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SCAN ME

Research Paper

ASSESSMENT OF COPPER TOLERANCE IN SELECTED CROPS

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Abstract

Heavy metal pollution is one of the major environmental problems we face today, mainly due to rise of anthropogenic activities. Activities such as mining, smelting, oil extraction, several manufacturing processes, electroplating, fertilizers, pigment and dyes manufacture and vehicular emissions are a source of heavy metals in contamination of soil and water. India is primarily an agrarian country, crops grown on heavy metal polluted soil adversely affects the growth of these crops and subsequently the consumers. In this paper the effect of copper on seed germination, root and shoot growth, fresh weight of common Indian crops has been studied. Our results show that seed germination and root and shoot growth is significantly reduced at high concentrations of the heavy metal. Copper being a micronutrient, increased the germination rate at lower concentrations producing a stimulating effect on the plants. According to our results, the morphological responses of selected plants to copper, we can conclude that highest growth was recorded for Vigna radiata L. to the other selected species. The germination and growth of Trigonella foenum – graecum L. and Oryza sativa L. seedlings were found to be most sensitive. The resistance of the plants can be represented as Vigna radiata L. > Vigna aconitifolia L. > Pennisetum glaucum L. > Sorghum vulgare L. > Trigonella foenum – graecum L. > Oryza sativa L.

Key words: copper, seed germination, toxicity, seedling growth, crop.

INTRODUCTION

Deteriorating environment is one of the major issues that we face today. There are several reasons for the occurrence of pollution, the major one being urbanization. Intensive urban growth and industrialization started increasing the level of pollution converting our landscapes into vessels of waste. Developmental activities have depleted our natural resources and generated huge amount of wastes leading to the pollution of air, water and soil. Heavy metals is a general collective term, which applies to the group

of metals and metalloids with atomic density greater than 4 g/cm^3, or 5 times or more, greater than water [1]. Heavy metals naturally present in trace amounts are arsenic, cadmium, chromium, cobalt, lead, mercury, nickel and selenium are toxic even at very low concentrations, whereas few heavy metals such as copper, zinc and iron are of biological importance to growth, however at high concentrations, can cause damage to the organism by accumulating and displacing vital nutrients in the tissues. Copper is an essential micronutrient for all living organisms, including plants [2]. Copper is largely used in the chemical industry, metal piping, mining and pesticide production known to cause anaemia, gastrointestinal irritation, liver and kidney damage. The effect of Cu toxicity is seen on germination root growth and vigour of plants, causing chlorosis, necrosis and discolouration of leaf [3].

MATERIAL AND METHODS

Seeds, Planting material and stock solution: Seeds of *Trigonella foenum-graceum* L. studied for the effects of Copper on seed germination were purchased from Pyramid Seeds at Namdeo Umaji Agritech (India) Pvt. Ltd. Seeds of *Vigna radiata* L.*, Vigna aconitifolia* L.*, Pennisetum glaucum* L., *Sorghum vulgare* L., and *Oryza sativa* L. were bought from a local seed dealer.

For the germination studies: Ten surface sterilized seeds uniform in colour, weight and size were placed on a Petri dish (9 cm diameter) on double-layered filter paper. The seeds were sterilized to kill the surface organisms by placing them in Bavistin solution prepared using 200mg of Bavistin dissolved in 100ml distilled water. The seeds were left in this solution for 5 minutes followed by a through rinse repeated twice, using sterile distilled water preceding the germination studies. The filter paper was moistened with varying concentrations of heavy metal solutions, 5mL on the first day followed by 2 ml on alternate days for 7 days. Triplicates of each treatment in a completely randomized designed were studied along with a separate control set using distilled water.

A 1000 ppm stock solution was prepared for Copper using 3.929g of Copper sulphate pentahydrate ($CuSO_4.5H_2O$) of analytical grade purchased from Loba Chemie. The stock solution was then diluted to prepare 1, 3, 5, 10, 50, 100, 200, 300, 500 ppm for the treatment of seeds. All the standards were prepared by non – serial dilutions.

Germination indices i.e. Total germination (GT) and Seedling vigour index (SVI) [4]

were selected and recorded for this study. Seed germination was observed after 24 hours, for a constant percentage of germination; other growth attributes viz. length of the root and shoot (cm) and fresh weight (g) of the seedlings was recorded on a digital balance after a period of 7 days.

Total Germination: the final Germination percentage is a measure of the time for a population of seeds to germinate in order to estimate its viability and is expressed as a percentage. The total germination (GT) was calculated using the following formula:

GT = no. of seeds germinated/total seeds x 100

Seedling Vigour Index: Seed vigour helps understand the potential for emergence and development of seedlings in field conditions. Compared to GT, SVI being more sensitive is an important component of germination studies as it provides a better understanding of seed damage and deterioration and response to stressors.

Seedling vigour index was calculated by following formula:

SVI = Germination % × Seedling length (cm)

Seedling length = RL + SL where RL is root length (cm), SL is shoot length.

To determine the significance between samples, a "Student's t – test" was carried out at $p<0.05$ level of significance. Box and Whiskers plot along with Histograms were used for data analysis. Statistical analysis was carried out using SPSS software version 11.0.

[III] RESULTS

3.1. Effect of Copper on total germination

Being a heavy metal copper also showed a similar trend that higher concentrations of Cu solutions affected the germination of selected plants (Table 1.11, Fig 1.59). Since it is a micronutrient the effect copper produced on germination was observed to be not as pronounced as the non - essential heavy metals. Amongst the six species the germination percentage of**:**

Vigna radiata L. did not show extreme effects of copper treatment germinating up to 500ppm with 75% germination. However a steady decline was observed from control to the highest concentration applied.

Vigna aconitifolia L. was least affected as it showed an 80% germination at 500ppm, gradual decrease in the percentage germination was observed from 1ppm onwards, with 95% at 1ppm.

Trigonella foenum – graecum L. had a steady rate of germination up to 300ppm. 90% germination was recorded at 1ppm reducing to 85% at 300ppm, a sudden drop was observed from 300ppm to 500ppm with 55% seeds germinating at the highest applied concentration.

In *Pennisetum glaucum* L. 60% germination was observed at 500ppm and the highest was 100% germination at 3ppm.

In *Sorghum vulgare* L. 90% of the seeds germinated at 100ppm and only 50% germinated at 500ppm.

Oryza sativa L. was noted to be the weakest plant of all six selected plants when subjected to higher concentrations of copper. Only 25% of the seeds germinated at 300ppm and 500ppm, whereas lower concentrations had a favourable effect on rice showing 90% germination at 100ppm.

Table 1.11: Effect of Copper on Total germination (%)

Ppm	*Vigna radiata* L.	*Vigna aconitifolia* L.	*Trigonella foenum – graecum* L.	*Pennisetum glaucum* L.	*Sorghum vulgare* L.	*Oryza sativa* L.
Control	100	100	100	100	100	100
1	85	95	90	85	95	100
3	75	85	80	100	85	100
5	80	90	90	80	80	95
10	90	90	90	85	80	95
50	90	85	90	85	75	95
100	85	90	95	75	90	90
200	85	90	90	70	85	35
300	75	90	85	60	70	25
500	75	80	55	60	50	25

Values are average of 30 samples.

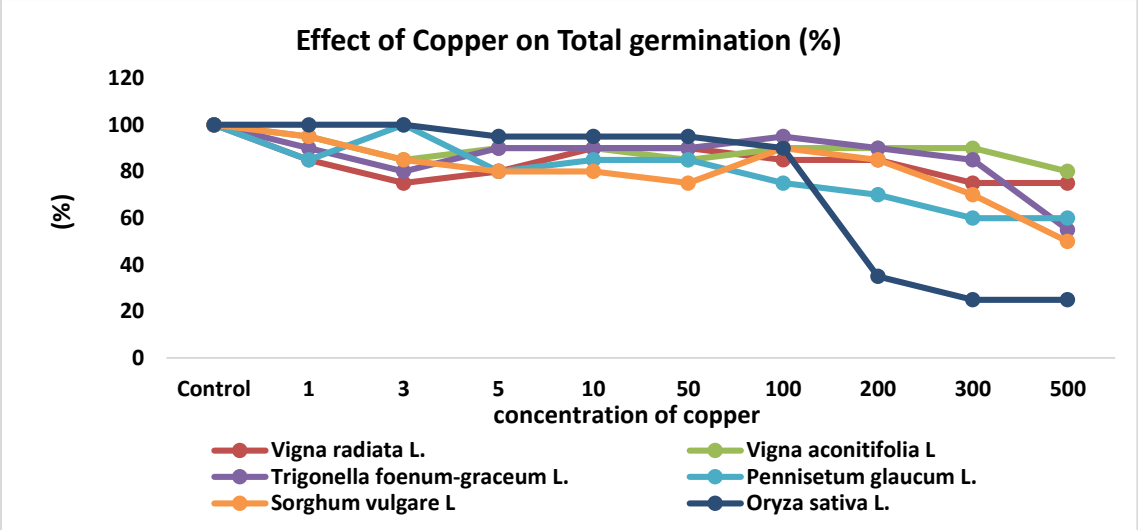

Fig 1.59: Trend graph showing the effect of copper on total germination (%)

3.2. Effect of Copper on Length of Radicle

Table 1.12: Effect of Copper on Length of Radicle (cm)

Ppm	*Vigna radiata* L.	*Vigna aconitifolia* L.	*Trigonella foenum – graecum* L.	*Pennisetum glaucum* L.	*Sorghum vulgare* L.	*Oryza sativa* L.
Control	11.54±1.88	11.08±2.62	3.9±0.90	7.63±3.10	8.21±1.96	5.75±0.97
1	4.77±0.78*	4.98±1.90*	3.09±1.59*	7.21±1.11#	4.27±0.92*	1.17±0.48*
3	5.51±1.00*	5.16±0.79*	2.05±0.89#	6.86±1.11**	2.95±0.0*	1.50±0.35*
5	6.85±2.01*	5.00±2.04*	2.88±1.37*	6.44±1.14**	2.57±0.49*	1.25±0.21*
10	7.62±1.47*	4.51±1.55*	2.48±0.75#	6.47±1.55**	1.59±0.37*	1.17±0.29*
50	4.12±1.03*	3.24±0.81*	1.59±0.80*	5.67±1.00#	1.65±0.68*	1.18±0.40*
100	4.24±1.16*	1.84±0.90*	0.86±0.26*	5.48±1.03*	0.86±0.49*	0.85±0.41*
200	1.66±0.55*	1.22±0.54*	0.62±0.37*	2.92±1.86*	0.52±0.23*	0.27±0.13*
300	1.48±0.53*	0.75±0.42*	0.40±0.16*	2.77±0.73*	0.20±0.14*	0.12±0.05*
500	0.87±0.37*	0.57±0.34*	0.35±0.19*	2.92±0.66*	0.18±0.13*	0.23±0.09*

*significant at p < .01 ** not significant at p < .05 #significant at p < .05 values are average of 30 samples

***Vigna radiata* L.:** Length of radicle in control was 11.54cm, followed by 7.62cm at 10 ppm. As shown in Fig 1.60, representing box and whisker plots for mung bean seeds, the length of radicle, increased gradually up to 10ppm after which a significant was observed with slight radicle emergence of 0.87cm at 500 ppm.

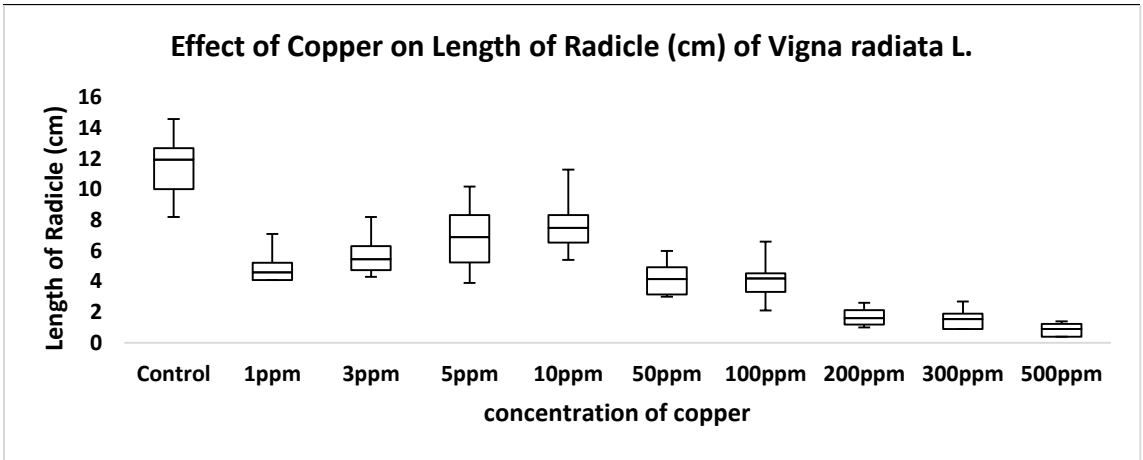

Fig 1.60: Effect of Copper on Length of Radicle (cm) of *Vigna radiata* L.

***Vigna aconitifolia* L.**: The control value was 11.08 cm. The highest length of radicle upon treatment was 5.16cm at 3ppm. The results obtained for 1ppm and 5ppm are fairly comparable with the lowest reading recorded of 0.57cm at 500ppm. (Fig 1.61)

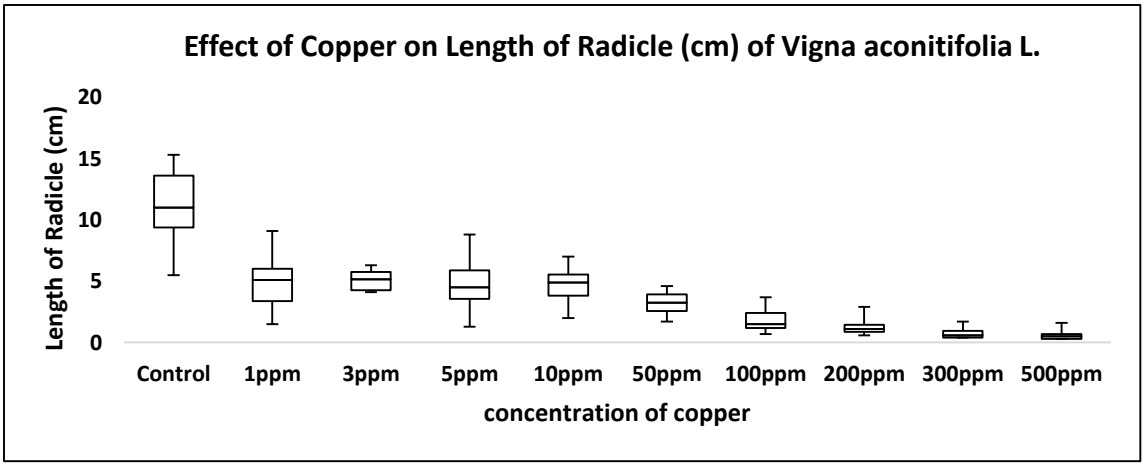

Fig 1.61: Effect of Copper on Length of Radicle (cm) of *Vigna aconitifolia* L.

***Trigonella foenum – graecum* L.:** Showed a minor but steady decrease in the length of radicle with an increase in concentration. 3.09 cm was measured length of radicle at 1ppm showing a 20% difference from control (3.90 cm). The minimum radicle length for Trigonella seedlings was 0.35cm. (Fig 1.62)

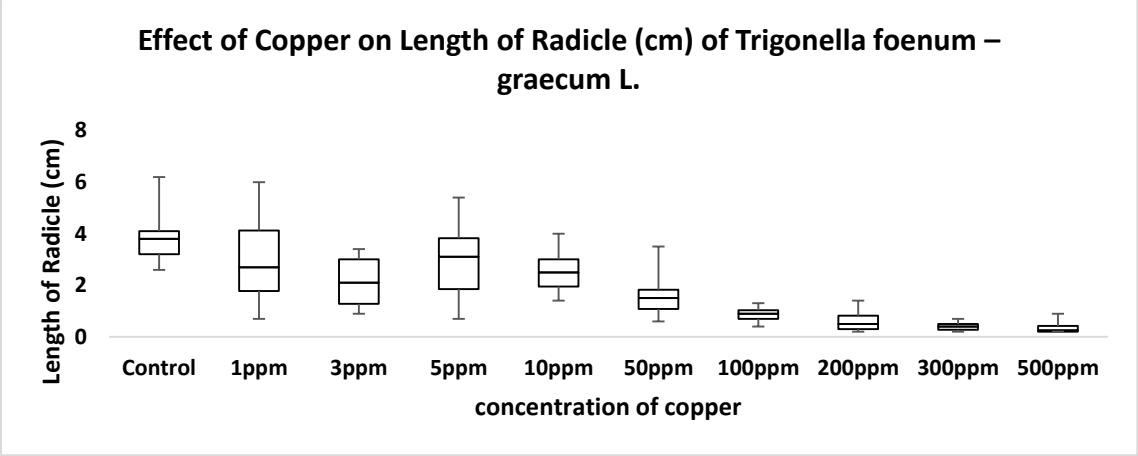

Fig 1.62: Effect of Copper on Length of Radicle (cm) of *Trigonella foenum – graecum* L.

***Pennisetum glaucum* L.:** Showed a marginal 5% difference from control at 7.63cm to 7.21 cm of radicle length at 1ppm. A significant radicle length was recorded up to 100ppm, a drastic decline was noted at 200ppm. At 300ppm least radicle growth was observed with a length of 2.77cm. (Fig 1.63)

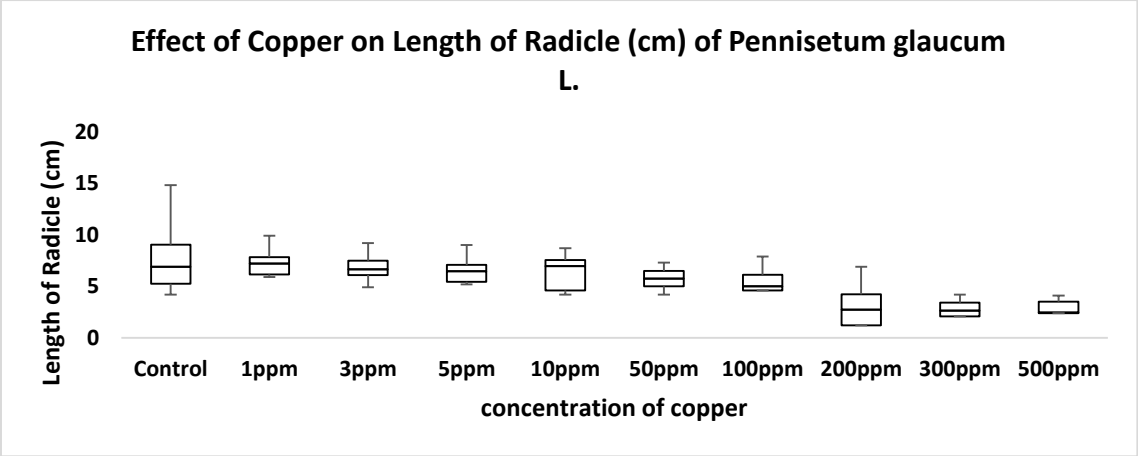

Fig 1.63: Effect of Copper on Length of Radicle (cm) of *Pennisetum glaucum* L.

***Sorghum vulgare* L.:** Highest length of radicle in *Sorghum vulgare* L. was for control 8.21 cm, dropping to 4.27 cm at 1ppm while the lowest value was 0.18 cm at 500ppm. Adverse effects of copper were observed from 100ppm onwards with severe inhibition of radicle emergence. (Fig 1.64)

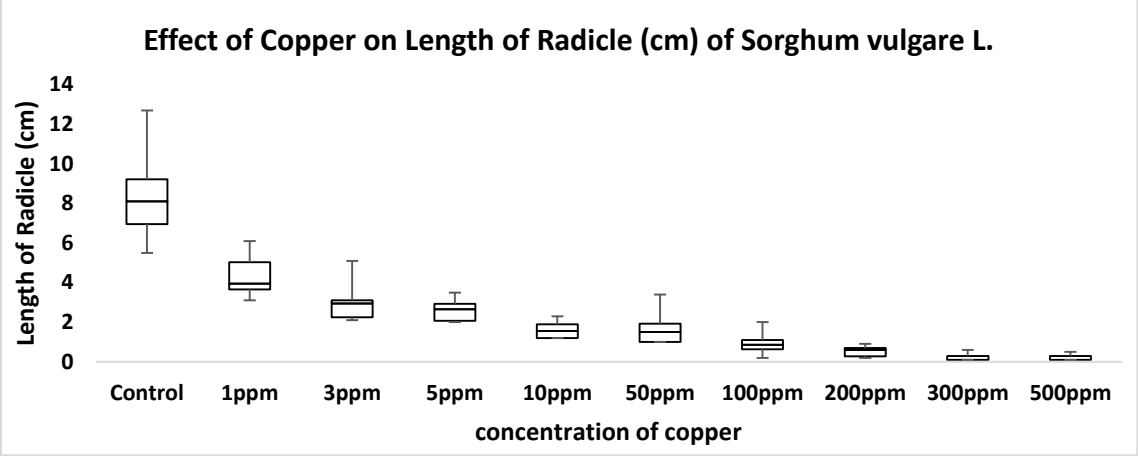

Fig 1.64: Effect of Copper on Length of Radicle (cm) of *Sorghum vulgare* L.

***Oryza sativa* L.:** Severe inhibition of radicle length was observed in rice seedlings. *Oryza sativa* had the highest length of 1.50cm upon treatment with copper at 3ppm and 0.23cm was the lowest length at 500ppm. The length of radicle from 1 to 50 ppm is fairly comparable, the decrease in length is significant from 100ppm onwards. (Fig 1.65)

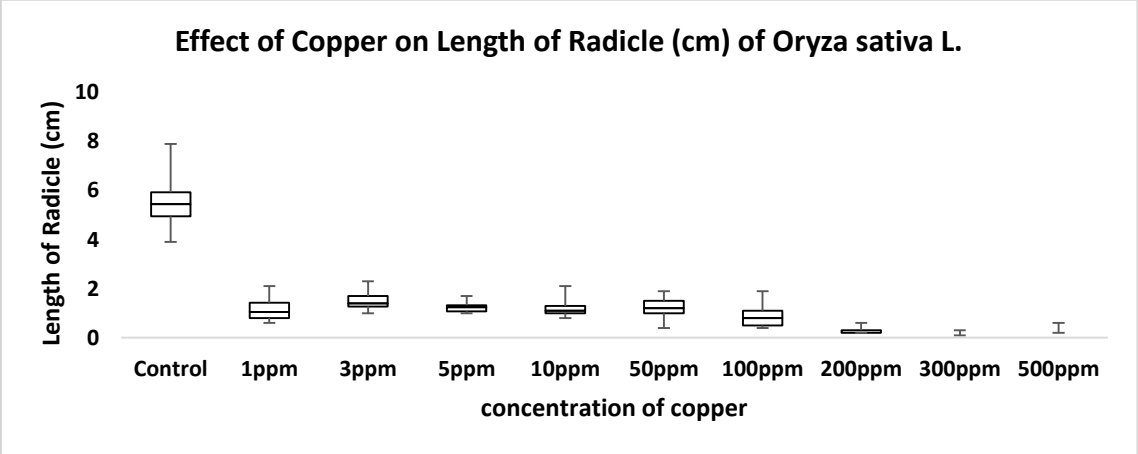

Fig 1.65: Effect of Copper on Length of Radicle (cm) of *Oryza sativa* L.

3.3. Effect of Copper on Length of Plumule

Table 1.13: Effect of Copper on Length of Plumule (cm)

Ppm	*Vigna radiata* L.	*Vigna aconitifolia* L.	*Trigonella foenum – graecum* L.	*Pennisetum glaucum* L.	*Sorghum vulgare* L.	*Oryza sativa* L.
Control	12.04±1.36	9.82 ±1.22	6.69±0.72	5.95±1.10	6.74±2.51	4.77±0.40
1	9.47±2.50*	11.06±2.43#	5.80±1.15*	5.98±1.30**	6.52±1.95**	3.74±0.63
3	11.76±3.82**	11.25±3.25**	5.46±1.45*	6.24±1.17**	5.83±0.77**	3.93±0.43
5	13.13±3.54**	10.80±2.06**	5.22±2.21*	6.61±1.21#	5.31±1.22#	4.02±0.59

10	11.63±1.63**	11.45±2.48#	5.44±0.89*	5.23±1.09#	6.55±1.24**	3.89±0.42
50	11.22±1.78**	10.82±2.42**	4.85±0.9*	5.90±2.16**	4.78±1.42*	3.94±0.40
100	12.64±2.81**	9.44±1.75**	5.24±1.0*	5.85±1.70**	2.19±0.91	3.68±0.53
200	9.50±2.08*	9.19±2.52**	1.63±0.43*	3.29±2.27*	1.99±0.75	1.75±0.47
300	10.68±3.05*	8.21±2.16#	1.18±0.30*	2.05±0.69*	0.68±0.49	0.58±0.44
500	10.13±2.33*	5.58±1.86*	0.96±0.21*	2.05±1.13*	0.91±0.41	0.59±0.49

*significant at p < .01 ** not significant at p < .05 #significant at p < .05 values are average of 30 samples

***Vigna radiata* L.:** Maximum length of plumule in *Vigna radiata* L. was 13.13 cm at 5 ppm and the lowest length noted was 9.50 cm at 200 ppm. Mung bean seedlings showed higher tolerance to copper compared to the other plants selected. (Fig 1.66).

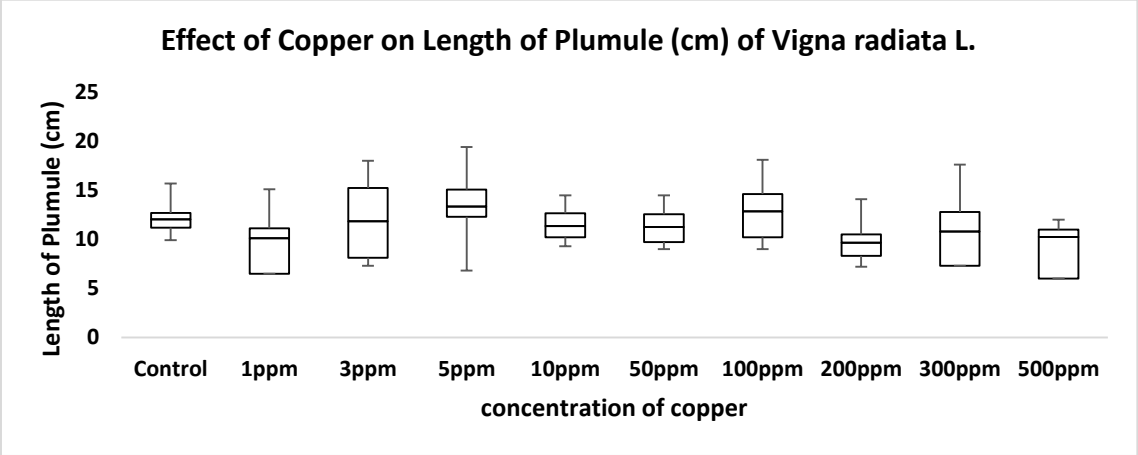

Fig 1.66: Effect of Copper on Length of Plumule (cm) of *Vigna radiata* L.

***Vigna aconitifolia* L.:** Highest length of plumule in *Vigna aconitifolia* L. was 11.45 cm at 10 ppm and the lowest length noted was 5.58 cm at 500ppm. Moth bean seedlings showed consistent length of plumule up to 300 ppm. (Fig 1.67)

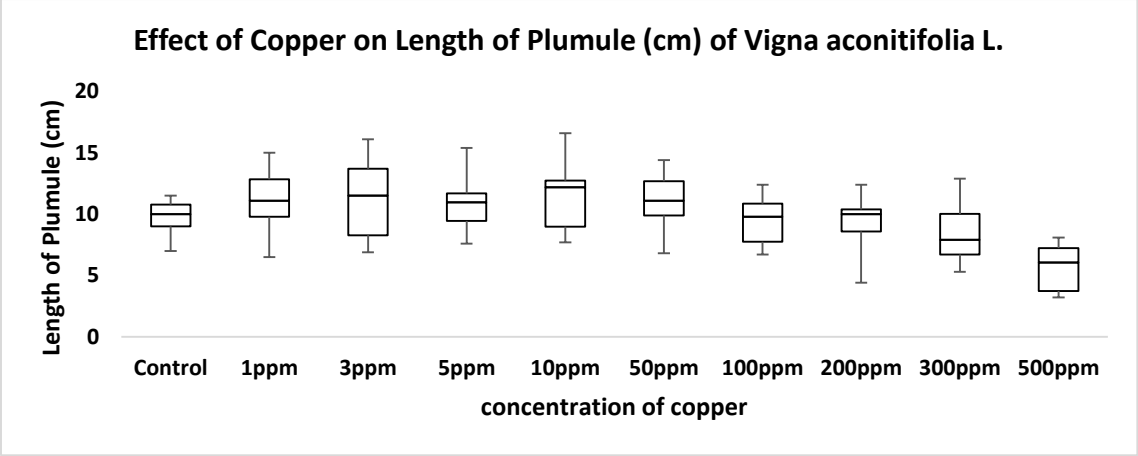

Fig 1.67: Effect of Copper on Length of Plumule (cm) of *Vigna aconitifolia* L.

***Trigonella foenum – graecum* L.:** The length of plumule for control value was 6.69 cm, maximum length of plumule upon treatment with copper for Trigonella seedlings was 5.80 cm and minimum value was 0.96cm at 500ppm. The seedlings showed severe inhibition of growth at 200ppm and thereafter. (Fig 1.68)

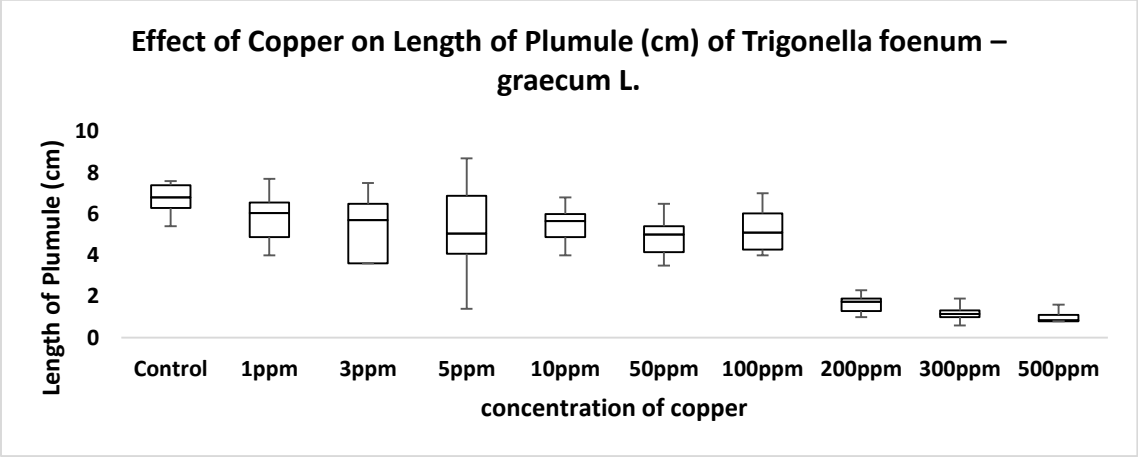

Fig 1.68: Effect of Copper on Length of Plumule (cm) of *Trigonella foenum – graecum* L.

***Pennisetum glaucum* L.:** Control value showed 5.95 cm plumule. An increase in length of plumule of 6.61cm at 5ppm and 2.50 cm was the lowest noted length at 500ppm. The length of plumule was comparable and consistent up to 100ppm after which a significant drop was observed. (Fig 1.69).

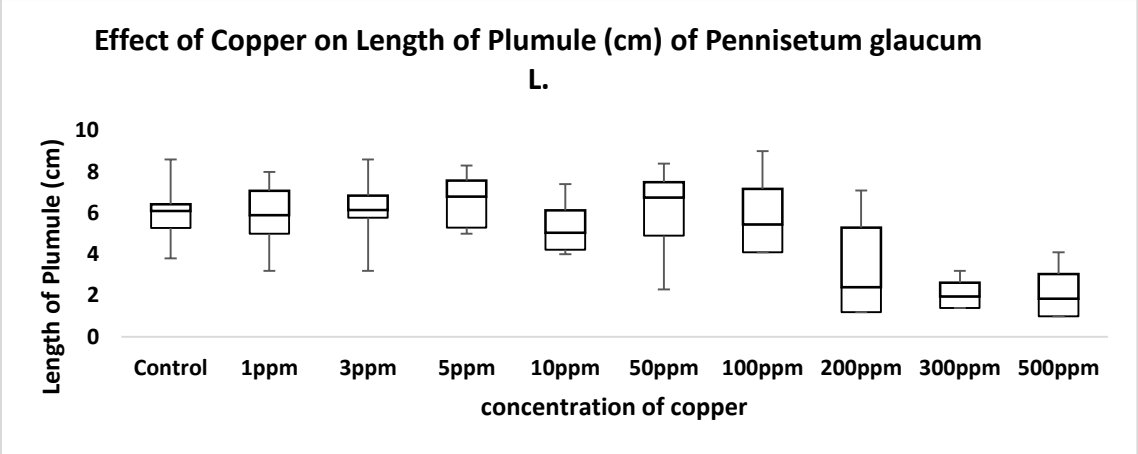

Fig 1.69: Effect of Copper on Length of Plumule (cm) of *Pennisetum glaucum* L.

***Sorghum vulgare* L.:** Sorghum seedlings showed a highest length of 6.55 cm at 10ppm and the lowest value of plumule was 0.68 cm at 300ppm. The control value being 6.74cm. (Fig 1.70).

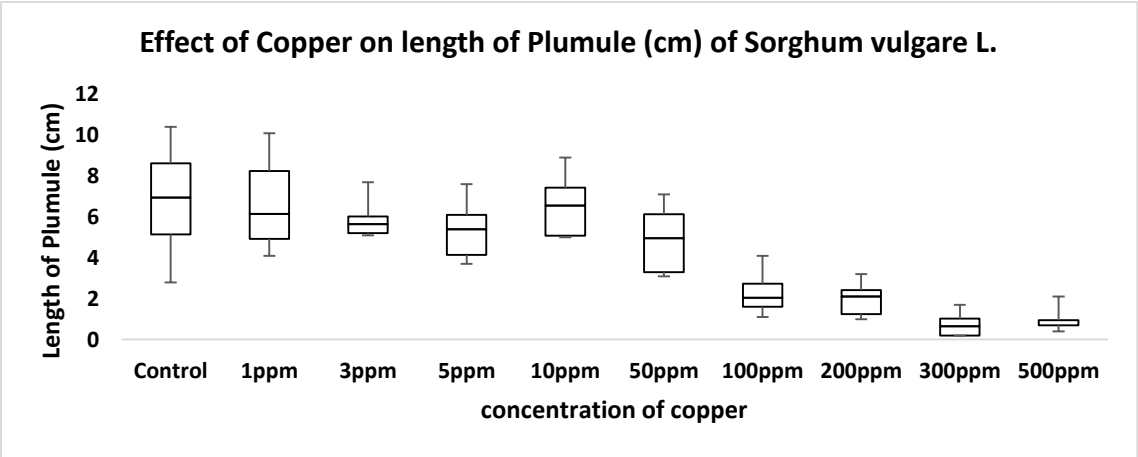

Fig 1.70: Effect of Copper on Length of Plumule (cm) of *Sorghum vulgare* L.

***Oryza sativa* L.**: Seedlings were severely inhibited at higher concentrations, having a plumule length of 0.58 cm at 300ppm. The length of plumule was 4.02 cm at 5 ppm. The control value was 4.77cm. (Fig 1.71)

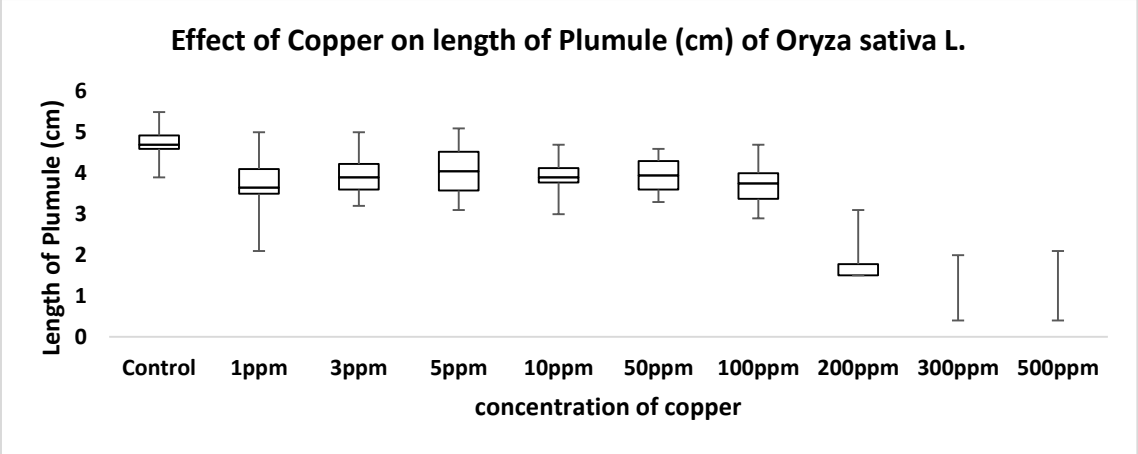

Fig 1.71: Effect of Copper on Length of Plumule (cm) of *Oryza sativa* L.

3.4. Effect of Copper on Fresh weight

Table 1.14: Effect of Copper on Fresh weight (g)

Ppm	*Vigna radiata* L.	*Vigna aconitifolia* L.	*Trigonella foenum – graecum* L.	*Pennisetum glaucum* L.	*Sorghum vulgare* L.	*Oryza sativa* L.
Control	0.2937±0.05	0.1747±0.03	0.1673±0.03	0.0663±0.01	0.1214±0.01	0.0451±0.004
1	0.1811±0.05*	0.1721±0.03**	0.1547±0.01*	0.0611±0.01**	0.1003±0.02*	0.0410±0.005**
3	0.2470±0.06*	0.1989±0.03#	0.1385±0.04#	0.0640±0.01**	0.1006±0.01*	0.0383±0.008**
5	0.2647±0.04**	0.1743±0.04**	0.1258±0.02#	0.0448±0.01*	0.1001±0.00*	0.0343±0.005**
10	0.2862±0.05**	0.1601±0.02**	0.1317±0.02*	0.0494±0.01*	0.0966±0.01*	0.0296±0.005**
50	0.2200±0.07*	0.1416±0.04*	0.1227±0.02#	0.0461±0.01*	0.0816±0.01*	0.0365±0.005**
100	0.2175±0.06*	0.1531±0.02#	0.1330±0.01*	0.0312±0.006*	0.0606±0.01*	0.0337±0.003**

200	0.2104±0.05*	0.1304±0.04*	0.1016±0.01*	0.0248±0.005*	0.0541±0.007*	0.0225±0.003#
300	0.2375±0.03*	0.1137±0.02*	0.0799±0.01*	0.0224±0.005*	0.0496±0.0*8	0.0131±0.004*
500	0.2126±0.03*	0.0927±0.02*	0.0714±0.01*	0.0188±0.004*	0.0439±0.004*	0.0200±0.001*

*significant at p < .01 ** not significant at p < .05 #significant at p < .05 values are average of 30 samples

***Vigna radiata* L.:** Upon treatment with copper solution it showed a highest weight 0.2862g at 10 ppm and the lowest weight was 0.1811g at 1ppm. The control value being 0.2937g. (Fig 1.72)

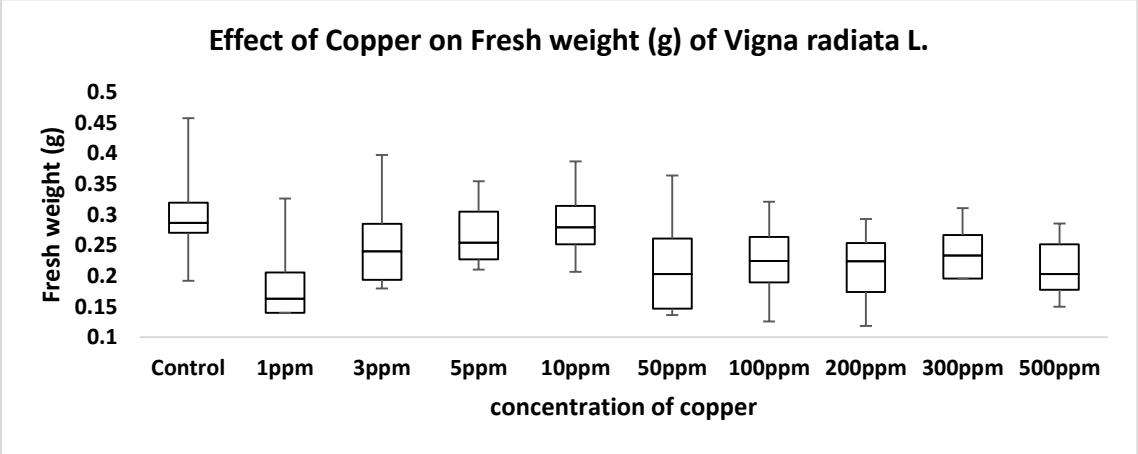

Fig 1.72: Effect of Copper on Fresh weight (g) of *Vigna radiata* L.

Vigna aconitifolia* L.*: A gradual decline in the fresh weight was noted from 0.1989g at 1ppm being the highest calculated average to 0.0927g being the lowest at 500 ppm. (Fig 1.73)

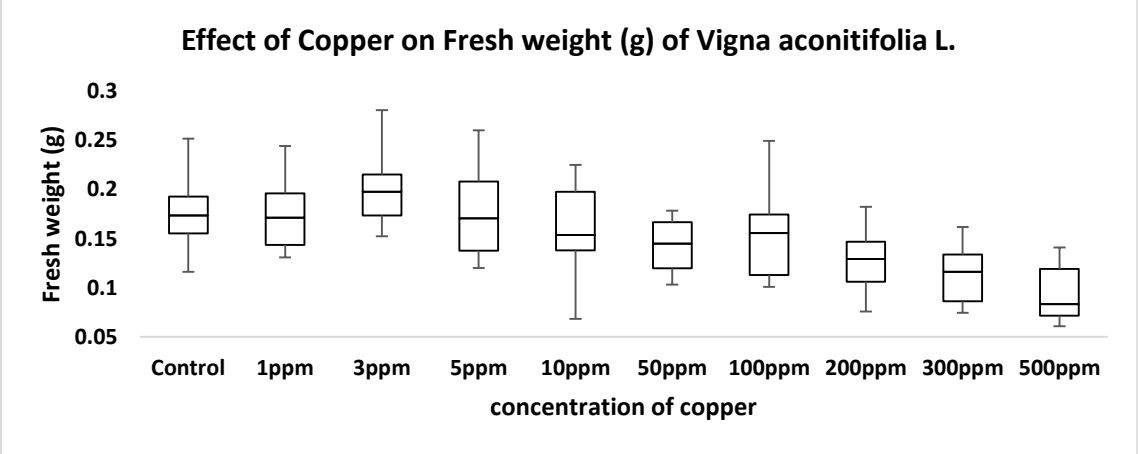

Fig 1.73: Effect of Copper on Fresh weight (g) of *Vigna aconitifolia* L.

Trigonella foenum – graecum: The control value was 0.1673g slightly higher than average fresh weight of Trigonella seedlings of 0.1547g at 1ppm of copper and a minimum fresh weight was recorded 0.071g at 500ppm. Severe reduction in fresh weight was observed at higher concentrations of 300 ppm and 500ppm. (Fig 1.74)

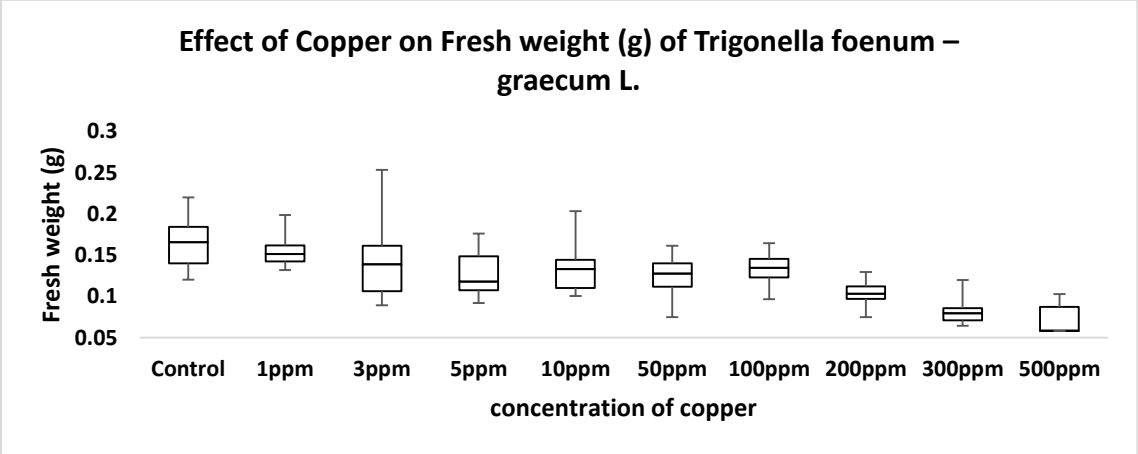

Fig 1.74: Effect of Copper on Fresh weight (g) of *Trigonella foenum – graecum* L.

***Pennisetum glaucum* L.**: Fresh weight of *Pennisetum glaucum* L. was 0.0640g at 3ppm and a minimum value recorded was 0.0188g at 500ppm. A favourable increase in fresh weight was observed at 3ppm in comparison to 1 ppm. The value of control was 0.0663g. (Fig1.75)

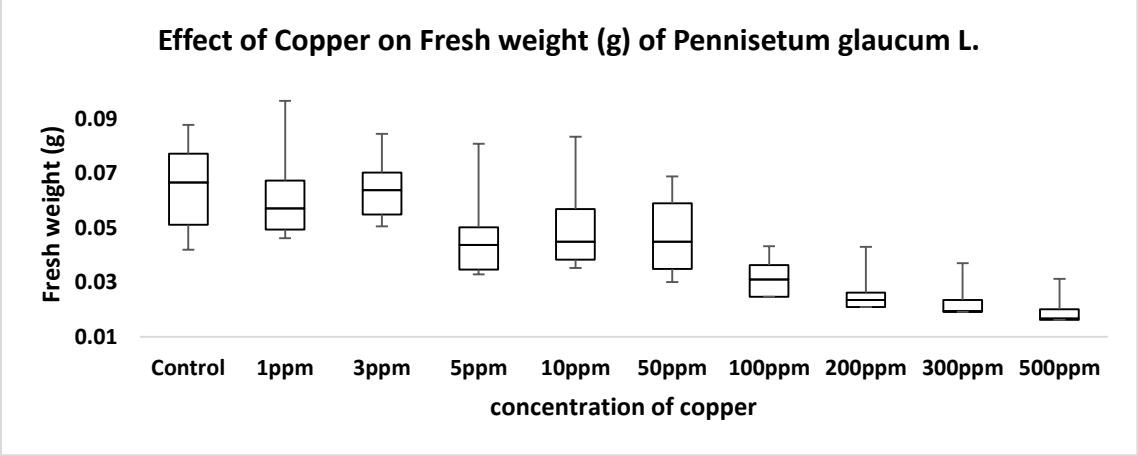

Fig 1.75: Effect of Copper on Fresh weight (g) of *Pennisetum glaucum* L.

***Sorghum vulgare* L.:** Maximum fresh weight recorded in *Sorghum vulgare* L. was 0.1214g for control and 0.1006g at 3ppm and minimum weight noted was 0.0439g at 500ppm. (Fig 1.76)

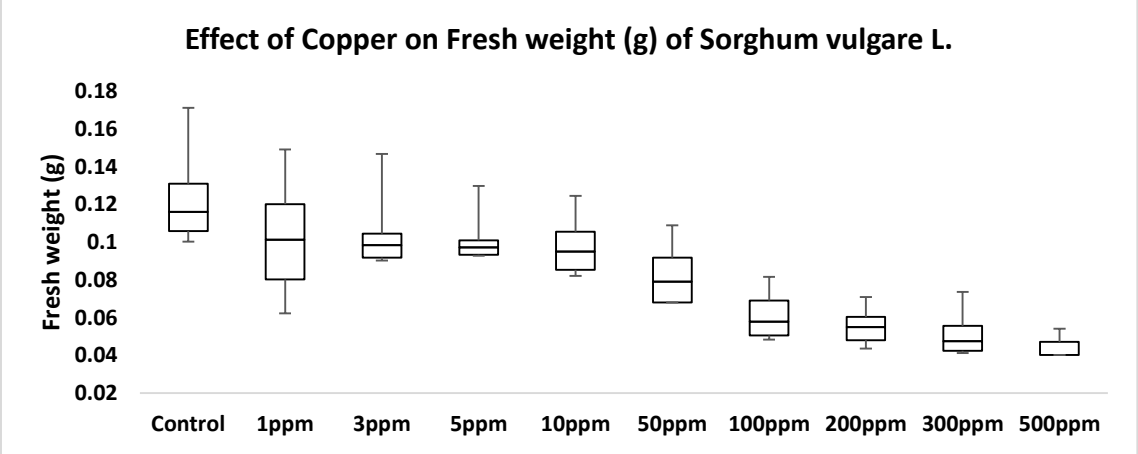

Fig 1.76: Effect of Copper on Fresh weight (g) of *Sorghum vulgare* L.

***Oryza sativa* L.:** Seedlings of *Oryza sativa* L. showed a gradual decrease in fresh weight from control through 500ppm. Control value was 0.0451g and 0.0410g was at 1ppm. The minimum fresh weight was 0.0200g at 500ppm. (Fig 1.77)

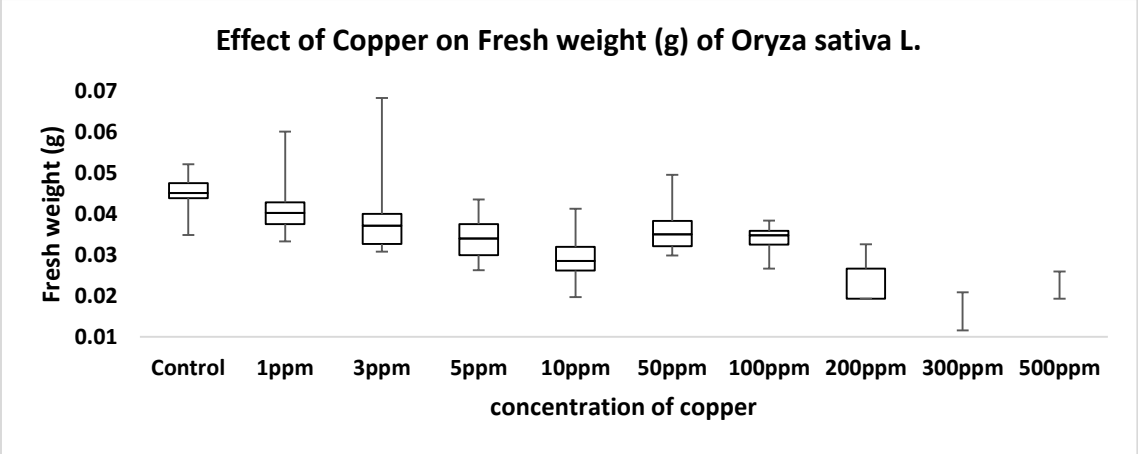

Fig 1.77: Effect of Copper on Fresh weight (g) of *Oryza sativa* L.

3.5. Effect of Copper on Seed Vigour Index (SVI)

Based on the data collected for the total germination percentage and early seedling growth which shows a decline in the overall length of radicle, plumule and fresh weight, the SVI calculations also show a decreasing value with an increase in the concentrations. (Table 1.15, Fig 1.78).

Vigna radiata L.: the control value was 2358, followed by 1799.10 at 5 ppm and a minimum of 759.75 at 500ppm.

A decline in the SVI was noted in *V. aconitifolia* L. from 1524.75 to 492.40 in concentrations 1 through 500 ppm. Control value was 2090.

Trigonella foenum – graecum L. was observed to be one the weakest plants, 800.55 was the highest calculated SVI value and 72.50 was the least at 500ppm. Control value was 1059. Despite the favourable increase in growth at lower concentrations, overall there was a decrease in SVI with an increasing concentration of copper.

In *Pennisetum glaucum* L. a drop in vigour from 1362.50 for control set to 298.5 at 500 ppm was calculated.

Sorghum vulgare L. showed a 94% difference from 1025.52 to 52.25 when the concentrations were increased from 1ppm to 500ppm. The highest SVI was calculated for control at 1495.50.

Oryza sativa L. also was amongst the weaker plants against copper stress, a drop was noted in the vigour of seeds 491.50 to 17.62 upon being treated with 1ppm of copper gradually advancing to 300 ppm concentration. Control value was 1034.50.

Table 1.15: Effect of Copper on Seed Vigour Index (SVI)

Ppm	*Vigna radiata* L.	*Vigna aconitifolia* L.	*Trigonella foenum – graecum* L.	*Pennisetum glaucum* L.	*Sorghum vulgare* L.	*Oryza sativa* L.
Control	2358	2090	1059	1362.50	1495.50	1034.50
1	1068.75	1524.75	800.55	1122	1025.52	491.50
3	1382.40	1394.85	601.20	1310	747.15	543.50
5	1799.10	1422.45	729	1044.80	630.40	501.60
10	1733.40	1436.85	713.25	994.92	651.60	481.65
50	1304.32	1195.52	579.60	983.45	482.62	487.35
100	1434.80	1015.65	579.97	850.12	274.95	408.15
200	949.45	937.80	202.50	434.70	213.77	71.05
300	912.75	807.30	134.72	289.50	62.30	17.62
500	759.75	492.40	72.05	298.50	52.25	20.50

values are average of 30 samples.

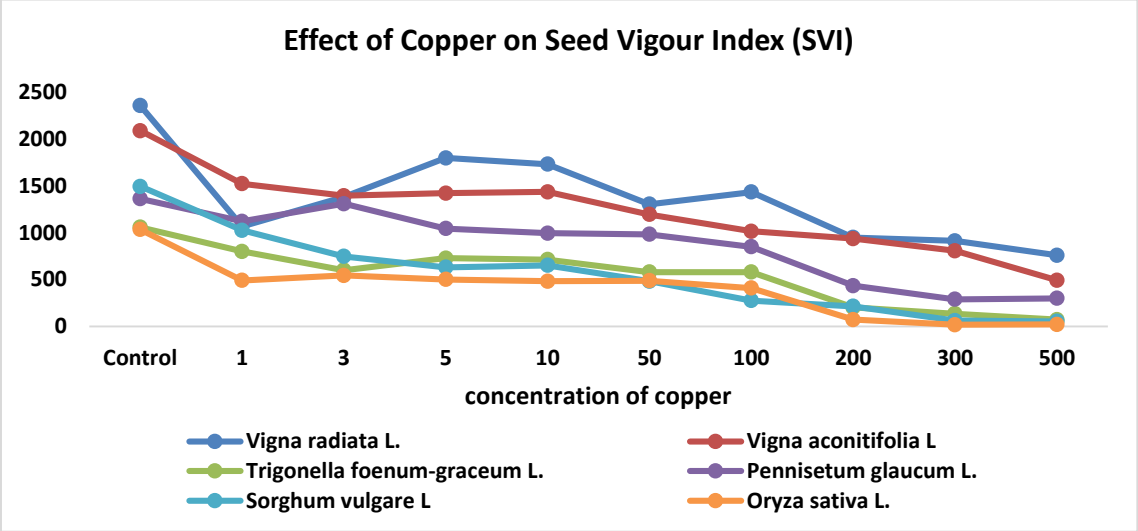

Fig 1.78: Trend graph showing the effect of Copper on Seed Vigour Index (SVI)

DISCUSSION

Copper being a micronutrient, increased the germination rate at lower concentrations producing a stimulating effect on the plants. Copper has a negative effect on plants at high concentrations such as shortening of length of radicle and plumule, decrease in fresh weight and low germination rate. The recorded values for copper treatment at lower concentrations up to 5 ppm showed a favourable effect of the emergence and length of the radicle. Based on the values recorded, 50ppm can be considered as the threshold thereafter a significant decline can be seen in the growth of radicle. Detrimental effects of copper were most pronounced at 500ppm with slight emergence of radicle for all the selected plants. A reduction of germination indices was reported in many studies due to cell membrane injury by water deficit stress for rice [5] and in

wheat [6]. Although a slight increase was observed in germination percentage and lateral roots in 5mg/L of copper concentration. Another study reported reduction in wheat plumule length, radicle length, number of lateral roots, fresh weight and dry weight with increased copper concentration. [7]

Copper was found to be beneficial to plant growth at 3ppm and 5ppm for all the selected species showing an increase in growth whereas copper was toxic at 100ppm and onwards, showing inhibition of root and shoot elongation. Based on the observations, severe inhibition was noted at 200ppm to 500ppm. Since the emerging radicle of seedlings subjected to higher concentrations of copper were inhibited, this affected the growth of plumule as well. 3ppm and 5ppm concentrations produced a favourable increase in the length of plumule for the plants selected. High phytotoxicity of copper on shoot length of wheat was reported at 500 ppm concentration [8]. Similarly, it was reported that increasing concentrations of copper significantly reduced the seedling growth in *Ipomoea batatas* [9].

The fresh weight of the seedling corresponds with the increase and decrease in radicle and plumule length at specific concentrations of copper. The fresh weight of seedlings of all plant species selected showed a minor increase at 3 ppm and 5 ppm whereas at higher concentrations a gradual decrease in weight was observed. Similar results were noted in *L. culinaris* plants at 100 ppm causing decreased the seedling growth [10]. The overall reduced can be attributed to the oxidative stress induced by heavy metals at a cellular level [11].

According to our results, the morphological responses of selected plants to copper, we can conclude that highest growth was recorded for *V. radiata* to the other selected species. The germination and growth of fenugreek and rice seedlings was found to be more sensitive.

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