Journal of Crop Nutrition Science
ISSN: 2423-7353 (Print) 2538-2470 (Online)
Vol. 7, No. 3, 2021
http://JCNS.iauahvaz.ac.ir
OPEN ACCESS

Evaluation Effect of Copper Foliar Application at Different Growth Stages of on Seed yield and Its Components of Cowpea (*Vigna Sinensis* L.)

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RESEARCH ARTICLE

ARTICLE INFO.
Received Date: *1 Jul. 2021*
Received in revised form: *2 Aug. 2021*
Accepted Date: *5 Sep. 2021*
Available online: *30 Sep. 2021*

To Cite This Article:
Shahram Ashabi, Mohammad Reza Dadnia. Evaluation Effect of Copper Foliar Application at Different Growth Stages of on Seed yield and Its Components of Cowpea (*Vigna Sinensis* L.). ***J. Crop. Nutr. Sci.,*** 7(3): 24-33, 2021.

ABSTRACT

BACKGROUND: Nutrients play a very important role in chemical, biochemical, physiological, metabolic, geochemical, biogeochemical, and enzymatic processes.
OBJECTIVES: This research was done to assess the effect of different concentration and stage of foliar application of Copper on seed yield, yield components and harvest index 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). This experiment had 36 plots.
RESULT: Result of analysis of variance revealed effect of different concentration and growth stage of foliar application of Copper on all measured traits was significant but interaction effect of treatments was not significant (instead number of pod per plant, seed yield and biologic yield). Mean comparison result of different concentration of foliar application of Copper indicated that maximum amount of number of pod per plant (14.83), number of seed per pod (11.96), seed weight (23.65 gr), seed yield (211.61 gr.m^{-2}), biologic yield (509.19 gr.m^{-2}), harvest index (41.55%) was noted for 300 gr.ha^{-1} Copper (Also it doesn't have significant difference with 150 gr.ha^{-1} Copper) and minimum of those belonged to control treatment. As for Duncan classification made with respect to different growth stage of foliar application of Copper the highest amount of number of pod per plant (14.18), number of seed per pod (12.04), seed weight (23.41 gr), seed yield (206.01 gr.m^{-2}), biologic yield (518.75 gr.m^{-2}), harvest index (40.75%) belonged to vegetative stage and lowest ones was for beginning of pod formation.
CONCLUSION: Finally based on result of current research according economic aspects use of 150 gr.ha^{-1} of copper foliar application in the vegetative stage at studied region is advised to producers.

KEYWORDS: *Crop production, Harvest index, Nutrition, Pod, Pulse.*

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1. BACKGROUND

Micronutrient deficiencies in crop 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).

2. OBJECTIVES

This research was done to assess the effect of different concentration and stage of foliar application of Copper on seed yield, yield components and harvest index 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.

3.3. Measured Traits

After physiological ripening seed yield, biologic yield, pod length, number of pods per m^2, number of pods per plant, number seeds per pod, seed weight and plant height was determined. Harvest index (HI) was calculated according to formula of Gardener *et al*. (1985) as follows: **Equ.1.** HI= (Seed yield/Biologic yield) ×100.

Table 1. Physical and chemical properties of studied field

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

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. Number of pod per plant

According result of analysis of variance effect of different concentration, growth stage of foliar application of Copper and interaction effect of treatments on number of pod per plant 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 (14.83) 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 (9.45) belonged to control treatment (Table 3). Between different growths stage of foliar application of Copper the maximum seed yield (14.18) was observed in vegetative stage and the lowest one (10.03) was found in beginning of pod formation (Table 4). Evaluation mean comparison result of interaction effect of treatments indicated maximum seed yield (15) was noted for 300 $gr.ha^{-1}$ Copper in vegetative stage and lowest one (9.8) belonged to none use of copper at beginning of pod formation (Fig.1). El-Habbasha *et al.* (2012) also reported that foliar application of humic acid on peas pods (*Cicer arientinum* L.) improve the growth and quality of pea crops. Foliar application of humic acid on beans (*Phaseolus vulgaris* L.), crops leads to increased plant growth, pods per plant, pod weight, protein rate and chlorophyll of plants through increased rate and extent of nutrients absorption (El-Bassiony, 2010). 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 Mung bean reported that zinc fertilizer significantly increased the number of pods per plant.

4.2. Number of seed per pod

Result of analysis of variance showed effect of different concentration and growth stage of foliar application of Copper on number of seed per pod was significant at 1% 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 number of seed per pod (11.96) 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 (8.08) 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 number of seed per pod belonged to vegetative stage (12.04) and beginning of pod formation (7.96) (Table 4). Some researchers such as Sujatha *et al*. (2008) confirmed mentioned result, because they showed that the application of vermicompost by improving the physical properties of the soil to increase absorption elements, improve the production led to increase the number of seeds per row and number of rows per ear.

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

S.O.V	df	No. pod per plant	No. seed per pod	Seed weight
Replication	2	4.07^{ns}	2.25^{ns}	8.04^{ns}
Different concentration of Copper foliar application (C)	3	154.18^{**}	12.34^{**}	90.11**
Copper foliar application at different growth stage (G)	2	119.24^{**}	10.07^{**}	102.3**
C × G	6	84.17^{**}	0.06^{ns}	1.44^{ns}
Error	22	2.95	0.94	8.62
CV (%)	-	14.22	9.41	13.6

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

Continue table 2.

S.O.V	df	Seed yield	Biologic yield	Harvest index
Replication	2	38.11^{ns}	10.45^{ns}	0.81^{ns}
Different concentration of Copper foliar application (C)	3	38510.7**	62018.6**	102.04^{*}
Copper foliar application at different growth stage (G)	2	22637.4**	510.46**	83.55^{*}
C × G	6	13058.6**	25493.2**	1.78^{ns}
Error	22	405.5	1573.6	10.47
CV (%)	-	10.51	8.21	8.16

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

4.3. Seed weight

According result of analysis of variance effect of different concentration and growth stage of foliar application of Copper on seed weight was significant at 1% probability level but interaction effect of treatments was not significant (Table 2). Evaluation mean comparison result revealed the maximum seed weight (23.65) 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 (18.81) belonged to control treatment (Table 3). Among different growth stage of foliar application of Copper maximum seed weight (23.41) was obtained for vegetative stage and minimum of that (19.57) was for beginning of pod formation (Table 4). In a research on wheat, it was determined that the application of vermicompost fertilizer would increase the seed weight of wheat (Bar-Tal *et al.*, 2004). Some researchers reported that increase 1000-seed weight due to the application of iron Nano fertilizer due to the optimal combination of micronutrient and main nutrient elements in the reproductive stages of the plant. Available main elemental led to improve the accumulation of assimilates in the seeds and produce heavier seeds (Bybordi and Mamedov, 2010).

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

Different concentration of Copper foliar application	No. pod per plant	No. seed per pod	Seed weight (gr)
None use of copper or control	9.45b	8.08c	18.81b
150 $gr.ha^{-1}$	13.67a	11.55a	22.42a
300 $gr.ha^{-1}$	14.83a	11.96a	23.65a
450 $gr.ha^{-1}$	10.35ab	9.75b	21.51ab

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

Continue table 3.

Different concentration of Copper foliar application	Seed yield ($gr.m^{-2}$)	Biologic yield ($gr.m^{-2}$)	Harvest index (%)
None use of copper or control	166.28c	450.29c	36.92c
150 $gr.ha^{-1}$	205.65a	498.61a	41.24a
300 $gr.ha^{-1}$	211.61a	509.19a	41.55a
450 $gr.ha^{-1}$	182.27b	473.08b	38.52b

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

4.4. 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.2). 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 4. Effect of Copper foliar application at different growth stage on measured traits

Copper foliar application at different growth stage (G)	No. pod per plant	No. seed per pod	Seed weight (gr)
Vegetative stage	14.18a	12.04a	23.41a
Beginning of flowering	12.01ab	11.16ab	21.79ab
Beginning of pod formation	10.03b	7.96b	19.57b

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

Continue table 4.

Copper foliar application at different growth stage (G)	Seed yield (gr.m^{-2})	Biologic yield (gr.m^{-2})	Harvest index (%)
Vegetative stage	206.01a	518.75a	40.75a
Beginning of flowering	198.12ab	486.14b	39.71ab
Beginning of pod formation	170.23b	443.5c	38.38b

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

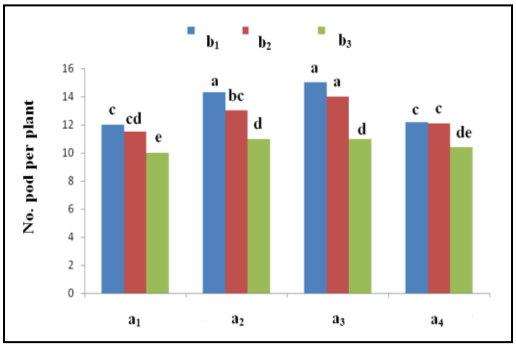

Fig.1. Mean comparison interaction effect of treatment on number of pod per plant.
Mean which have at least once common letter are not significant different at the 5% level using (DMRT)

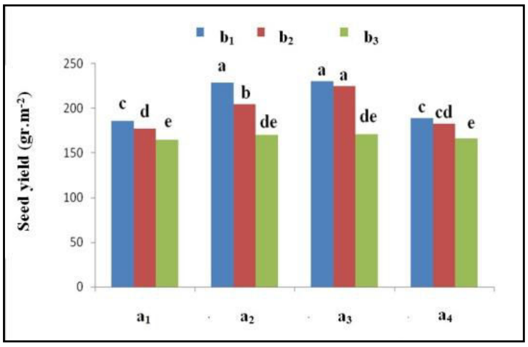

Fig.2. Mean comparison interaction effect of treatment on seed yield.
Mean which have at least once common letter are not significant different at the 5% level using (DMRT)

4.5. Biologic yield

Result of analysis of variance showed effect of different concentration, growth stage of foliar application of Copper and interaction effect of treatments on biologic 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 biologic yield (509.19 $gr.m^{-2}$) 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 (450.29 $gr.m^{-2}$) belonged to control treatment (Table 3). Between different growths stage of foliar application of Copper the maximum biologic yield (518.75 $gr.m^{-2}$) was observed in vegetative stage and the lowest one (443.50 $gr.m^{-2}$) was found in beginning of pod formation (Table 4). Evaluation mean comparison result of interaction effect of treatments indicated maximum biologic yield (511 $gr.m^{-2}$) was noted for 300 $gr.ha^{-1}$ Copper in vegetative stage and lowest one (420 $gr.m^{-2}$) belonged to none use of copper at beginning of pod formation (Fig.3).

Micronutrients improve plant growth, that matter is probably due to improved carbon uptake, increased synthesis of metabolites and maintenance of the plant water status. These results were consistent with findings of this study. On the other hand, increase in dry matter due to application of zinc element can enhance biosynthesis of auxin, increase chlorophyll content and enhance enzymatic activity of phosphoenol-pyruvate carboxylase and ribulose bis-phosphate carboxylase, decrease sodium accumulation in plant tissues and increase uptake of nitrogen and phosphorus (Saeedi Aboueshaghi and Yidwi, 2015).

4.6. Harvest index

Harvest index indicates the rate of photosynthetic distribution between the reproductive and vegetative organs, and indicating the transfer of dry matter to a part of the plant that is harvested (Cavender *et al*., 2003). According result of analysis of variance effect of different concentration and growth stage of foliar application of Copper on harvest index was significant at 5% probability level but interaction effect of treatments was not significant (Table 2). Evaluation mean comparison result revealed the maximum harvest index (41.55%) 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 (36.92%) belonged to control treatment (Table 3). Among different growth stage of foliar application of Copper maximum harvest index (40.75%) was obtained for vegetative stage and minimum of that (38.38%) was for beginning of pod formation (Table 4).

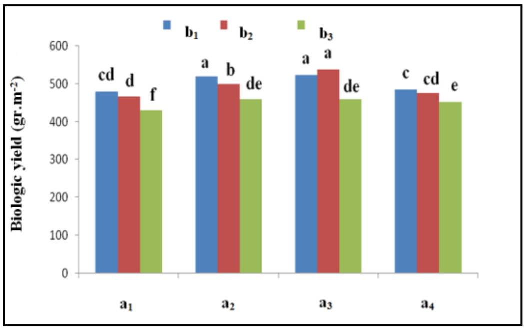

Fig.3. Mean comparison interaction effect of treatment on biologic yield.
*Mean which have at least once common letter are not significant different at the 5% level using (DMRT)

5. CONCLUSION

Finally based on result of current research according economic aspects use of 150 gr.ha^{-1} of copper foliar application in the vegetative stage at studied region is advised to producers.

ACKNOWLEDGMENT

The authors thank all colleagues and other participants, who took part in the study.

FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

FUNDING/SUPPORT: This study was done by support of Department of Agronomy, Islamic Azad University, Ahvaz Branch.

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