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*Full Length Research Paper*

# Seed germination and seedling growth of haricot bean (*Phaseolus vulgaris* L.) cultivars as influenced by copper sulphate

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**An experiment was conducted in the laboratory at Ambo university, Ambo to investigate the effect of copper sulphate levels on germination and seedling growth of three haricot bean cultivars: *Atendaba, Argene* and *Nassier.* Five copper sulphate levels (0, 0.5, 1.0, 1.5, and 2.0 mM) were evaluated in a factorially arranged Complete Randomized Design with four replications. The study revealed that cultivars differed significantly in germination, shoot and root lengths, shoot and root fresh weights, and seedling and root dry weights. No significant difference was observed in shoot dry weight, seedling and shoot vigor indices, tolerance index, and root phytotoxicity. The cultivar *Nassier* showed high tolerance index, and low shoot and root phytotoxicity compared to other cultivars. The copper sulphate levels did not induce differences in germination. The seedling, shoot and root fresh and dry weights; seedling, shoot and root vigour indices; and tolerance index decreased significantly with increased copper sulphate concentration. Phytotoxicity of shoot and root increased significantly with increase in copper sulphate concentrations.**

**Key words:** Copper sulphate, germination, *Phaseolus vulgaris* L. , phytotoxicity, tolerance index, vigour index.

## INTRODUCTION

Haricot bean (*Phaseolus vulgaris* L.) is the second most important grain legume cultivated as cash crop in Ethiopia (CSA, 2011) and is mainly produced in the rift valley area of the country characterized by high industriliztion and urbanization. The use of pesticides in the production of this crop is most common, as the crop is raised both at small holder level and on commercial scale. Copper sulphate is generally used as a fertilizer or fungicide, by farmers to prevent fungal diseases. Copper is a heavy metal (Dharam *et* al., 2007; Jay et al., 2011; Hema et al., 2013) and has been reported to possess both negative and positive effects on plants. The status of native copper concentration in the soil, together with the frequency of application and quantity of copper sulfate applied, will determine the effects of copper sulfate on plants (Saravanan et al., 2001). With the rapid industrialization, urbanization and intensive agriculture, increasing contamination of heavy metals like copper in soil has become a major concern. This could be due to mining and metal production, electric goods, kitchenware, alloys, pestirides and pigments, insecticides, fungicides, ice-makers, industrial emissions, copper IUCDs, and phosphate fertilizer production; which find its way to agricultural soils and water bodies through the disposal of copper containing waste water, sludge and combustion of fossil fuel. This situation however, is given less consideration in most developing countries. Copper is an

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essential micronutrient for plants, but its excess use results in metabolic disorders and growth inhibition in most plants (Gupta et al., 2001). Excessive level of copper metals in the soil environment adversely affects the germination of seeds, plant growth, alter the level of biomolecules in the cells and interfere with the activities of many key enzymes related to normal metabolic and developmental processes (Rahoui et al., 2010). Hyper accumulation of heavy metals can lead to toxic effects in human, animal, plant and other microorganisms (Tkaczuk, 2005). Excess concentration of copper can cause disruption of natural aquatic and terrestrial ecosystems (Meagher, 2000). Copper apparently accumulates in roots, and cause damage by toxicity (Adriano, 1986). The negative effects of copper on plants and animals contributed much to create an interest of scientists to focus on heavy metals research particularly to its toxicological importance (Azevedo and Lea, 2005).

As of now, limited information is available on the phytotoxic effect of copper sulphate on haricot bean cultivars under Ethiopian conditions. Hence, this study was undertaken with a view to investigate the effect of copper on seed germination, and seedling growth of *P. vulgaris* L .

## MATERIALS AND METHODS

To evaluate the effect of graded levels of copper sulphate on germination and early growth of haricot bean, a factorial experiment was conducted in 2013 in Complete Randomized Design, with four replications in Plant Sciences and Horticulture laboratory, Ambo University. Three haricot bean cultivars viz., *Atendaba*, *Argene* and *Nassier* were treated with four levels of copper sulphate solutions ( 0.5, 1.0, 1.5, 2.0 mM), besides deionized water as a control. The seeds of varieties under test were sterilized with 5% sodium hypochlorate solution for 10 min to avoid fungal contamination, then the seeds were washed with deionized water to proceed with germination study.

Twenty seeds were placed per petridish uniformly and covered with lid to prevent loss of moisture through evaporation. The treatments were applied every 72 h starting from 1$^{st}$ day, and seeds were allowed to germinate for 10 days at room temperature. The germination percentage was recorded on the 10th day. Germination was considered to have occurred when radicles attained a length of 2 mm. Germination percentage was calculated according to ISTA (1999):

$$\text{Germination percentage} = \frac{\text{No. of germinated seeds}}{\text{Total No. of seeds sown}} \times 100$$

On the 10th day, 10 seedlings in each Petri plate were sampled to measure the root length and shoot length using a scale. Shoot and root fresh weights of the same plants were measured using sensitive balance, and root and shoot dry weights were recorded after oven drying for 72 h at 50°C.

The seedling vigor index (SVI) was determined as per Hosseein and Kasra (2011) as shown below:

Seedling Vigor Index = % of Germination × Seedling dry weight (g)

Tolerance index (T.I.) was determined as per Iqbal and Rahmati (1992) method:

T.I. = (Mean root length in metal solution / Mean root length in distilled water) × 100

The percentage of phytotoxicity on shoot and root of seedlings was calculated following the formula given by Chou and Lin (1976):

$$\text{Phytotoxicity of Shoot (\%)} = \frac{\text{Shoot length of control - Shoot length of treatment}}{\text{Shoot length of control}} \times 100$$

$$\text{Phytotoxicity of Root (\%)} = \frac{\text{Root length of control - Root length of treatment}}{\text{Root length of control}} \times 100$$

Statistical analysis of the data was performed using one-way ANOVA by SAS statistical software (Version 9). Based on ANOVA, results mean separations were performed by Duncan's multiple range test at 5% level.

## RESULTS AND DISCUSSION

### Percent germination, and shoot and root growth

The results revealed that there was no significant interaction between cultivars and copper sulphate concentrations in all the parameters measured. The germination percentage revealed significat difference ($p<0.05$) between cultivars. The highest germination percentage (98.33) was recorded for *Argene* cultivar, followed by *Atendaba* (95.83%) and *Nassier* (88.33%), demonstrating the existance of genetic variability among cultivars. Similar finding has been reported by Souguir et al. (2008) who investigated the potential genotoxicity of $Cu^{+2}$ in *Vicia faba* and *Pisum sativum*; and Stoinova et

**Table 1.** Effect of copper sulphate on germination percentage and seedling growth of haricot bean cultivars.

| Cultivars | Germination (%) | Shoot length (cm) | Root length (cm) | Shoot fresh weight (g) | Root fresh weight (g) | Shoot dry weight (g) | Root dry weight (g) | Seedling weight (g) |
|---|---|---|---|---|---|---|---|---|
| Atendaba | 95.83 ab | 8.95 c | 2.69 b | 0.553 b | 0.157 ab | 0.050 a | 0.0198 a | 0.0802 a |
| Argene | 98.33 a | 14.20 a | 3.38 ab | 0.605 ab | 0.154 b | 0.059 a | 0.0188 b | 0.0725 b |
| Nassier | 88.33 b | 12.74 b | 4.13 a | 0.625 a | 0.162 a | 0.062 a | 0.0199 a | 0.0814 a |
| **SE(m) ±** | 10.03 | 1.01 | 1.65 | 0.007 | 0.001 | 0.0007 | 0.0004 | 0.0001 |

Means with similar letters in each column are not significant at 5% level of probability.

al. (2007) who reported the existance of genetic variability among barley cultivars for copper ion stress.

The analysis of varience indicated that cultivars differed significantly in shoot and root lengths, shoot and root fresh weights, root dry weight , and seedling dry weight, though there was no significant difference in shoot dry weight. The highest shoot length (14.2 cm) was recorded for *Argene* cultivar, while the lowest shoot length (8.85 cm) was for *Atendaba* cultivar. The highest value of root length, shoot and root fresh weight, root dry weight, and seedling dry weight were obtained in *Nassier* cultivar (Table 1). Though, *Nassier* cultivar has low germination percentage compared with *Argene* cultivar, it has the highest root length which has a profound influence on stress tolerance. Houshmandfar and Moraghebi (2011) reported that cultivar tolerant to heavy metal stress have larger root length, and other related growth traits. Therefore, *Nassier* cultivar, with higher root length could show greater tolerance to hyper copper stress.

Haricot bean cultivars did not exhibit differences in seedling vigour index, shoot vigour index, tolerance index, and root phytotoxicity (Figures 1 and 2). *Nassier* cultivar gave significantly higher root vigour index compared to *Atendaba* and *Argene* cultivars. Besides, *Nassier* also showed relatively high tolerance index, and low shoot and root phytotoxicity. The phytotoxicity of shoot varied with cultivars, and the highest (25.4%) was recorded for *Atendaba*, and the least for *Nassier* (18.78%). Nevertheless, there was no significant difference ($p<0.05$) among cultivars for phytotoxicity of roots. The cultivar *Nassier* had relatively low phytotoxicity of roots, that described as it was better in tolerating hyper copper concentration, than *Atendaba* and *Argene* cultivars.

## Germination percentage and early seedling growth

There was no significant variation in germination percentage at different levels of copper concentration (Table 2). As the concentration of copper sulphate increased from control to 1 mM, germination percentage increased from 93.03 to 95.83%. Low concentration of copper has growth promotary effect, while higher concentrations have germination inhibitory effect (Hema et al., 2013). Similarly, Sharma et al. (2000) reported that low concentration of copper has stimulatory effect on plant growth, while higher concentrations were inhibitory and toxic to plant growth. Our finding is in agreement with Jay et al. (2011) who reported that germination percentage of mung bean was not significantly affected with different levels of cupper sulphate; and Morteza (2011) who found non-significant differences with different levels of copper sulphate on germination of *Agropyron elongatum*. Liu et al. (2009) reported that the lowest concentrations of copper were the least harmful to the germination of seeds, while with increasing concentrations of the solutions the germination of the seeds declined.

The shoot and root lengths, shoot fresh and dry weights, root fresh and dry weights, and seedling dry weight decreased significantly with increasing copper sulphate concentrations. The highest shoot length (14.87 cm) and root length (8.16 cm) were obtained in control, and the lowest shoot length (9.47 cm) and root length (1.60 cm) were with 2 mM concentration of copper sulphate. These findings are in line with those of Jay et al. (2011) on mung bean cultivar, and Dharam et al. (2007) on wheat who reported a reduction of plumule and radicle length with increase in copper sulphate concentration. The reduction in the shoot length could be due to excess accumulation of copper salt in the cell wall, which modifies the metabolic activities and limits the cell wall elasticity (Naseer, 2001). However, an increase in the levels of heavy metals like copper in root environment can cause reduction in absorption of water and nutrients, prevention of enzyme activities, reduction of cell metabolism, and cessation of growth, acceleration of senescence and even mortality of plant (Souguir et al., 2008; Peralta et al., 2000; Cheng and Huang, 2006).

Shoot and root fresh weights, shoot and root dry weights, and seedling dry weight decreased significantly with increase in copper sulphate concentration as compared to control. Dharam et al. (2007) also reported that fresh weight, and dry weight of seedlings decreased with increasing copper concentration in wheat.

## Seedling vigour, tolerance and phytotoxicity

It was observed that copper sulphate concentration have a significant effect on seedling vigour index, shoot and

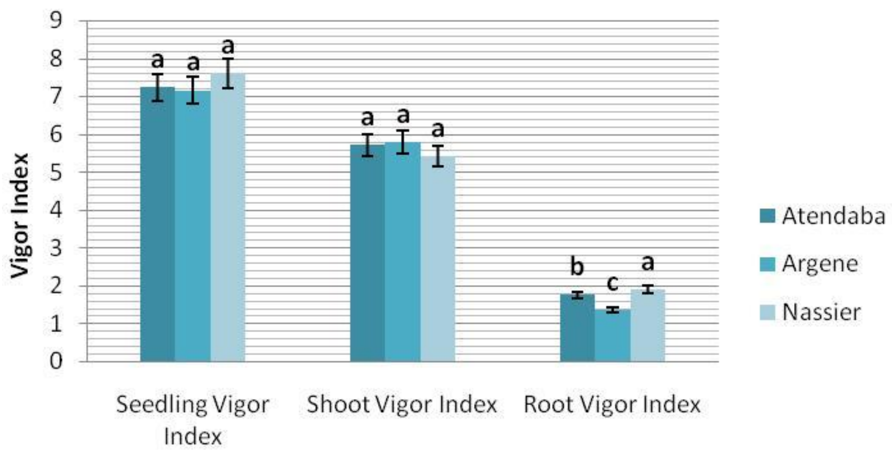


**Figure 1.** Cultivar vigor index.

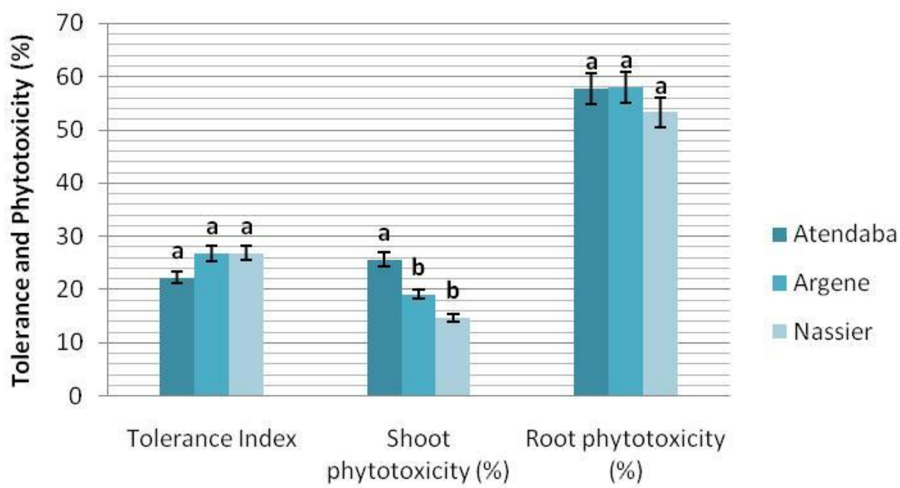


**Figure 2.** Cultilvar tolerance index and phytotoxicity percentage.

**Table 2.** Mean effect of copper sulphate on germination and seedling growth of haricotbean.

| Copper sulphate (mM) | Germination (%) | Shoot length (cm) | Root length (cm) | Shoot fresh weight (g) | Root fresh weight (g) | Shoot dry weight (g) | Root dry weight (g) | Seedling weight (g) |
|---|---|---|---|---|---|---|---|---|
| Control | 93.06 a | 14.87 a | 8.16 a | 0.788 a | 0.218 a | 0.080 a | 0.0252 a | 0.1053 a |
| 0.5 | 94.4 a | 13.35 b | 3.13 b | 0.647 b | 0.169 b | 0.068 b | 0.0192 b | 0.0877 b |
| 1 | 95.83 a | 11.98 c | 2.34 bc | 0.575 bc | 0.131 c | 0.058 c | 0.0169 c | 0.0745 c |
| 1.5 | 94.44 a | 10.11 d | 1.77 c | 0.511 cd | 0.130 c | 0.051 cd | 0.0153 c | 0.0659 d |
| 2 | 93.06 a | 9.47 d | 1.60 c | 0.451 d | 0.108 c | 0.044 d | 0.0127 d | 0.0566 e |
| SE(m)± | 10.03 | 1.01 | 1.28 | 0.09 | 0.03 | 0.01 | 0.002 | 0.0001 |

Means with similar letters in each column are not significant at 5% level of probability.

root vigour indices, and tolerance index (Figures 3 and 4). The highest value for each trait was noticed on control treatment. An increase in concentrations of copper sulphate progressively decreased vigour indices and tolerance index as compared to control. The result of this finding was in agreement with Isak (2013) on wheat.

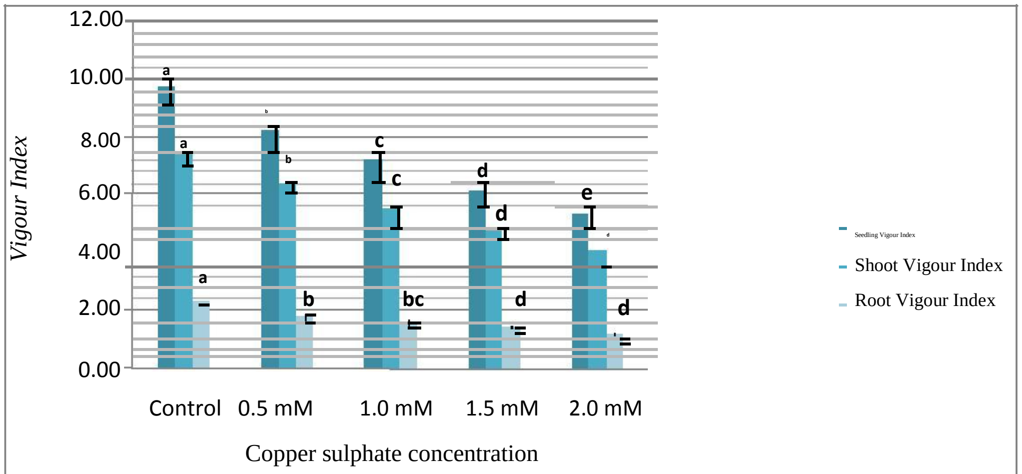


**Figure 3.** Effect of copper sulphate concentration on Vigour index of *Phaseolus vulgaris L.*

Copper sulphate concentration

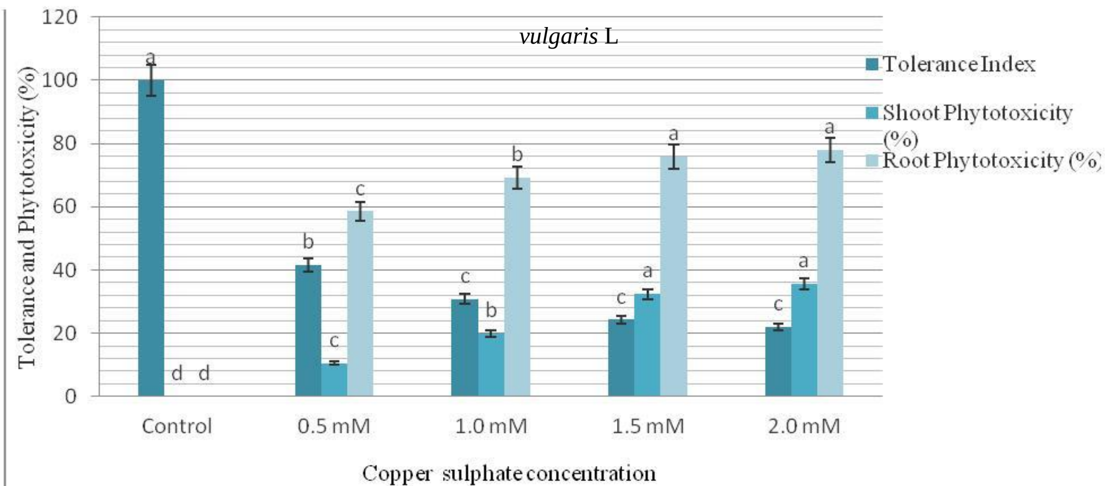


**Figure 4.** Effect of copper sulphate concentration on tolerance index and phytotoxicity of *Phaseolus vulgaris L.*

The phytotoxicity on shoot and root increased as the copper concentration increased. The lower shoot and root phytotoxicity was recorded with control treatment and lower concentration (0.5 mM), whereas it increased at higher concentration (2 mM). The increase in the concentration of copper significantly affect the phytotoxicity percent of both shoot and root of haricot bean. The highest phytotoxixity in shoot (35.6%) and root (77.87%) were recorded with 2 mM. Recently, Isak (2013) reported that root and shoot phytotoxicity reduced at lower concentration, and increased at higher concentration on wheat.

## Conclusion

Haricot bean cultivars varied in their germination percentage and tolerance to different concentrations of copper sulphate. Cultivar *Nassier* exhibited copper tolerance at seedling growth stages compared to other genotypes, though it has low germination percentage. An increase in copper sulphate concentration has inhibitory effect on different growth parameters of haricot bean.

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