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Assess Effect of Copper Foliar Application at Different Growth Stages on Protein Yield and Crop Production of Cowpea under Warm and Dry Climate Condition

Shahram Ashabi[1], Mohammad Reza Dadnia*[2]

1- *Msc. Graduated, Department of Agronomy, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.*

2- *Assistant Professor, Department of Agronomy, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.*

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ABSTRACT

BACKGROUND: Mineral fertilizers played a great role towards improving crop yields but main constraint in achieving proven crop potential is imbalanced use of fertilizers, particularly low use of microelements like copper as compared to N.

OBJECTIVES: This research was consisted to determine effect of different concentration and stage of foliar application of Copper on qualitative traits of Cowpea.

METHODS: This research was carried out via factorial experiment based on randomized complete blocks design with three replications along 2017 year. The treatments included different concentration of Copper foliar application (a_1: none use of copper or control, a_2: 150 $gr.ha^{-1}$, a_3: 300 $gr.ha^{-1}$, a_4: 450 $gr.ha^{-1}$) and Copper foliar application at different growth stage (b_1: apply at vegetative stage, b_2: beginning of flowering stage, b_3: beginning of pod formation).

RESULT: According result of analysis of variance effect of different concentration and growth stage of foliar application of Copper (instead copper percentage) on all measured traits was significant but interaction effect of treatments was not significant (instead seed yield). Evaluation mean comparison result revealed the maximum plant height (157.14 cm), seed yield (211.61 $gr.m^{-2}$), protein percentage (31.56%), protein yield (66.59 $gr.m^{-2}$) and Copper percentage (21.29%) was noted for 300 $gr.ha^{-1}$ Copper (Also it doesn't have significant difference with 150 $gr.ha^{-1}$ Copper) and minimum of mentioned traits belonged to control treatment. Between different growths stage of foliar application of Copper the maximum amount of studied characteristics was observed in vegetative stage and the lowest ones was found in beginning of pod formation.

CONCLUSION: It is recommended to farmers due to compliance with environmental aspects and less consumption of chemical inputs to use 150 $gr.ha^{-1}$ of copper foliar application in the vegetative stage.

KEYWORDS: *Black-eyed pea, Legume, Micro elements, Plant height, Qualitative trait.*

***Corresponding Author:** Mohammad Reza Dadnia ✉ Rezadadnia@yahoo.com

1. BACKGROUND

Balanced supply of essential nutrients is one of the most important factors in increasing crop yields. The functions of copper in the plants are to metabolize nitrogen and carbohydrate and to synthesize lignin. It also affects flavour and coloring of the vegetables and their storage ability, which aids to the prevention of diseases. In Cu deficient soil, roots are vulnerable to fungal and bacterial attack (Sharif *et al*. 2019). Macro and micronutrients deficiencies have been reported for different soils and crops (Hussain *et al*., 2006). Ozcan *et al*. (2017) by evaluate macro and micro element contents of oat grains reported the highest Zn and Cu contents of oat grains were found in TL76 (37.68 $mg.kg^{-1}$) and TL67 (8.67 $mg.kg^{-1}$). Abd El-Wahab (2008) stated that micronutrients such as Fe, Mn and Zn have important roles in plant growth and yield of aromatic and medicinal plants. Copper is an essential plant nutrient that plays an efficient role in chlorophyll development, and protein formation from amino acids and gives rigidity to plant because copper strengthens plant cell wall. In tall plants Cu is essential for more than 30 enzymes which acts as redox catalysts like nitrate reductase, cytochrome oxidase or act as dioxygen carrier like heamocynin (Mohamed and Taha, 2003). Copper also has an influence on the metabolic processes of plant like photosynthesis and reduction of respiration in pollen capability and its deficiency increases infertility of spikelet in lot of unfilled grains (Dobermann and Fairhurst, 2000). Agaalipour *et al*. (2012) studied the application of biofertilizers, instead of chemical fertilizer, for optimal cowpea nutrition and reported that there were significant differences between rates of factor A (Nitragin biofertilizers + Yashil chemical fertilizer) and rates of chemical fertilizers and interaction between different levels of factor (A and B) for grain yield, number of grain per pod, number of grain per plant, number of pod per plant, 1000- grain weight and harvest index at 5% level of probability. Application 52.5 $kg.ha^{-1}$ urea highly increased on traits. Generally application of biofertilizers with chemical fertilizers had maximum increase in all traits under study. Foliar application of Cu significantly increases the grain yield of wheat (Karamanos, *et al*., 2004). Usman Ali *et al*. (2004) by evaluate the effect of copper on wheat growth and soil composition through its soil and foliar application soil application of copper reported soil application of copper better than foliar application in increasing the yield and its components of wheat. Also foliar application of copper at 4 $mg.kg^{-1}$ was better than 2 $mg.kg^{-1}$.

2. OBJECTIVES

This research was consisted to determine effect of different concentration and stage of foliar application of Copper on qualitative traits of Cowpea.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research was carried out via factorial experiment based on randomized complete blocks design with three replications along 2017 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 treatments included different concentration of Copper foliar application (a_1: none use of copper or control, a_2: 150 gr.ha^{-1}, a_3: 300 gr.ha^{-1}, a_4: 450 gr.ha^{-1}) and Copper foliar application at different growth stage (b_1: apply at vegetative stage, b_2: beginning of flowering stage, b_3: beginning of pod formation). This experiment had 36 plots. Each plot consisted of 5 lines with a distance of 60 cm and 5 meters length. The distance between the shrubs on every row was 20 cm.

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 recommendations of Iranian Soil and Water Research Institute at the planting stage. The light-disk harrow was used to mix the soil and the fertilizer after soil fertilization. The furrower was used to make furrows at a distance of 60 cm. 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

Soil depth (cm)	Cu (ppm)	P (ppm)	K (ppm)	N (%)	pH	EC (ds.m^{-1})	O.C (%)	Soil texture
0-15	1.1	5	221	5.4	7.1	4	0.63	Clay loam
15-30	0.9	4.41	217	5.2	7.0	3.82	0.55	Clay loam

3.3. Measured Traits

In order to determine the yield of two planting lines from each plot harvested and after the removal of marginal effect were carried to the research laboratory and were placed in the oven tool 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. To measure the seed nitrogen content and straw nitrogen content the Kjeldahl method was used. So, to calculate the seed protein content the following formula was used (Bremner *et al*., 1983): **Equ.1.** Seed protein content (%)= Nitrogen percentage × 5.8. Protein yield was determined by multiply seed yield to seed protein percentage.

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

Result of analysis of variance showed effect of different concentration and growth stage of foliar application of Copper on plant height was significant at 5% probability level but interaction effect of treatments was not significant (Table 2). Mean comparison result of different concentration of foliar application of Copper indicated that maximum plant height (157.14 cm) was noted for 300 gr.ha^{-1} Copper (Also it doesn’t have significant difference with 150 $gr.ha^{-1}$ Copper) and minimum of that (111.12 cm) belonged to control treatment (Table 3). As for Duncan classification made with respect to different growth stage of foliar application of Copper maximum and minimum amount of plant height belonged to vegetative stage (158.74 cm) and beginning of pod formation (115.43 cm) (Table 4). Aminifar *et al*. (2017) by investigate the effects of different fertilizer applications and sesame/cowpea intercropping systems on soil fertility reported the highest yield of sesame (1292.6 $kg.ha^{-1}$), and cowpea (3772.4 $kg.ha^{-1}$) were obtained from their sole cropping. Among the applications of fertilizer, the highest yield of sesame (950.49 $kg.ha^{-1}$) and cowpea (2582.50 $kg.ha^{-1}$) belonged to bioorganic and biofertilizer treatments, respectively. It seems that 50:50 sesame-cowpea intercropping (M_3) and application of 30 $kg.ha^{-1}$ N + 50 $kg.ha^{-1}$ P + 150 $kg.ha^{-1}$ bio-organic fertilizer (F_4), may reduce application of chemical fertilizers and be beneficial to sesame-cowpea intercropping system.

Table 2. Result of analysis of variance effect of treatments on measured traits

S.O.V	df	Plant height	Seed yield	Protein percentage	Protein yield	Copper percentage
Replication	2	0.85^{ns}	38.11^{ns}	0.14^{ns}	11.26^{ns}	0.08^{ns}
Different concentration of Copper foliar application (C)	3	625.18^{*}	38510.7^{**}	95.01^{*}	885.5^{*}	56.9^{*}
Copper foliar application at different growth stage (G)	2	534.04^{*}	22637.4^{**}	72.3^{*}	439.7^{*}	0.72^{ns}
C × G	6	5.17^{ns}	13058.6^{**}	0.304^{ns}	199.4^{ns}	0.54^{ns}
Error	22	100.19	405.5	7.02	85.6	6.83
CV (%)	-	7.35	10.51	9.51	5.36	13.06

$^{ns, *}$ ^{and} **: no significant, significant at 5% and 1% of probability level, respectively.

4.2. Seed yield

According result of analysis of variance effect of different concentration, growth stage of foliar application of Copper and interaction effect of treatments on seed yield was significant was significant at 1% probability level (Table 2). Assessment mean comparison result indicated in different concentration of foliar application of Copper the maximum seed yield (211.61 $gr.m^{-2}$) was noted for 300 $gr.ha^{-1}$ Copper (Also it doesn't have significant difference with 150 $gr.ha^{-1}$ Copper) and minimum of that (166.25 $gr.m^{-2}$) belonged to control treatment (Table 3). The results of Hosseinpour *et al*. (2015) confirm that foliar application of micronutrients (Copper and Iron) had a significant effect on seed number per ear, 100-seed weight and seed yield. Between different growth stage of foliar application of Copper the maximum seed yield (206.01 $gr.m^{-2}$) was observed in vegetative stage and the lowest one (170.23 $gr.m^{-2}$) was found in beginning of pod formation (Table 4).

Divyashree *et al*. (2018) stated that foliar application of microelements such as copper increased seed yield and iron concentration. Evaluation mean comparison result of interaction effect of treatments indicated maximum seed yield (220 $gr.m^{-2}$) was noted for 300 $gr.ha^{-1}$ Copper in vegetative stage and lowest one (160 $gr.m^{-2}$) belonged to none use of copper at beginning of pod formation (Fig.1). Rafi'i Shirvan and Asghari Pour (2008) reported that Copper foliar application at the vegetative growth stage increases yield by increasing the length of flowering and pod formation period, increasing the number of seeds per pod, leaf area and dry weight. If copper foliar application at the rate of 300 $gr.ha^{-1}$ is done in the vegetative stage, the crop enters the reproductive phase with a higher potential. Therefore, the plant has a higher potential for grain production and this increases grain yield. It seems that micronutrients such as copper increase grain yield by increasing photosynthesis and improving leaf area duration. Absorption of more nutrients by the plant increases the growth and biochemical activities and led to increase crop production (Hosseinpour *et al*., 2015).

Table 3. Effect of different concentration of Copper foliar application on measured traits

Different concentration of Copper foliar application	Plant height (cm)	Seed yield ($gr.m^{-2}$)	Protein percentage (%)	Protein yield ($gr.m^{-2}$)	Copper percentage (%)
None use of copper or control	111.12c	166.28c	23.45c	38.92d	18.36c
150 $gr.ha^{-1}$	153.18a	205.65a	30.98a	63.50a	20.98a
300 $gr.ha^{-1}$	157.14a	211.61a	31.56a	66.59a	21.29a
450 $gr.ha^{-1}$	123.01b	182.27b	25.61b	46.61c	19.37b

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT)

4.3. Protein percentage

Result of analysis of variance showed effect of different concentration and growth stage of foliar application of Copper on protein percentage was significant at 5% probability level but interaction effect of treatments was not significant (Table 2). Evaluation mean comparison result revealed the maximum protein percentage (31.56%) was noted for 300 $gr.ha^{-1}$ Copper (Also it doesn’t have significant difference with 150 $gr.ha^{-1}$ Copper) and minimum of that (23.45%) belonged to control treatment (Table 3). Among different growth stage of foliar application of Copper maximum protein percentage (30.21%) was obtained for vegetative stage and minimum of that (24.45%) was for beginning of pod formation (Table 4). Lotfi *et al*. (2018) to study the possibility of reducing the consumption of nitrogen fertilizer using biological fertilizer on yield and yield components of cowpea reported protein content of seed inoculated with biofertilizer nitroxin was 24.96 percent which is one percent more than non-inoculated seeds. The amount of protein obtained in application of nitrogen was also more than non-application treatment and the difference between them was significant. The interaction of urea fertilizer and bio-fertilizer, resulted in highest and the lowest yield in seeds inoculated with 50 kg of urea fertilizer and no fertilizer application biological fertilizer + no fertilizer urea in 2046 and 1336 kg per hectare, respectively. Copper is an essential plant nutrient that plays an efficient role in chlorophyll development, and protein formation from amino acids and gives rigidity to plant because copper strengthens plant cell wall. In tall plants Cu is essential for more than 30 enzymes which acts as redox catalysts like nitrate reductase, cytochrome oxidase or act as dioxygen carrier like heamocynin (Mohamed and Taha, 2003).

4.4. Protein yield

According result of analysis of variance effect of different concentration and growth stage of foliar application of Copper on protein yield was significant at 5% probability level but interaction effect of treatments was not significant (Table 2). Assessment mean comparison result indicated in different concentration of foliar application of Copper the maximum protein yield (66.59 gr.m^{-2}) was noted for 300 gr.ha^{-1} Copper (Also it doesn't have significant difference with 150 gr.ha^{-1} Copper) and minimum of that (38.92 gr.m^{-2}) belonged to control treatment (Table 3). Between different growths stage of foliar application of Copper the maximum protein yield (62.21 gr.m^{-2}) was observed in vegetative stage and the lowest one (41.56 gr.m^{-2}) was found in beginning of pod formation (Table 4). Sarbandi and Madani (2014) reported that application of micronutrients 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.

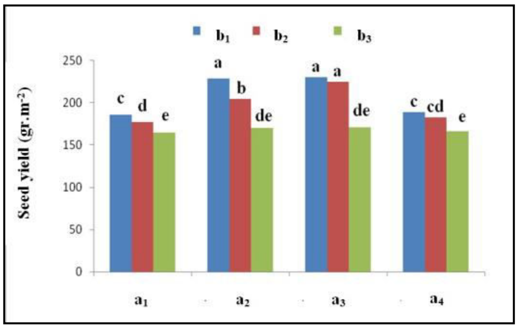

Fig.1. Mean comparison interaction effect of treatment on seed yield
*Mean which have at least once common letter are not significant different at the 5% level using (DMRT)
Different concentration of Copper foliar application (a_1: none use of copper or control, a_2: 150 gr.ha^{-1}, a_3: 300 gr.ha^{-1}, a_4: 450 gr.ha^{-1})
Copper foliar application at different growth stage (b_1: apply at vegetative stage, b_2: beginning of flowering stage, b_3: beginning of pod formation)

4.5. Copper percentage

Result of analysis of variance showed effect of different concentration of foliar application of Copper on copper percentage was significant at 5% probability level but effect of growth stage and interaction effect of treatments was not significant (Table 2). Mean comparison result of different concentration of foliar application of Copper indicated that maximum copper percentage (21.29%) was noted for 300 gr.ha^{-1} Copper (Also it doesn't have significant difference with 150 gr.ha^{-1} Copper) and minimum of that (18.36%)

belonged to control treatment (Table 3). Khalaj *et al*. (2020) by evaluate the effect of foliar application of iron and magnesium nano-chelate fertilizers on Morphophysiological characteristics of *Vigna sinensis* L., reported the highest stem height, distance between the first pods from the soil surface and leaf area index were observed in the nano-iron treatment 0.25 $gr.l^{-1}$ and 1% nano magnesium. The iron composition of 0.25 $gr.l^{-1}$ and nano magnesium had the highest number of lateral branches (5.46 branches per plant) and sheath length (16.56 cm). While the Greenness index traits and the amount of iron and magnesium in leaves, had the highest efficiency in 0.5 grams per liter iron in both nano and normal forms, with normal magnesium treatment. However, the highest grain protein (22.35%) was produced by using nano-iron at 0.25 $gr.l^{-1}$ alone. Also, the highest yield resulted from the application of Fe (0.5 $gr.l^{-1}$) × nano magnesium with mean of 2377.73 $kg.ha^{-1}$ and the lowest values were for the nano magnesium (792.55 $kg.ha^{-1}$). One of the reasons for the observed increase in yield in Fe (0.5 $gr.l^{-1}$) × nano magnesium was the increase in the number of pods per plant and the weight of 1000 seeds. Zahedi *et al*. (2018) by study the effect of drought stress and application of potassium on seed yield, some biochemical characteristics, and the content of micronutrients (such as Copper, Zinc and Manganese) in cowpea reported application of 120 mg potassium in stress conditions caused an increase in the concentration of Cu, Zn and, Mn. Findings seem to suggest that potassium improves growth characteristics in cowpea by decreasing the undesirable consequences of drought stress. Therefore, application of potassium sulfate is recommended as a strategy to mitigate the effects of draught stress in cultivation of cowpea.

Table 4. Effect of Copper foliar application at different growth stage on measured traits

Copper foliar application at different growth stage (G)	Plant height (cm)	Seed yield ($gr.m^{-2}$)	Protein percentage (%)	Protein yield ($gr.m^{-2}$)	Copper percentage (%)
Vegetative stage	158.74a	206.01a	30.21a	62.21a	20.21a
Beginning of flowering	134.18b	198.12ab	29.31ab	58.03b	20.01a
Beginning of pod formation	115.43c	170.23b	24.45b	41.56c	20.00a

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT)

5. CONCLUSION

It is recommended to farmers due to compliance with environmental aspects and less consumption of chemical inputs to use 150 $gr.ha^{-1}$ of copper foliar application in the vegetative stage.

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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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