

Impact of Copper on Germination, Growth and Biochemical Change in Blackgram (*Vigna mungo* (L.) Hepper)

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ABSTRACT

Pollution is an undesirable change in physical, chemical and biological characteristic of our environment that will harmfully affect the human life and living organisms. Environment is defined as the total circumstances surrounding an organism, or group of organism especially the combination of external physical, conditions that affect and influence the growth, development and survival of organism. The environment is the sum total of biotic and abiotic conditions influencing the response of organisms. Copper is widely prevalent in our environment and was considered as an essential element for all living organisms including plants. The present investigation has been carried out to assess the Impact of copper on seed germination and biochemical changes of *Vigna mungo* (L.) Hepper. For this purpose; best cultivars were procured and healthy seeds were collected and surface sterilized before sowing. Seedlings were raised in petri plates lined with filter paper. The seedlings were irrigated with respective concentration of copper (0, 5, 10, 25, 50, 100, 200, 250, 500, 1000 mg l⁻¹). As the result of germination studies it indicate that when there was a progressive increase in copper level there was a gradual decline in rate of germination, seed germination index and root tolerance index value when compared to control. The seedling growth parameters also showed the same level. The copper concentration above 100 mg l⁻¹ (200, 250, 500 and 1000 mg l⁻¹) proved to be lethal to the crop. Based on the results of screening test of cultivars, the cultivar VBN-8 proved to be more tolerant and VBN-3 showed poor seed germination and growth characteristic under copper exposure.

Key words: Biochemical changes, Blackgram (*Vigna mungo*), Copper, Cultivar and Seed germination.

INTRODUCTION

The contamination of the large areas of agronomic soils by heavy metals appears to increase plant uptake causing to their bioaccumulation affinity and toxicity leading to enormous losses in plant productivity and harmful health (Dermirevska-Kepova et al. 2004, Younis et al. 2018). Heavy metals are one of the environment contaminants that are increasing the rate of pollution (Benavides et al. 2005). Heavy metal pollution has become very acute and is considered to be hazardous (Järup 2013). The extreme level of heavy metals in the soil environment unfavourably affect the germination of seeds, growth of plant, alter the level of biomolecules in the cells and affect with the activities of many key enzymes associated to normal metabolic and developmental progressions (Kuriakosa and Prasad 2008, Kachout et al. 2009, Rahoui et al. 2010). The effect of stress differs with its strength, period and phenophases of crop. In the

majority of germplasm assessment studies for drought tolerance, seed yield is the major criteria for changes of biochemical in crops (Baroowa et al. 2016). Heavy metals are naturally present in the environment at different levels in soil, water and air which cause pollution after reaching certain concentration (Heidari and Sarin 2011). Heavy metal accumulation in plants leads to loss of agricultural yields which result in hazardous effects. Heavy metal stress in plant have an adverse effect on growth and development of the plant by showing some physiological and biochemical damage (Liu et al. 1994, Du et al. 2004). Plants can use metals in mineral form only because as there is no direct metal available to plants (Appenroth 2009). The micro nutrients are indispensable for biogenesis, proper functioning of nucleic acid and chlorophyll as well as stress resistance. Some heavy metal plays an important role in plant cell as micronutrient, while others have stimulating provoking effects on plants

even in low concentration (Kovacs 2009).

The current understanding of the more important features concerning copper toxicity and tolerance in plants, and brings information of recent findings on copper trafficking including copper detoxification factors, copper transporters and copper chaperones (Yruela 2005). Copper (Cu) is an essential redox-active transition metal that is involved in many physiological processes in plants because it can exist in multiple oxidation states in-vivo. Under physiological conditions Copper (Cu) exists as Cu^{2+} and Cu^+ . Copper acts as a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signaling (Marschner 1995, Raven et al. 1999). Copper functions as structural and catalytic compound for several enzymes and protein. Excess copper absorbed by plant leading to reduce growth, chlorosis and damage to plasma membrane permeability, leading to iron leakage. Excess copper induces a wide range of biochemical effect and metabolic disturbances in plants, which are responsible for a strong inhibition of growth. Copper concentration in the environment is seriously low (20^{-3} ppm) in the contaminated soil and sediments less (<2 ppm) in natural water (Baccouch 1998). Copper plays a key role in many metabolic mechanisms but it can be toxic when the copper (Cu) content in tissues is slightly higher than its optimal levels. Some plant species have capacity to grow in the metal contaminated soil and accumulate elevated amount of heavy metals (hyper-accumulation) as an eco-physiological adaptation in metaliferous soil (Singh et al. 2008). Blackgram was able to sustain homeostasis at lower concentrations of Cr by triggering the cascade of enzymes following cellular detoxification mechanism (Rath and Das 2021). All the heavy metals are potentially lethal at high concentrations (Mohaptra et al. 2021). For healthier accepting towards the tempted heavy metal stress, copper (Cu) element was selected for usage in the present study.

Keeping in view, this present experiment was designed to study the impact of copper toxicity on the seedling growth and biochemical changes of blackgram (*Vigna mungo* (L.) Hepper). The research experiment comprises of laboratory level and the

seed germination studies were performed. A varietal screening test was carried out, such as: (i) To find the tolerant and sensitive cultivar of black gram under copper treatments, (ii) To determine the lethal dose of copper on germination and seedling growth of black gram, (iii) To record the morphological parameters, (iv) To determine the extent of copper induced morphological changes, and (v) To analyses the biochemical constituent of Black gram seedling under different concentration of copper.

MATERIAL AND METHODS

The present studies have been carried out to evaluate the Impact of copper on seed germination and biochemical changes of *Vigna mungo* (L.) Hepper. The research experiment comprises of laboratory studies. The germination studies were performed in the Post Graduate Research Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India. The certified seeds of black gram cultivars viz., VBN-3, VBN-4, VBN-5, VBN-8, were obtained from, the National Pulses Research Institute, Vamban of Pudukottai District and Regional Rice Institute, Aaduthurai of Thanjavur District in Tamilnadu. The seeds of uniform size, colour and weight were choosen for the experiment and stored in metal tins as suggested by (Rao 1976). Copper salt were dissolved in distilled water to obtain various concentration of copper (0, 5, 10, 25, 50, 100, 200, 250, 500, 1000 mg l^{-1}).

The germination studies were carried out according to the Top Paper method recommended by International Seed Testing Association (1976). Four cultivars were selected and surface sterilizes with 0.1% mercuric chloride for two minutes. The seed were washed thoroughly for 5 to 10 minutes under running water to avoid surface contamination. Twenty five seeds of each cultivar were equispacedly arranged in each petri plates lined with filter paper. An equal volume of various concentration of copper solution (viz., 0, 5, 10, 25, 50, 100, 200, 250, 500, 1000 mg l^{-1}) were irrigated into respective plates. Seeds germinated in distilled water (0 mg l^{-1}) served as control. The metal concentration above 100 mg l^{-1} was proved to be lethal. Five replicates were maintained for each concentration including control and for all the four cultivars. The plastics plates were

incubated in Yorco seed germinator at 28°C. The number of seeds germinated in each treatment and control were counted daily up to the 10th day and the germination percentage was calculated. The protrusion of radical through the seed coat was taken as criterion for germination. The various germination characteristics of black gram cultivars like germination percentage and seed germination index were recorded on the 10th day of the treatment suggested by Chou et al. (1978). For the measurement of seedling growth five replicates from each concentration were selected at random. Various morphological parameters like shoot length, root length and number of lateral roots were recorded on the 10th day of the treatment. For measurement of seedling growth, five seedlings from each plate were selected at random including the control. The germination percentage rate was calculated by using the formulae proposed by Abdul-Baki and Anderson (1973). Vigour index of the seedlings were calculated by using the formulae proposed by Abdul-Baki and Anderson (1973). Seed Germination Index was calculated by using the formula given by Carley and Watson (1968). The tolerance index of the seedlings can also be calculated by using the formulae proposed by Turner and Marshal (1972). Beside the morphological parameters certain biochemical constituents such as Chlorophyll a and b, Carotenoids and Proteins activity was analyzed following Arnon (1949), Carotenoids by Kirk and Allen (1965) and Protein content by Lowry et al. (1951) methods.

Statistical analysis

Experiment was conducted with three replications and six treatments. Statistical analysis was conducted using one-way analysis of variance (ANOVA) using SPSS 12.0 software. Comparisons of means were performed by the Fisher's Protected LSD test at $p \leq 0.05$.

RESULTS

Blackgram (*Vigna mungo* (L.) Hepper) belongs to the family Leguminosae (COPR 1981). It is the major pulses crop cultivated in many parts of India which is commonly used for preparation of various foods. Black gram protein gives 340 calories per 100 g of dry weight. It contains 20-25% more protein compared to cereals. It is a good source of fiber in

addition it contains hemicellulose (43-50%), cellulose (35-76%), lignin (15-07%), cutin (5-24%) and silica (2-83%). They also provide energy, essential, mineral, vitamins, and several compounds consumed beneficial for good health. Due to the growing demand of black gram, it is selected due to resistant adverse climatic condition to improve the soil fertility (Aykroyd and Poughty 1964, Molly Thomas 1990, Tiwari and Singh 2012, Singh 2017).

Seed germination rate

Seed germination percentage was found to be maximum at (100%) in VBN-8 cultivars at 5 mg l⁻¹ of copper concentration (Table 1). The minimum germination percentage was found (35%) in VBN-3 cultivars at 100 mg l⁻¹ concentration of copper treatment, respectively. The maximum percentage of reduction over control value was observed (50%) in VBN-3 cultivars at 100 mg l⁻¹ concentration of copper treatment and the minimum value was noted (5.26%) at 5 mg l⁻¹ concentration of copper treatment in VBN-8 cultivars.

Germination index

The Germination Index of black gram cultivars under various treatments was given in Table 2. The highest germination index (855) was found in VBN-8 cultivar at 5 mg l⁻¹ of copper concentration. The lowest (362) was in VBN-3 at 100 mg l⁻¹ concentration of copper treatment. The maximum percentage of reduction over control was recorded (48.14%) in VBN-3 cultivar at 100 mg l⁻¹ copper treatment and the minimum (4.12%) at 10 mg l⁻¹ in VBN-4 cultivar. The variance between the cultivars and the concentrations were found to be highly significant.

Root tolerance index

Highest root tolerance index value (1.160) at 10 mg l⁻¹ copper was in VBN-8 cultivar and lowest (0.562) at 100 mg l⁻¹ concentration in VBN-3 cultivar (Table 3). The F – test value calculated for the variance between the cultivars and the concentration were found to highly significant ($p < 0.1$).

Morphological studies

Root length (cm plant⁻¹)

The seedling growth parameters like root, shoot

Table 1. Germination rate of blackgram at different exposure of copper.

Ca (mg l ⁻¹)	Cultivars			
	VBN -3	VBN -4	VBN - 5	VBN - 8
0	70	90	80	95
5	75(+7.14)	95(+5.56)	85(+6.25)	100(+5.26)
10	65(-7.14)	85(-5.56)	75(-6.25)	90(-5.26)
25	60(-14.29)	75(-16.67)	70(-12.50)	80(-15.79)
50	50(- 28.57)	65(-27.78)	60(-25.60)	70(-24.32)
100	35(-50.00)	50(-44.44)	45(-43.75)	85(-42.11)

F value for the variance between cultivars **536.37** **

F value for the variance between concentration **147.78** **

0 mg l⁻¹ control

Percentage of reduction over control values are given in parentheses (+ / -)

** Significant at 1 per cent level

Table 2. Germination index of blackgram as affected by copper treatments

Ca (mg l ⁻¹)	Cultivars			
	VBN -3	VBN -4	VBN - 5	VBN - 8
0	698	801	790	832
5	730(+4.58)	825(+3.00)	810(+2.53)	855(+2.76)
10	654(-15.90)	768(-4.12)	742(-6.80)	790(-5.05)
25	587(-15.90)	653(-18.48)	626(-20.76)	686(-17.55)
50	435(-37.68)	545(-31.96)	505(-36.08)	592(-28.85)
100	362(-48.14)	472(-41.07)	445(-43.67)	502(-39.66)

F value for the variance between cultivars **1025.97** **

F value for the variance between concentration **101.98** **

0 mg l⁻¹ control

Percentage of reduction over control values are given in parentheses (+ / -)

** Significant at 1 per cent level

length and numbers of lateral roots were recorded. The Root length of black gram cultivars was recorded in the (Table 4). The maximum value of root length were recorded in VBN-8 cultivars (6.10) at 5 mg l⁻¹ concentration of copper and lowest value (1.69) was found in 100 mg l⁻¹ concentration of copper treatment in VBN-3 cultivars. The maximum percentage of reduction over control value was found (40.91%) in 100 mg l⁻¹ copper concentration in VBN-3 cultivars and the maximum value was observed (4.75%) 10 mg l⁻¹ copper concentration in VBN-8 cultivars. The F- test value calculated for the variance between

Table 3. Root tolerance index of blackgram under copper treatment

Ca (mg l ⁻¹)	Cultivars			
	VBN -3	VBN -4	VBN - 5	VBN - 8
0	-	-	-	-
5	1.099	1.140	1.123	1.160
10	0.933	0.952	0.951	0.963
25	0.852	0.875	0.863	0.884
50	0.704	0.783	0.755	0.800
100	0.562	0.659	0.608	0.662

F value for the variance between cultivars **712. 18****

F value for the variance between concentrations **12. 44****

0 mg l⁻¹ control

Percentage of reduction over control values are given in parentheses (+ / -)

** Significant at 1 per cent level

Table 4. Root length (cm plant⁻¹) of blackgram cultivars under different exposure of copper

Ca (mg l ⁻¹)	Cultivars			
	VBN -3	VBN -4	VBN - 5	VBN - 8
0	2.86	4.46	3.72	5.26
5	3.20(+11.89)	5.01(+12.33)	4.24(+13.19)	6.10(+15.97)
10	2.72(-4.90)	4.16(-6.73)	3.54(-4.84)	5.10(-4.75)
25	2.50(-12.59)	3.80(-14.80)	3.12(-13.71)	4.65(-11.60)
50	2.24(-21.68)	3.21(-28.03)	2.81(-24.46)	4.21(-19.96)
100	1.69(-40.91)	2.71(-39.24)	2.45(-34.14)	3.48(-33.84)

F value for the variance between cultivars **87.79** **

F value for the variance between concentration **113.48** **

0 mg l⁻¹ control

Percentage of reduction over control values are given in parentheses (+ / -)

** Significant at 1 per cent level

cultivars and the concentration were found to be highly significant (p < 0.01).

Number of lateral roots (cm plant⁻¹)

Highest number of lateral roots was recorded in VBN-8 cultivars (21.48) at 5 mg l⁻¹ concentration of copper and the lowest number of lateral roots observed in VBN-3 (3.18) at 100 mg l⁻¹ concentration of copper treatment (Table 5). The maximum percentage of reduction over control value was found in VBN-8 (74.10%) at 100 mg l⁻¹ and the minimum reduction (12.70%) at 10 mg l⁻¹ concentration of copper exposure in VBN-3 cultivars.

Shoot length (cm plant⁻¹)

Longest shoot length was recorded in VBN-8 cultivars (11.02) at 5 mg l⁻¹ concentration of copper and the lowest shoot length observed in VBN-3 (5.02) at 100 mg l⁻¹ concentration of copper treatment (Table 6). Maximum percentage of reduction over control was found in VBN-8 (36.12%) at 100 mg l⁻¹ and the minimum reduction value was recorded (3.94%) at 10 mg l⁻¹ concentration of copper exposure in VBN-3 cultivars.

Biochemical constituents*Photosynthetic pigments (mg g⁻¹ fresh weight)*

The effect of different concentration of copper on the photosynthetic pigments contents of black gram seedlings was given in Table 7. Chlorophyll a, chlorophyll b, total chlorophyll viz (0.748, 0.656 and 1.404, respectively) and carotenoids (0.472) contents were recorded maximum at 0 mg l⁻¹ concentration in VBN-8 cultivar. The lowest values (0.454, 0.440 and 0.301, respectively) were recorded at 100 mg l⁻¹ concentration in VBN-3 cultivars. The highest percentage of reduction over control value for chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content was observed (36.50, 31.02 and 33.26 %, respectively) at 100 mg l⁻¹ concentration in VBN-3 cultivar. The F-Test values calculated for the variance between genotypes and between concentrations were found to be highly significant.

Protein (mg g⁻¹ fresh weight)

The effect of different concentration of copper on the protein content of the root, stem and leaf of black gram cultivars is given in the (Table 8). The highest protein of groundnut was higher in root (2.224), stem (3.81) and leaf (4.499) at 0 mg l⁻¹ in VBN-8. The lowest protein content of root (1.024), stem (2.33) and leaf (2.433) was observed in VBN-3 cultivars at 100 mg l⁻¹ copper concentration. The maximum percentage of reduction over control value was observed for root, stem and leaf (47.16%, 3.94% and 41.85%) in VBN-3 cultivar at 100 mg l⁻¹ concentration. The maximum percentage of reduction over control value was observed for root, stem and leaf (8.00%, 9.10% and 7.29%) at 10 mg l⁻¹ concentration in VBN-8 cultivars. The F-test values calculated for the variance between the cultivars and the treatments were significant at 1 per cent level.

Table 5. Number of lateral roots (plant⁻¹) of blackgram under copper treatment

Ca (mg l ⁻¹)	Cultivars			
	VBN -3	VBN -4	VBN - 5	VBN - 8
0	12.28	17.58	16.50	19.72
5	13.59(+10.26)	18.56(+5.57)	17.48(+5.94)	21.48(+8.92)
10	10.72(-12.70)	15.28(-13.08)	14.40(-12.73)	18.52(-6.09)
25	7.50(-38.93)	12.10(-31.17)	11.70(-29.09)	15.50(-21.40)
50	5.20(-57.65)	9.78(-44.37)	8.50(-48.48)	12.25(-37.88)
100	3.18(-74.10)	6.32(-64.05)	5.00(-66.06)	8.78(-55.48)

F value for the variance between cultivars **553.21 ****F value for the variance between concentrations **169.04 ****0 mg l⁻¹ control

Percentage of reduction over control values are given in parentheses (+ / -)

** Significant at 1 per cent level

Table 6. Shoot length (cm plant⁻¹) of blackgram cultivars under different exposure of copper

Ca (mg l ⁻¹)	Cultivars			
	VBN -3	VBN -4	VBN - 5	VBN - 8
0	7.86	9.22	8.80	10.52
5	8.25(+4.96)	9.78(+6.07)	9.20(+4.55)	11.02(+4.75)
10	7.55(-3.94)	8.89(-3.58)	8.51(-3.30)	10.12(-3.80)
25	7.20(-8.40)	8.30(-9.98)	8.02(-8.86)	9.50(-9.70)
50	6.50(-17.30)	7.42(19.52)	7.10(-19.32)	8.72(-17.11)
100	5.02(-36.12)	6.35(-31.13)	6.25(-28.98)	7.30(-30.61)

F value for the variance between cultivars **286.70 ****F value for the variance between concentrations **138.20****0 mg l⁻¹ control

Percentage of reduction over control values are given in parentheses (+ / -)

** Significant at 1 per cent level

DISCUSSION

The pollution is the contamination of earth's environment with material that interferes with human health, the quality of life or the natural functioning of ecosystems (Förstner and Wittmann 2012). Heavy metals are of the great interest for research purpose with admiration to toxicological prominence to human health, animals and plants (Almeida et al. 2007, Azevedo and Lea 2005). Legume seeds from the ample source of protein and other rich nutrients compounds, such as triterpenoid, inositol phosphates, isoflavones and saponins. Commonly, legumes are

Table 7. Photosynthetic pigments (mg g⁻¹ fresh weight) of blackgram cultivars as affected by copper exposure

Ca (mg l ⁻¹)	VBN - 3				VBN - 8			
	Chl a	Chl b	Total Chl	Carotene	Chl a	Chl b	Total Chl	Carotene
0	0.715	0.640	1.355	0.451	0.748	0.656	0.404	0.472
10	0.682(4.61)	0.602(5.94)	1.264(6.71)	0.420(6.867)	0.715(4.41)	0.620(5.49)	1.325(5.63)	0.445(5.72)
25	0.625(12.59)	0.556(13.12)	1.181(12.84)	0.385(14.63)	0.642(14.17)	0.578(11.89)	1.220(13.10)	0.406(13.98)
50	0.512(28.39)	0.502(21.56)	1.014(25.17)	0.342(24.17)	0.530(29.14)	0.515(21.49)	1.045(25.57)	0.375(20.56)
100	0.454(36.50)	0.440(31.25)	0.894(-34.02)	0.301(33.26)	0.478(36.10)	0.468(28.66)	10.946(32.62)	0.340(27.97)

F value for the variance between cultivars F_1 38.181** F_2 360.294** F_3 3379.98** F_4 737.766**

F value for the variance between concentrations F_1 8.116** F_2 16.72** F_3 146.64** F_4 80.363**

F_1 (chl.a) – chlorophyll.a; F_2 (chl.b) – chlorophyll. b; F_3 (Total chl); F_4 Carotene (carotenoids)

0 mg l⁻¹ control

** Significant at 1 per cent level

Table 8. Copper exposure on protein content (mg g⁻¹ fresh weight) of blackgram seedlings

Ca (mg l ⁻¹)	VBN-3			VBN-8		
	Root	Stem	Leaf	Root	Stem	Leaf
0	1.938	3.700	4.184	2.224	3.811	4.499
10	1.744(10.01)	3.340(9.73)	3.729(10.87)	3.046(8.00)	3.464(9.10)	4.171(7.29)
25	1.511(22.03)	2.976(19.57)	3.308(20.93)	1.898(14.66)	3.118(18.18)	3.826(14.96)
50	1.312(32.30)	2.699(27.05)	2.816(32.70)	1.676(24.64)	2.835(25.61)	3.468(22.92)
100	1.024(47.16)	2.333(36.94)	2.433(41.85)	1.317(40.78)	2.512(34.08)	3.082(31.49)

F value for the variance between cultivars F_1 240.81** F_2 709.41** F_3 77.07**

F value for the variance between concentrations F_1 255.94** F_2 54.36** F_3 64.69 **

0 mg l⁻¹ control

** Significant at 1 per cent level

grown-up in marginal agricultural lands which are prone to climatic variations. The value addition to the seed through seed improvement techniques is necessary (Zhung et al. 2019). The enhancement in quality and vigour of the seed by seed improvement is stood mainly to stability in the metabolic functions, rapid hydrolysis of reserve food substantial through germination for efficient energy source, maintenance of the seed coat membrane reliability, activating of antioxidant mediated protection response to conquering pathogens and developing inherent resistance to served abiotic and biotic traits that are deterring crop growth. The study focused on the up gradation of seed quality through different seed enhancement techniques specifically for legume crops (Channappagoudar et al. 2022). Black gram is an significant nutritious legume plant, the loess plateau with robust drought tolerance and extensive adaptability. To understand the adaptation mechanism of black gram under imitation levels of

drought stress, and the growth appearances, physiological parameters including of chlorophyll content, reactive oxygen species, and antioxidant enzymes were dignified and the changes were paralleled with control (Jothimani and Arulbalachandran 2020). The plant *Vigna mungo* seedling are in the progression of rise in tolerance to mercuric chloride management (Mohaptra et al. 2021). The response of the drought stress on morphological, physiological and biochemical characters was evaluated in blackgram genotypes in a pot culture experiment showed that the photosynthesis, transpiration rate, stomatal conductance, peroxidase activity and proline content could be beneficial to screen for drought tolerance in the blackgram (Gurumurthy et al. 2019). To the heat stress coupled with the moisture stress through the reproductive phase of the late sown black gram crop obliged it to complete the phenophases to some extent previous than the normal sown one. The stress

prompted reduction in yield owing to controlled photosynthetic activity and nitrogen assimilation were evident from abridged chlorophyll contents and nitrate reductase activity (Banerjee et al. 2021). Effect of deleterious of Copper and Zinc provided either individually or mixture in the nutrient media was examined in order to evaluate the effect of metal interaction in *Vigna mungo* (L.). The combined effect of Copper and Zinc metal was more noticeable in comparison to their individual effects (Solanki et al. 2011). The Copper have time dose dependent on safflower seedlings (Gautam et al. 2016). The effect of copper as a micronutrient for enhanced plant growth, protein content and antioxidant enzyme activity of mungbean (*Vigna radiate*) under the influence of different concentration of copper was reported by Verma et al. (2011).

The effect of copper as a micronutrient for enhanced plant growth, protein content and antioxidant enzyme activity of mungbean (*Vigna radiata*) under the influence of different concentrations of copper which promoted the seedlings growth at less than 50 μM concentration (Verma et al. 2011). The maximum seed germination, morphological study and biochemical content in maximum tolerant variety (Tindivanam - 9) and susceptible variety (VBN-5) under different concentration of chromium treatment. The control seeds showed a maximum effect in all the growth parameters. Chromium induced in higher concentration treatment showed minimum effect in all the varieties. The optimum concentration showed a maximum effect in all the variety, especially T-9 variety more effectively in all the parameters. The biochemical studies like chlorophyll-a, chlorophyll-b and total chlorophyll content protein and reducing sugar were found to be more and more in untreated concentration, (control) the biochemical contents gradually decreased as the concentration increased (Saminathan 2013). Pot culture experiments were carried out with black gram seeds with different concentrations of chromium solution. The study revealed that increase in chromium reduces the growth and yield in the plants (Sundaramoorthy et al. 2018). The impact of stress varies with its intensity, duration and phenophases of crop. In majority of germplasm evaluation studies for drought tolerance, seed yield is the major criteria. The

response of drought stress on morpho-physiological and biochemical characters was assessed in black gram genotypes in a pot culture experiment (Gurumurthy et al. 2019).

The result of the analysis of variance showed that the varying levels of copper had a significant effect on germination percentage and germination index. With increase in concentration of copper from 0 to 100 mg l^{-1} the germination percentage and the speed of germination rate declined. The reduction was more significant in the VBN-8 that in other cultivars resistant to heavy metal on stages of seed germination. Seedling growth is the key to establishing plants under resistive condition (Almansouri et al. 2001). Implication of both combined metals of copper and zinc induced considerable changes in germination, growth, productivity, biochemical changes and mineral contents of groundnut (Parveen 2017). Rahman Khan and Khan (2010) also reported that the germination percentage and the seed germination rate were reduced in the percentage of nickel and cobalt. The inhibitory effect of copper is apparent at the concentrations of 10 and 25 mg l^{-1} onwards. The maximum reduction in germination rate and germination index was observed at 100 mg l^{-1} concentration of copper. It is obviously from the result of the present investigation that the rate of the germination index where retorted with increasing metal concentration. According to Shafiq et al. (2008) decreasing seed germination of plant can be attributed to accelerated break down of stored food materials in seed, reduction in seed germination can also attributed to the alternation of selection permeability of cell membrane. The result of the present study was supported by earlier reports of Mahmood et al. (2005) in maize due to copper and zinc Rahman Khan and Khan (2010) in Chickpea due to nickel and cobalt, Akinci and Akinci (2010). Early seedling growth means root and shoot length which is critical for plant production. It determines the crop stand density (Gelmond 1978). Copper should inhibitory effect on seedling growth both root and shoot length was significantly reduced. Root length and shoot length showed a linear trend between the cultivars and concentration of copper treatments. There was a progressive declined in root length with progressive increasing in metal

concentration of copper treatment. However, the low concentration of copper treatment showed a slight increasing in root and shoot length. This may be due to the fact that copper serve as micronutrients. Adverse effect of heavy metal especially copper on roots are related to server reduction in the elongation growth of the longest root as well as root plasma of the seedlings (McBride 2001), significant in inhabitation of root length and number later root of all crop seedling at higher concentration of copper also reported by various members, in maize under cobalt treatment (Jaeel et al. 2009). The results are in consonance with the findings of Imtiyaz et al. (2014) duets cobalt and lead treatment, Solanki et al. (2011) duets zinc and copper.

The dry weight of seedlings varied significantly with progressively increase in copper concentration. At low levels of copper (5 and 10 mg l⁻¹) treatment there was a slight increase in dry weight of seedlings. This may be due to the fact that their metals serve as micronutrients at low levels. Due to synergistic effect of these metals the dry weight of seedlings decreased according to the concentration applied. There was a gradual decline with increase in metal concentration. The concentration above 5 and 10 mg l⁻¹ of copper showed a progressive decline in the dry weight of seedling under copper exposure. The differential response of black gram cultivars to dry weight of copper treatment is comparable to the differential toxicity of cadmium on rice cultivar (Bagchi 1976). Mercury on Cucurbit (Sharma 1984), (Singh et al. 2013) and cobalt and lead in soya bean cultivators (Imtiyaz et al. 2014) and (Farooqi et al. 2009). Chlorophyll is the key chemical compound present in the plants which is necessary for photosynthesis. Various abiotic stress decreases the chlorophyll content in plants. In the present study when the black gram plants were treated by copper at low concentration of copper (5 and 10 mg l⁻¹) the chlorophyll a, b and carotenoids are increased. The further increase in copper level significantly decreased the chlorophyll and carotenoid content. The increase in photosynthetic pigments was obviously duets copper and zinc which act as structural and catalytic components of proteins enzymes as a co factor for normal development of pigment biosynthesis (Balashouri and Prameladevi 1995). The results are in agreement with the reports

of Vijayarengan and Lakshmanachary (1994) and Samantaray et al. (1998) in *Vigna radiata*. The net result of copper uptake in different parts of black gram cultivars resulted in reduction of plant height, number of lateral roots and biochemical constituents. The VBN-8 cultivar showed a maximum reduction in all the parameters when compared to VBN-3. Hence the results of the present investigation, it can be consider that cultivar VBN-8 was proved to be more tolerant than other three cultivars. The VBN-3 is sensitive to copper treatment so it can be recommended to cultivate the VBN-8 cultivar of black gram in copper contaminated soul to get better growth and yield. The identified copper tolerant cultivar VBN-8 can be used as potential germplasm for future studies. Further studies are warranted to intend the hidden facts on the metabolism of copper tolerance of black gram cultivar.

CONCLUSIONS

The present investigation has been carried out to assess the Impact of copper on seed germination and biochemical changes of (*Vigna mungo* (L.) Hepper). For this purpose; best cultivars were procured and healthy seeds were collected and surface sterilized before sowing. Seedlings were raised in petri plates lined with filter paper. The seedlings were irrigated with respective concentration of copper (0, 5, 10, 25, 50, 100, 200, 250, 500, 1000 mg l⁻¹). This study was focused to determine the tolerant and sensitive cultivars to copper treatment, to find the lethal concentration of copper at which the crop was not able to survive, to find the germination studies under the copper treatment and to find the biochemical changes under copper treatment. As the result of germination studies it indicate that when there was a progressive increase in copper level there was a gradual decline in rate of germination, seed germination index and root tolerance index value when compared to control. The seedling growth parameters also showed the same level. The copper concentration above 100 mg l⁻¹ proved to be lethal to the crop. Based on the results of screening test of cultivars, the cultivar VBN-8 proved to be more tolerant and VBN-3 showed poor seed germination and growth characteristic under copper exposure. The toxic effect of heavy metals on plant growth depends

on the amount of toxic metal taken up. Further research is needed to determine the yield of crop and the level of molecular basis studies for tolerance on the crop plant. In further more research must be followed on the growth and development of plants cultivated in unregulated waste amended agricultural soil to avoid food security problem.

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