O.V	df	Plant height	Pod length	Leaf area index	Chlorophyll index
Replication	2	1.71^{ns}	0.62^{ns}	0.253^{ns}	10.5^{ns}
Methanol (M)	2	523.05^{*}	14.807^{**}	1.504^{**}	175.07^{**}
Zinc Chelate (Z)	2	438.66^{*}	20.31^{**}	1.03^{*}	163.2^{**}
M × Z	4	1.05^{ns}	0.08^{ns}	0.06^{ns}	0.38^{ns}
Error	16	93.29	1.40	0.14	15.35
CV (%)	-	7.33	10.19	9.9	7.2

^{ns}, * and ** are non-significant and significant at 5 and 1% probability levels, respectively.

Continue table 2.

S.O.V	df	Protein content	Protein yield	Seed yield
Replication	2	1.153^{ns}	2.68^{ns}	188.3^{ns}
Methanol (M)	2	28.12^{*}	200.34^{**}	30742.3^{**}
Zinc Chelate (Z)	2	23.95^{*}	194.81^{**}	26852.1^{**}
M × Z	4	0.609^{ns}	2.39^{ns}	10574.33^{**}
Error	16	6.83	14.85	398.56
CV (%)	-	9.28	7.13	10.51

^{ns}, * and ** are non-significant and significant at 5 and 1% probability levels, respectively.

4.2. Pod length

Result of analysis of variance revealed effect of Methanol and Zinc Chelate on pod length was significant at 1% probability level but interaction effect of treatments was not significant (Table 2). Mean comparison result of different level of Methanol indicated that maximum pod length (13.12 cm) was noted for 20%vol. and minimum of that (10.01 cm) belonged to control treatment (Table 3). As for Duncan classification made with respect to different level of Zinc Chelate maximum and minimum amount of pod length belonged to 4 per thousand concentration (12.99 cm) also it doesn't have significant differences with 2 per thousand (12 cm) and control (9.86 cm) (Table 4). This finding, were accordance with results of Fatahe (2006).

4.3. Leaf area index

At the beginning of growth, the leaf area of the plant is made up by young leaves with high photosynthetic capacity, i.e., high efficiency of fixation of atmospheric CO_2.

Table 3. Mean comparison effect of different levels of Methanol on measured traits

Treatment	Plant height (cm)	Pod length (cm)	Leaf area index	Chlorophyll index
None use of Methanol or control	105.59c	10.01c	3.23b	49.19b
10% Vol	136.2b	11.71b	3.68ab	55.64ab
20% Vol	153.49a	13.12a	4.43a	58.33a

*Similar letters in each column show non-significant difference at 5% probability level in Duncan test.

Continue table 3.

Treatment	Protein content (%)	Protein yield (gr.m^{-2})	Seed yield (gr.m^{-2})
None use of Methanol or control	24.27c	40.58c	167.24c
10% Vol	28.64b	52.72b	184.1b
20% Vol	31.51a	68.71a	218.08a

*Similar letters in each column show non-significant difference at 5% probability level in Duncan test.

As the plant develops, leaf senescence enhances, reducing the photosynthetic efficiency of the leaves besides increasing respiratory losses, compromising the NAR and LAR, and consequently the RGR (Wilson, 1981). Leaf area index (LAI) is the main physiological determinant of crop yield. It describes the surface growth and light use during crop period (Ullah *et al*., 2013). According result of analysis of variance effect of Methanol and Zinc Chelate on leaf area index was significant at 1% and 5% probability level, respectively but interaction effect of treatments was not significant (Table 2). According result of mean comparison maximum of leaf area index (4.43) was obtained for 20%vol. Methanol and minimum of that (3.23) was for control treatment (Table 3). Evaluation mean comparison result indicated in different level of Zinc Chelate the maximum leaf area index (4.15) was noted for 4 per thousand concentrations also it doesn't have significant differences with 2 per thousand (4.09) and minimum of that (3.1) belonged to control treatment (Table 4). Leaf area index is the component of crop growth analysis that accounts for the ability of the crop to capture light energy and is critical to understanding the function of many crop management practices. Leaf area index can have importance in many areas of agronomy and crop production through its influence on: light interception, crop growth weed control, crop-weed competition, crop water use, and soil erosion. To measure LAI, scientists generally have cut a number of plants at the soil surface, separated leaves from the other plant parts, and measured the area of individual leaves to obtain the average leaf area per plant. The product of leaf area per plant and the plant population gives the LAI. Alternatively, LAI could be measured none destructively with this procedure if area of individual leaves was determined by some combination of leaf length and width measurements (Shirkhani and Nasrolahzadeh, 2016).

4.4. Chlorophyll index

Result of analysis of variance showed effect of Methanol and Zinc Chelate on chlorophyll index was significant at 1% probability level but interaction effect of treatments was not significant (Table 2). Assessment mean comparison result indicated in different level of Methanol the maximum chlorophyll index (58.33) was noted for 20%vol. and minimum of that (49.19) belonged to control treatment (Table 3). Compare different level of Zinc Chelate showed that the maximum and the minimum amount of chlorophyll index belonged to 4 per thousand concentrations (59.37) and control (48.58) treatments (Table 4). Thalooth *et al*. (2005) stated that cadmium consumption has reduced chlorophyll content in sunflower. Dhopte and Manuel (2002) reported that adding zinc to the medium increased cadmium toxicity and increased chlorophyll and photosynthesis rate. They concluded that zinc reduces the harmful effects of cadmium by improving photosynthesis and thus improves the photosynthesis and internal interactions. Staggenborg *et al*. (2008) reported that the consumption of zinc in water stress conditions had a positive and significant impact on growth, yield and yield components of plants. The use of cadmium in the barley causes leaves chlorosis, roots browning and reduction of the amount of chlorophyll in leaves and as the iron concentration was more than critical level, reduction of chlorophyll was attributed to reduction of photosynthetic compounds. The essential micronutrients required by the plant should not be overlooked.

Table 4. Mean comparison effect of different levels of Zinc on measured traits

Treatment	Plant height (cm)	Pod length (cm)	Leaf area index	Chlorophyll index
None use of Zinc or control	104.7b	9.86b	3.1b	48.58c
2 per thousand	143.25a	12a	4.09a	55.19b
4 per thousand	147.34a	12.99a	4.15a	59.37a

*Similar letters in each column show non-significant difference at 5% probability level in Duncan test.

Continue table 4.

Treatment	Protein content (%)	Protein yield ($gr.m^{-2}$)	Seed yield ($gr.m^{-2}$)
None use of Zinc or control	22.84b	38.89b	170.3b
2 per thousand	30.35a	59.33a	195.51a
4 per thousand	31.23a	63.58a	203.61a

*Similar letters in each column show non-significant difference at 5% probability level in Duncan test.

4.5. Protein content

According result of analysis of variance effect of Methanol and Zinc Chelate on the protein content was significant at 5% probability level but interaction effect of treatments was not significant (Table 2). Thalooth *et al*. (2009) indicated that zinc element would not significantly increase the protein content of seeds, but Sawan *et al*. (2001) indicated to the positive effect of Zinc on the increase in seed protein. Evaluation mean comparison result revealed in different level of Methanol the maximum protein content (31.51%) was noted for 20%vol. and minimum of that (24.27%) belonged to control treatment (Table 3). Between different levels of Zinc Chelate the maximum protein content (31.23%) was observed 4 per thousand concentrations (also it doesn't have significant differences with 2 per thousand) and control (22.84%) treatments (Table 4). Jalilshesh-Bahre and Movahedi Dhnavi (2014) reported that zinc and manganese significantly increased protein content of the seeds. These elements constitutes the structure of some enzymes, are involved in synthesis of amino acids, which are essential in synthesis of proteins. Therefore, application of these elements (manganese) increases protein content of the seeds. Protein percentage increases by application of micronutrients since these elements (especially manganese) are involved in metabolism of nitrogen and synthesis of enzymes. These elements also regulate enzymatic activity of a large number of enzymes. Enzymes are synthesized from protein (Jamshidi *et al.*, 2016).

4.6. Protein yield

Result of analysis of variance revealed effect of Methanol and Zinc Chelate on protein yield was significant at 1% probability level but interaction effect of treatments was not significant (Table 2). Mean comparison result of different level of Methanol indicated that maximum protein yield (68.71 $gr.m^{-2}$) was noted for 20%vol. and minimum of that (40.58 $gr.m^{-2}$) belonged to control treatment (Table 3). Mabhot (2017) reported that Nano-chelate zinc with large specific surface area, high solubility and a certain complex is highly absorbed by plants. This increases photosynthesis capacity of the plant and allocates more photosynthetic products to reproductive organs and increases seed yield, which directly or indirectly increases protein content of the seeds. Sarbandi and Madani (2014) reported that application of micronutrients (manganese and zinc) significantly improved yield, yield components, biological yield and protein percentage, the highest protein percentage belonged to micro fertilizer treatment and the lowest protein percentage belonged to control. As for Duncan classification made with respect to different level of Zinc Chelate maximum and minimum amount of protein yield belonged to 4 per thousand concentration (63.58 $gr.m^{-2}$) also it doesn't have significant differences with 2 per thousand (59.33 $gr.m^{-2}$) and control (38.89 $gr.m^{-2}$) (Table 4). Marschner (1993) reported that, by increasing consumption of Iron and zinc in corn, we can witness that the total amount of carbohydrate and seed protein is increased, and as a result the gain weight, number of seeds and at last yield will be increased as well. Bakhash-Klarastaghi *et al.* (2007) also showed that application of zinc fertilizer relatively increased seed yield and protein content of the seed.

4.7. Seed yield

Result of analysis of variance revealed effect of Methanol, Zinc Chelate and interaction effect of treatments on seed yield was significant at 1% probability level (Table 2). Mean comparison result of different level of Methanol indicated the maximum and the minimum amount of seed yield belonged to 20%vol. (218.08 $gr.m^{-2}$) and control treatment (167.24 $gr.m^{-2}$) (Table 3). Soleymani and Shahrajabian (2016) reported that application of zinc fertilizer treatment led to the highest biological yield and seed yield due to the positive effect of zinc on biosynthesis of auxin and positive effect of iron on photosynthesis and improved plant growth. Among different level of Zinc Chelate maximum seed yield (203.61 $gr.m^{-2}$) was obtained for 4 per thousand concentrations (also it doesn't have significant differences with 2 per thousand) and control (170.3 $gr.m^{-2}$) treatments (Table 4). Shojaei and Makariyan (2015) by evaluate the effect of three levels of zinc fertilizers (control, 5, 10 g per liter of zinc oxide) on yield and its components of Mungbean reported that zinc fertilizer significantly increased the number of pods per plant. Evaluation mean comparison result of interaction effect of treatments indicated maximum seed yield (235.76 $gr.m^{-2}$) was noted for 20%vol. Methanol and 4 per thousand concentration Zinc Chelate (also it doesn't have significant differences with 2 per thousand) and lowest one (149.5 $gr.m^{-2}$) belonged to control treatment (Table 5).

Table 5. Mean comparison interaction effect of treatment on measured traits

Methanol	Zinc Chelate	Seed yield ($gr.m^{-2}$)
Noneuse of Methanol or control	Noneuse of Zinc or control	149.5d
	2 per thousand	178.3c
	4 per thousand	180.69c
10% Vol	Noneuse of Zinc or control	171.9d
	2 per thousand	186.5b
	4 per thousand	190.73b
20% Vol	Noneuse of Zinc or control	183.43bc
	2 per thousand	231.73a
	4 per thousand	235.76a

*Similar letters in each column show non-significant difference at 5% probability level in Duncan test.

5. CONCLUSION

Generally based on result of this study use 20%vol. Methanol and 2 per thousand concentration Zinc Chelate produce the maximum amount of mentioned characteristics and can be advice to farmers.

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FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

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