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Response of Growth Indices to Copper Foliar Application at Different Growth Stages of Cowpea

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: 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 all crops 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.

OBJECTIVES: The current study was conducted to evaluate the effect of different concentration and stage of foliar application of Copper on growth curves and crop production 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 crop growth rate) on all measured traits was significant at 1% probability level but interaction effect of treatments was not significant (instead seed yield). The highest seed yield with an average of 220 $gr.m^{-2}$ of foliar application of 300 $gr.ha^{-1}$ of copper in the vegetative and early flowering stage (which was not statistically significant with the treatment of foliar application of 150 $gr.ha^{-1}$ of copper in the vegetative stage) and The lowest one was obtained from the non-foliar application of copper in the early stage of pod formation at the rate of 160 $gr.m^{-2}$.

CONCLUSION: Generally, due to the positive response of the cowpea to the copper in terms of growth, seed yield and its components, the use of copper fertilizer in fields where the concentration of this element in their soil is less than critical level, consumption of 150 $gr.ha^{-1}$ of copper foliar application in the vegetative stage in Ahvaz region is recommended.

KEYWORDS: *Dry weight, Leaf area, Phenology, Seed yield, Spray.*

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1. BACKGROUND

Micronutrient deficiencies in crop plants become important worldwide because over growing population of world is affected by lower level of micronutrient in human food (Welch and Gramham, 1999) and poor content of essential nutrients and micronutrients in grains of modern high yielding wheat cultivars are mostly recognized (Fan *et al*., 2008). Copper (Cu) is one of eight essential plant micronutrients. Copper is required for many enzymatic activities in plants and for chlorophyll and seed production. Deficiency of copper can lead to increased plant susceptibility to disease, one example being ergot which can cause significant yield loss in small grains. Most Minnesota soils supply adequate amount of copper for crop production. However, copper deficiency can occur in high organic matter and sandy soils (Sutradhar *et al*., 2017). The amount of copper available to plants varies widely among soils. Copper in the soil is held with clay minerals as a cation (Cu^{2+}) and in association with organic matter. Some silicate minerals and carbonate contain copper as impurities (Sutradhar *et al*., 2017). 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 all 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 is not mobile in organic soils as it is attracted to soil organic matter and clay minerals. Copper deficiencies often occur in soils with peaty soils with greater concentrations of organic matter. Copper binds with organic matter more tightly than any other of the crop micronutrients. Crops sensitive to copper deficiency grown on peat soils with organic matter content more than 8% are likely to show copper deficiency symptoms (Sutradhar *et al*., 2017). 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). Copper use efficiency is improved if the fertilizer is water soluble and the particle size of the fertilizer is small. A single application of copper can last for many years. Foliar application of copper can also be an effective way to correct copper deficiency in small grains and vegetable crops. The growth stage and application time has a major influence on the effectiveness of the treatment (Sutradhar *et al*., 2017). Copper mobilizes from old leaves to younger parts of the plant to some extent with the degree of mobilization greater when Cu is more available to the plant and movement is related to leaf senesce (Loneragan, 1981). Mature plants deficient in Cu have delaying heading, and empty or partially-filled heads due to lack of viable pollen and also senesce (Graham, 1975). Copper is naturally present in soil in several soluble (hydroxy and carbonate) and insoluble (oxide and sulphide) forms and with the soluble form differing in its availability to plants dependent on other soil properties predominantly soil pH, clay content and the presence of organic matter (Fernandes and Henriques, 1991). Plant roots take up Cu from soil solution as water soluble Cu^{2+} in the soil or from fertiliser. Uptake of Cu from the soil and into the plant depends on (Fernandes and Henriques, 1991): a. limited movement of the nutrient via mass flow or diffusion (from the soil to the root) b. the chemical availability of the nutrient c. growth of roots through the soil (root interception) d. active and passive uptake of the nutrient at the root surface itself. Foliar fertilization with micronutrients has been intensively used in the late years because this practice allows the application of minerals at the appropriate time during plant development (according to plant needs), it allows uniformity in nutrient distribution and increase in the nutrient absorption, and consequently it avoids losses in the environment (Ruiz-Garcia and Gomez-Plaza, 2013). Foliar application of Cu is an alternative to soil-applied Cu fertilizer. Foliar application has the advantage of allowing Cu to be applied strategically based on seasonal progress and the occurrence of visual symptoms. The most common form of foliar fertilizer is $CuSO_4$ (25% Cu). Alternative forms are copper oxychloride (52% Cu) and chelated-Cu (15% Cu) (Brennan, 1990). The tolerant genotypes could be recommended for cultivation in moderately salt affected areas (Roy *et al.*, 2014). Previously, experiments on the selection of salt tolerance cultivars of sorghum were conducted using copper foliar application improved physiological factors included amylose, amylopectin, starch and proteins (Faraco, 2015). Addition of fertilizers to supplement the natural soil fertility is essential for modern crop production, and precise management of nutrient elements is essential for a sustainable agriculture production (Barker and Pilbeam, 2006). Each plant needs to certain fertilizers according to its needs and soil analyze results. Also microelements are the critical elements for plants; however, microelements play the important role in crop productivity where it is used in low rate. Optimum plant nutrition and maximum yield is achieved when nutrient elements are available for plant during the growing season (Malakooti and Tabataei, 1998).

2. OBJECTIVES

The current study was conducted to evaluate the effect of different concentration and stage of foliar application of Copper on growth curves and crop production 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 the recommendations of the 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})	OC (%)	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 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. By measuring three factors including leaf area, leaf dry weight and total dry weight, the physiological parameters of growth including LAI, NAR, CGR and RGR were obtained using the following equations. 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. Crop growth rate, net assimilation rate and relative growth rate were measured according fallowing formula (Buttery, 1970; Enyi, 1962):

Equ.1. CGR (gr.m^{-2}.day^{-1}) = TDW_2-TDW_1/T_2-T_1

TDW_1= Primary dry weight (g), TDW_2= Secondary dry weight (gr)

T_1= initial sampling time, T_2= Secondary sampling time

Equ.2. NAR (gr.m^{-2}.day^{-1}) = CGR× $LnLA_2$-$LnLA_1$/LA_2-LA_1

CGR = Growth rate in grams per day per square meter

LA_1 = Initial leaf area, LA_2 = Secondary leaf area

Equ.3. RGR (gr.gr^{-1}.day^{-1}) = [Ln (TDW_2) – Ln (TDW_1)]/T_2-T_1

RGR= relative growth rate in gram per gram per day

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. Total dry weight (TDW)

According result of analysis of variance effect of different concentration and growth stage of foliar application of Copper on total dry weight (along beginning of flowering until pod formation) was significant at 1% probability level but interaction effect of treatments was not significant (Table 2). The results of this experiment were consistent with the findings of Sadeghipour and Aghaei (2012) in cowpea beans, who reported an increase in dry matter, compared to the control treatment by use of micro element. They stated that the use of micro element improves plant growth, which is probably due to improved carbon uptake, increased synthesis of metabolites and maintenance of water content in plant tissues. Mean comparison result of different concentration of foliar application of Copper indicated that maximum total dry weight (516.01 $gr.m^{-2}$, 605.14 $gr.m^{-2}$ belonged to Flowering and pod formation stage, respectively) 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 (390.15 $gr.m^{-2}$, 485.31 $gr.m^{-2}$ belonged to Flowering and pod formation stage, respectively) was for control treatment (Table 3). Saeedin (2016) evaluated the correlation between biological yield and seed yield of cowpea and reported a positive and significant correlation between mentioned traits. Its seem biological yield increased because of accumulation of photosynthetic products (source products) and high potential of seeds (reservoir) for absorption and accumulation of dry matter. Therefore, any increases in seed yield also increases the biological yield. However, less dry matter is accumulated in case of micronutrient deficiency, which decreases the biological yield. As for Duncan classification made with respect to different growth stage of foliar application of Copper maximum and minimum amount of total dry weight belonged to Vegetative stage, (also it doesn’t have significant difference with apply Copper at Beginning of flowering) and Beginning of pod formation (Table 4). Roknil (2013) concluded that the rapid increase in dry matter begins with an increase in leaf area and peaks when the plant reaches a maximum leaf area.

Table 2. Result of analysis of variance effect of treatments on total dry weight

S.O.V	df	Beginning of flowering	Beginning of pod formation
Replication	2	154.01^{ns}	95.4^{ns}
Different concentration of Copper foliar application (C)	3	29810.31**	19360.16**
Copper foliar application at different growth stage (G)	2	38516.5**	27941.4**
C × G	6	699.03^{ns}	1057.8^{ns}
Error	22	2703.47	1800.07
CV (%)	-	11.21	7.6

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

Table 3. Effect of different concentration of Copper foliar application on total dry weight along different growth stage

Treatment	Total dry weight ($gr.m^{-2}$)	
Different concentration of Copper foliar application	**Flowering stage**	**Pod formation stage**
None use of copper or control	390.15c	485.31c
150 $gr.ha^{-1}$	508.2a	591.02a
300 $gr.ha^{-1}$	516.01a	605.14a
450 $gr.ha^{-1}$	440.71b	550.75b

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

4.2. Leaf area index (LAI)

Result of analysis of variance revealed effect of different concentration and growth stage of foliar application of Copper on leaf area index (along beginning of flowering until pod formation) was significant at 1% probability level but interaction effect of treatments was not significant (Table 5). According result of mean comparison maximum amount of leaf area index was obtained for 300 $gr.ha^{-1}$ Copper (Also it doesn't have significant difference with 150 $gr.ha^{-1}$ Copper) and minimum of that was for control treatment in all growth stages (Table 6). It seems that the reason for higher leaf area index in copper fertilizer due to great effect of different levels of copper on vegetative growth. Therefore, leaf area development is provided by improving the number, size and area of leaves with the nutrients, which increases the leaf area index (Eskandari and Mozafari, 2012). Evaluation mean comparison result indicated in different growth stage of foliar application of Copper the maximum leaf area index was noted for vegetative stage (also it doesn't have significant difference with apply Copper at beginning of flowering) and minimum of that belonged to beginning of pod formation (Table 7). Pouryousef *et al.* (2010) reported that the use of 20 tons of animal manure per hectare had the greatest effect on the leaf area index and shoot development of Asparagus plant, which was due to the finer and richer nature and microbial population of animal manure than chemical fertilizer.

Table 4. Effect of Copper foliar application at different growth stage on total dry weight

Treatment	Total dry weight ($gr.m^{-2}$)	
Copper foliar application at different growth stage (G)	**Flowering stage**	**Pod formation stage**
Vegetative stage	510.1a	600.05a
Beginning of flowering	496.14a	584.11a
Beginning of pod formation	385.05b	490.22b

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

4.3. Crop growth rate (CGR)

According result of analysis of variance effect of different concentration of Copper foliar application on crop growth rate (along beginning of flowering until pod formation) was significant at 1% probability level but effect of apply copper at different growth stage and interaction effect of treatments was not significant (Table 8). It seems indicate that increasing the levels of micro and macro elements in the plant increases the crop growth rate and consequently increases the yield. Probably in the present experiment, a large part of the differences in crop growth rate is related to differences in leaf area index because changes in crop growth rate depend on changes in two parameters of leaf area index and net assimilation rate. Assessment mean comparison result indicated in different concentration of foliar application of Copper the maximum crop growth rate (15.01 $gr.m^{-2}.day^{-1}$) 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 (11. $gr.m^{-2}.day^{-1}$) belonged to control treatment (Table 9).

Table 5. Result of analysis of variance effect of treatments on leaf area index

S.O.V	df	Flowering stage	Pod formation stage
Replication	2	0.04^{ns}	0.11^{ns}
Different concentration of Copper foliar application (C)	3	38.59**	18.94**
Copper foliar application at different growth stage (G)	2	23.60**	40.07**
C × G	6	0.02^{ns}	0.01^{ns}
Error	22	0.18	0.1
CV (%)	-	12.22	13.86

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

Table 6. Effect of different concentration of Copper foliar application on leaf area index along different growth stage

Treatment	Leaf area index	
Different concentration of Copper foliar application	Flowering stage	Pod formation stage
None use of copper or control	2.95c	2.27c
150 $gr.ha^{-1}$	3.71a	2.82a
300 $gr.ha^{-1}$	3.84a	2.91a
450 $gr.ha^{-1}$	3.4b	2.41bc

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

Table 7. Effect of Copper foliar application at different growth stage on leaf area index

Treatment	Leaf area index	
Copper foliar application at different growth stage (G)	**Flowering stage**	**Pod formation stage**
Vegetative stage	3.82a	2.91a
Beginning of flowering	3.79a	2.81a
Beginning of pod formation	2.81b	2.11c

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

Table 8. Result of analysis of variance effect of treatments on crop growth rate

S.O.V	df	Flowering to Pod formation stage
Replication	2	0.81^{ns}
Different concentration of Copper foliar application (C)	3	149.3**
Copper foliar application at different growth stage (G)	2	0.11^{ns}
C × G	6	0.42^{ns}
Error	22	3.95
CV (%)	-	14.48

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

Randall *et al*. (2013) also reported that the highest crop growth rate was achieved in the treatment of micro fertilizer application. They stated that the reason for this was the more vegetation (photosynthetic surface), led to increase radiation absorption and dry matter production per unit area, and ultimately led to increased crop growth rate.

4.4. Net assimilation rate (NAR)

Result of analysis of variance revealed effect of different concentration and growth stage of foliar application of Copper (along beginning of flowering until pod formation) on net assimilation rate was significant at 1% probability level but interaction effect of treatments was not significant (Table 10). Mean comparison result of different concentration of foliar application of Copper indicated that maximum net assimilation rate (5.8 gr.m^{-2}.day^{-1}) 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 (3.51 gr.m^{-2}.day^{-1}) was for control treatment (Table 11). Sadeghipour and Aghaei (2012) reported that the application of micronutrients increased the absorption and construction (assimilation) by 50%. Between different growth stage of foliar application of Copper the maximum net assimilation rate (5.24 gr.m^{-2}.day^{-1}) was observed in vegetative stage (also it doesn't have significant difference with apply Copper at beginning of flowering) and the lowest one (3.38 gr.m^{-2}.day^{-1}) was found in beginning of pod formation (Table 12).

Table 9. Effect of different concentration of Copper foliar application on crop growth rate along different growth stage

Treatment	Crop growth rate ($gr.m^{-2}.day^{-1}$)
Different concentration of Copper foliar application	**Flowering to Pod formation stage**
None use of copper or control	11.88c
150 $gr.ha^{-1}$	14.52a
300 $gr.ha^{-1}$	15.01a
450 $gr.ha^{-1}$	13.47b

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

Table 10. Result of analysis of variance effect of treatments on net assimilation rate

S.O.V	df	Flowering to Pod formation stage
Replication	2	0.27^{ns}
Different concentration of Copper foliar application (C)	3	60.38**
Copper foliar application at different growth stage (G)	2	44.09**
C × G	6	0.54^{ns}
Error	22	0.45
CV (%)	-	12.11

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

Table 11. Effect of different concentration of Copper foliar application on net assimilation rate along different growth stage

Treatment	Net assimilation rate ($gr.m^{-2}.day^{-1}$)
Different concentration of Copper foliar application	**Flowering to Pod formation stage**
None use of copper or control	3.51c
150 $gr.ha^{-1}$	5.2a
300 $gr.ha^{-1}$	5.8a
450 $gr.ha^{-1}$	3.7bc

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

Table 12. Effect of Copper foliar application at different growth stage on net assimilation rate

Treatment	Net assimilation rate ($gr.m^{-2}.day^{-1}$)
Different concentration of Copper foliar application	**Flowering to Pod formation stage**
Vegetative stage	5.24a
Beginning of flowering	5.04a
Beginning of pod formation	3.38b

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

Nadim *et al*. (2012) reported among the iron, zinc, barium and copper treatments, the highest effect was obtained from copper treatment.

Table 13. Result of analysis of variance effect of treatments on seed yield

S.O.V	df	Seed yield
Replication	2	38.11^{ns}
Different concentration of Copper foliar application (C)	3	38510.7^{**}
Copper foliar application at different growth stage (G)	2	22637.4^{**}
C × G	6	13058.6^{**}
Error	22	405.5
CV (%)	-	10.51

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

Table 14. Effect of different concentration of Copper foliar application on seed yield along different growth stage

Treatment	Seed yield ($gr.m^{-2}$)
None use of copper or control	166.28c
150 $gr.ha^{-1}$	205.65a
300 $gr.ha^{-1}$	211.61a
450 $gr.ha^{-1}$	182.27b

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

Table 15. Effect of Copper foliar application at different growth stage on net seed yield

Treatment	Seed yield ($gr.m^{-2}$)
Vegetative stage	206.01a
Beginning of flowering	198.12a
Beginning of pod formation	170.23b

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

4.5. Seed yield

According result of analysis of variance effect of different concentration, growth stage of foliar application of Copper and interaction effect of treatments (along beginning of flowering until pod formation) on seed yield was significant was significant at 1% probability level (Table 13). 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 14). 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 (also it doesn't have significant difference with apply Copper at beginning of flowering) and the lowest one (170.23 $gr.m^{-2}$) was found in beginning of pod formation (Table 15).

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).

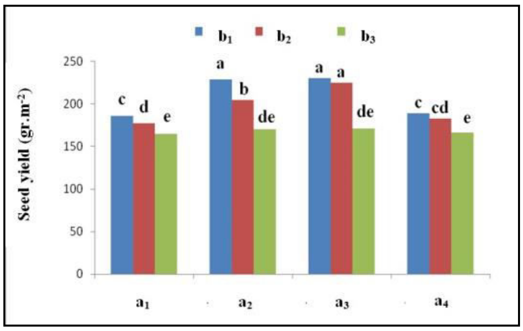

Fig.1. Mean comparison interaction effect of treatment on seed yield
Mean which have at least once common letter are not significant different at the 5% level using (DMRT)

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).

5. CONCLUSION

Generally, due to the positive response of the cowpea to the copper in terms of growth, seed yield and its components, the use of copper fertilizer in fields where the concentration of this element in their soil is less than critical level, consumption of 150 gr.ha^{-1} of copper foliar application in the vegetative stage in Ahvaz region is recommended.

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FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

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