# CHAPTER-4

# EFFECT OF EXCESS COPPER ON TEA SEED GERMINATION AND EARLY SEEDLING GROWTH

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## EFFECT OF EXCESS COPPER ON TEA SEED GERMINATION AND EARLY SEEDLING GROWTH

Seed germination and early seedling growth are regarded as critical phases, which are regulated by interactions of hormones and environmental factors and are known to be greatly influenced by stressful conditions (Shah and Dubey, 1995; Teisseire and Guy, 2000). The major effects of heavy metals on seeds are noticed as decrease in germination percentage, reduced root and shoot elongation, decrease in dry root and shoot mass, deformation and colour change (Sethy and Ghosh, 2013).

Until about the middle of the past century, tea was traditionally propagated from seeds. Seeds were generally produced in stands of well-spaced mature seed bearers (approximately 200 trees per hectare) in 'seed bari' or commercial seed orchards (Hall, 2000). However inconsistency in performance of the populations led to the development of a strong vegetative propagation method which provided a way of cloning to obtain homogenous populations of selected parents. The first scientific attempt for development of seed cultivars from clonal parents as inputs in north east India was made in 1939 at the Tocklai experimental Station, Jorhat, Assam (Chen *et al.*, 2012). Altogether 14 clonal seed progenies have been released as biclonal seed stock for commercial exploitation by the tea industries of north east India that were developed through crossing between two selected clonal parents (Barman, 2011; Mondal, 2014).

After the release of the clonal seeds, commercial seed 'baries' have been established for production of hybrid clonal seeds which were extensively used by the tea industries in their plantations (Chen *et al.*, 2012). Unprunned trees at the 'baries' can attain a height of 30 to 40 feet. The fragnant white bloossoms are succeeded by soft green lobed capsule, each of which contains 1-3 small, almost spherical seeds. In a fully ripened capsule, the lobes dehisce which allows the mature seeds (10-20 mm diameter) to fall on the ground (Hall, 2000). These seeds are required to be promptly collected from the ground of the seed orchards due to the fact that tea seeds being recalcitrant have low viability (Bhattacharjee and Singh, 1994; Mondal, 2014). After collection, seeds are passed through a rotary type shifter to eliminate very small seeds (Mondal, 2014). The remaining seeds are then placed in containers of water for grading. The healthy ones will sink within 24 h while the damaged seeds will float on the surface. The healthy seeds should be placed in a seedling nursery as soon as possible because viability starts declining after only ten days (Hall, 2000).

The tea seeds have a hard testa outside and the embryo is covered in between two large cotyledons. The viability of tea seeds can be maintained by surface sterilizing with mercuric chloride solution (0.01%) for 15 min and subsequent cold storage at $4^0$C (Mondal, 2014). Seeds are generally stored in moist charcoal for a few days, although it is advisable to use the healthy fresh seeds for propagation as far as possible (Singh, 1999).

Although several reports are available over effect of the metal toxicity on tea plants, almost no report exists on how heavy metals affect seed physiology. While keeping in mind the rising concerns over heavy metal stress affecting agriculture produce, in this chapter we focus our attention to the effect of copper ions on tea seeds of different clonal seed cultivars. We studied the percent seed germination and early seedling growth under different concentrations of copper.

## 4.1. MATERIALS AND METHODS

### 4.1.1. Collection and maintenance of plant materials

Three different clonal seed cultivars of tea (TS-462, TS-463 and TS-520), that were released by the Tocklai Experimental Station (Jorhat, Assam) were used during the present study. Freshly harvested tea seeds of the selected cultivars were obtained from Gayaganga Tea Estate located in the Terai plains of the Darjeeling district of West Bengal (Fig. 4.1). The selection was based on the extent of their cultivation in tea gardens of the Terai and Dooars region of Darjeeling district. All the cultivars mentioned above were selected for plantation in the experimental garden, Department of Botany, University of North Bengal, based on their growing suitability and environmental aspects as observed over the years under field conditions at Tocklai Experimental Station, Jorhat, Assam. The growth conditions were maintained as recommended by Bezbaruah and Singh (1988). The general


**Fig. 4.1:** (A) Gayaganga Tea Estate from where seeds were collected during the present study; (B) Tea seedling nursery.

characteristics of the cultivars used during the work are enlisted in Table 4.1. The selected clonal seed stocks were raised in the nursery of Gayaganga Tea Estate (Fig. 4.1) and also in the experimental garden of Department of Botany, University of North Bengal.

**Table 4.1: Biclonal seed stocks (cultivars) used in the study and their general characteristics**

| Biclonal seed stock number | Year of release* | Crossed between | Characters |
|---|---|---|---|
| TS-462 | 1980 | TV 1 (Assam) x 124.48.8 | Medium leaf, uniform growth, vigorous yield, fairly drought tolerant, suitable for both CTC and orthodox manufacture. |
| TS-463 | 1984 | TV 1 (Assam) x TV 19 (Cambod) | High yielding potential, fairly uniform in growth habit. The stock is suitable for both CTC and orthodox manufacture. |
| TS-520 | 1992 | TV19 (Cambod) x TV 20 (Cambod) | Uniform growth habit, high yielding potential. The stock is suitable for both CTC and orthodox manufacture. |

* Released by Tocklai Experimental Station, Jorhat, Assam situated in North East India.

### 4.1.2. Stress induction

To determine the percent germination in the treated and untreated seeds of the tea cultivars (TS-462, TS-463 and TS-520); metal application procedure as described by Munzuroglu and Geckil (2002) was followed with some modifications. Initially, after collecting the seeds, the surface of the seeds were sterilized with 0.01% $HgCl_2$ and washed thoroughly at least twice with distilled water. The clean seeds were then taken in a bag of cheese cloth and soaked in distilled water for 24 h. After the soaking, the bag was emptied into the water and the floating seeds were removed with a strainer. The sunken seeds were then collected after draining off the water and sowed in small earthen pots (6 cm diameter) containing moist sterilized sand. The pots were placed in dark in a growth chamber at $25^0$C. Rupturing of seeds occurred approximately after 5 d following which the seeds were placed in sand wetted with $CuSO_4$ solution. Sixteen different concentrations (0.5 mM – 8.0 mM) with 0.5 mM increments of copper sulphate solutions were prepared freshly in sterile distilled water and used for application in duplicate sets. Control sets were treated with sterile distilled water. Fresh application was done every 48 h to maintain the moisture and concentration. Hoagland and Knop's nutrient solution was applied once every 6 days in all experimental and control sets instead of the copper solution or distilled water. The germinated seedlings were sampled periodically and different parameters such as percent germination, root and shoot lengths and dry weights were measured upto 27 days. The experiment was repeated thrice. A set of plants (exposed to 6.5 mM $Cu^{2+}$ concentration) were allowed to grow until 60 days for observing the progressive deformations in the roots over a longer period.

#### 4.1.3. Assessment of stress response

##### 4.1.3.1. Percent germination

The pots were kept at 25$^0$C in the growth chamber and the germination was noted every 24 h. Seeds were considered to be germinated at one mm of radical emergence. The number of seeds germinated was counted and percent germination was calculated.

##### 4.1.3.2. Root and shoot length

The lengths of the shoots as well as roots of each variety were measured with a centimeter scale after removing the germinated seedlings from soil. The seedlings were sampled at 15, 18, 21, 24 and 27 d after treatment and deformities, if any were noted.

##### 4.1.3.3. Dry weight of roots and shoots

The dry weight of treated and untreated shoots and roots of the tested cultivars were measured after 27 d of treatment. This time duration was followed based on observations obtained during preliminary studies which showed that this time was necessary to test appropriate inhibitory effects. For this, sample roots and shoots were immediately cut off carefully from the grown seedlings and then allowed to dry in an oven at 80$^0$C. After 24 h the samples were removed from the oven, cooled and weighed.

## 4.2. RESULTS

### 4.2.1. Effect of different concentration of copper on percent seed germination

Figure 4.2 summarizes the effect of different concentrations of copper on seed germination of three tested tea seed cultivars of Northeast India. In general there was a progressive reduction in seed germination as metal concentration increased in all the tested cultivars. Minimum seed germination was recorded when 8 mM copper solution was applied. Germination percentage was found to be lowest in TS-463 (15%) and highest in TS-462 plants (25%). Germination in control plants which received only distilled water also followed the same trend, that is, TS-462 showed maximum germination (84%) followed by TS-520 (79%) and TS-463 (74%). Tea seeds allowed germinating in presence of excess copper showed distinct changes in percent seed germination and in morphology of germinated parts. These obvious changes were a part of the plants inherent capacity of tolerance or to adapt to stress situations.

### 4.2.2. Effect of different concentration of copper on shoot elongation

The data corresponding to the shoot growth of three cultivars of tea seedlings at different concentration of $Cu^{2+}$ (0.5 mM to 8.0 mM) reported in this work is shown in Tables 4.2, 4.3 and 4.4. There was progressive decrease in shoot lengths with increasing Cu concentrations (Figs. 4.3.1 and 4.3.2). The control seedlings after 27 days showed 14.2 cm of growth, however, those treated with 4 mM Cu recorded 8.1 cm and those treated with 8 mM Cu recorded only 2.9 cm of shoot length in TS-520 variety. The other cultivars showed similar trends. TS-463 showed least growth among the tested cultivars. Maximum reduction in length of shoot (88.2%) was observed in TS-463 variety followed by TS-520 (79.5%) and TS-462 (67.1%) (Fig. 4.4).

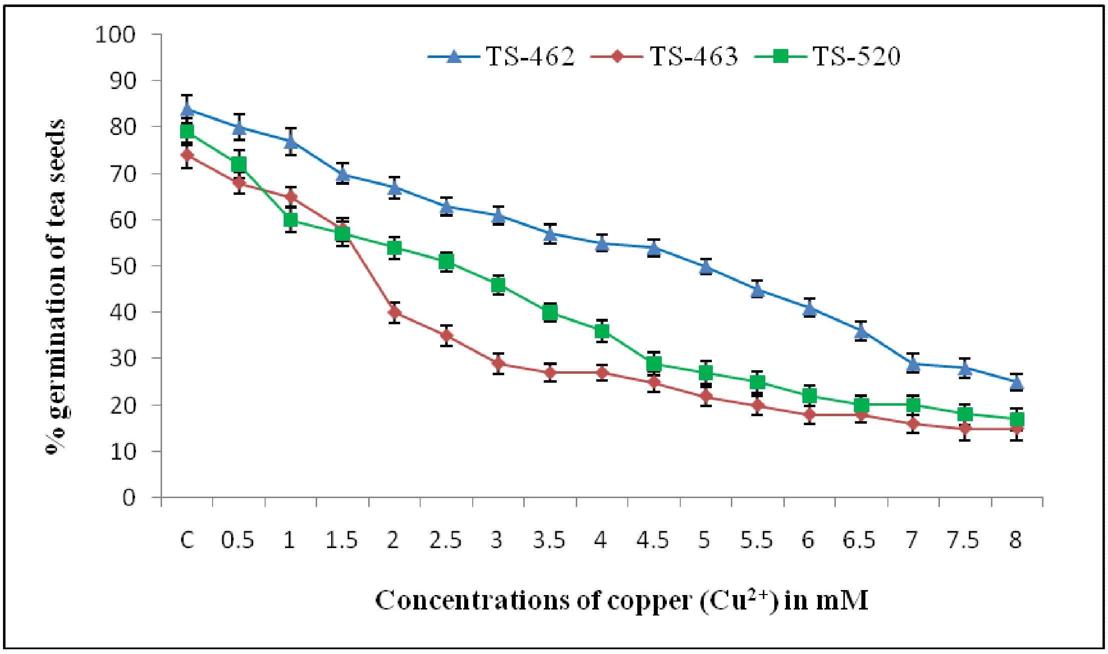


**Fig. 4.2:** Percent germination of seeds of tea cultivars (TS-462, TS-463 and TS-520) exposed to different concentrations of $CuSO_4$ solution. Data are mean of three replicates ± SE. C = Control.

**Table 4.2: Shoot elongation of TS-462 after 15, 18, 21, 24 and 27 days of treatment with different concentrations of copper solution**

| Concentration of $CuSO_4$ (mM) | Shoot length (in cm) | | | | |
|---|---|---|---|---|---|
| | **15 d** | **18 d** | **21d** | **24d** | **27d** |
| **Control** | 7.4±0.5 | 8.9±0.5 | 10.3±0.6 | 11.5±0.7 | 13.7±0.8 |
| **0.5** | 7.1±0.4 | 8.8±0.4 | 10.1±0.5 | 11.9±0.5 | 12.6±0.6 |
| **1.0** | 6.9±0.4 | 7.2±0.5 | 9.2±0.3 | 11.3±0.6 | 11.8±0.4 |
| **1.5** | 6.6±0.3 | 6.8±0.4 | 8.9±0.4 | 10.8±0.4 | 11.1±0.5 |
| **2.0** | 5.5±0.4 | 6.1±0.2 | 9.3±0.4 | 10.4±0.5 | 10.8±0.7 |
| **2.5** | 4.8±0.2 | 5.9±0.3 | 8.7±0.3 | 9.9±0.4 | 10.1±0.4 |
| **3.0** | 4.1±0.3 | 5.7±0.3 | 7.9±0.4 | 9.5±0.3 | 9.8±0.6 |
| **3.5** | 3.8±0.3 | 5.1±0.6 | 6.9±0.3 | 9.1±0.3 | 9.5±0.4 |
| **4.0** | 3.4±0.1 | 4.7±0.5 | 6.7±0.4 | 8.6±0.2 | 9.1±0.5 |
| **4.5** | 3.0±0.2 | 4.3±0.3 | 5.6±0.5 | 7.3±0.4 | 8.8±0.5 |
| **5.0** | 2.9±0.2 | 4.1±0.4 | 5.4±0.5 | 7.1±0.3 | 8.2±0.2 |
| **5.5** | 2.5±0.3 | 3.9±0.4 | 5.2±0.3 | 6.0±0.3 | 7.6±0.3 |
| **6.0** | 2.4±0.2 | 3.6±0.2 | 5.0±0.2 | 5.8±0.2 | 7.1±0.4 |
| **6.5** | 2.1±0.2 | 3.1±0.3 | 4.4±0.3 | 5.2±0.4 | 6.5±0.3 |
| **7.0** | 1.9±0.1 | 2.9±0.2 | 3.8±0.5 | 4.9±0.3 | 6.0±0.4 |
| **7.5** | 1.5±0.2 | 2.5±0.3 | 3.6±0.4 | 4.2±0.2 | 5.2±0.4 |
| **8.0** | 1.2±0.3 | 2.3±0.4 | 3.5±0.3 | 3.7±0.5 | 4.5±0.5 |
| **CD (5%)** | 0.20 | 0.21 | 0.23 | 0.25 | 0.26 |

*Data are mean of three replications; Data after ± indicate standard error values.

**Table 4.3: Shoot elongation of TS-463 after 15, 18, 21, 24 and 27 days of treatment with different concentrations of copper solution**

| Concentration of $CuSO_4$ (mM) | Shoot length (in cm) | | | | |
|---|---|---|---|---|---|
| | **15d** | **18d** | **21d** | **24d** | **27d** |
| **Control** | 5.5±0.5 | 6.5±0.5 | 7.5±0.4 | 9.8±0.6 | 11.9±0.7 |
| **0.5** | 5.1±0.4 | 6.3±0.3 | 7.1±0.5 | 9.5±0.5 | 10.6±0.6 |
| **1.0** | 4.4±0.3 | 6.0±0.5 | 6.8±0.4 | 8.8±0.4 | 9.0±0.6 |
| **1.5** | 4.3±0.5 | 5.6±0.4 | 6.6±0.4 | 8.5±0.5 | 8.8±0.5 |
| **2.0** | 4.2±0.3 | 5.3±0.5 | 6.5±0.3 | 7.0±0.5 | 7.6±0.5 |
| **2.5** | 4.1±0.4 | 5.2±0.3 | 6.3±0.3 | 6.9±0.3 | 7.2±0.4 |
| **3.0** | 3.7±0.4 | 5.1±0.2 | 6.1±0.3 | 7.7±0.4 | 6.8±0.5 |
| **3.5** | 3.3±0.3 | 4.2±0.3 | 5.9±0.2 | 7.3±0.4 | 6.0±0.5 |
| **4.0** | 3.1±0.3 | 3.9±0.4 | 5.7±0.3 | 7.1±0.4 | 5.2±0.3 |
| **4.5** | 2.9±0.2 | 3.7±0.3 | 4.9±0.2 | 6.4±0.3 | 4.9±0.4 |
| **5.0** | 2.4±0.2 | 3.2±0.2 | 4.3±0.1 | 6.0±0.3 | 4.5±0.2 |
| **5.5** | 2.1±0.2 | 2.9±0.3 | 4.2±0.1 | 5.8±0.3 | 4.0±0.4 |
| **6.0** | 1.8±0.2 | 2.8±0.3 | 4.0±0.2 | 4.8±0.2 | 3.5±0.2 |
| **6.5** | 1.3±0.3 | 1.5±0.2 | 1.9±0.1 | 2.3±0.2 | 2.5±0.3 |
| **7.0** | 1.1±0.1 | 1.2±0.2 | 1.8±0.2 | 2.1±0.1 | 2.3±0.2 |
| **7.5** | 0.9±0.2 | 1.1±0.3 | 1.3±0.1 | 1.9±0.1 | 2.0±0.3 |
| **8.0** | 0.7±0.2 | 0.9±0.2 | 1.1±0.1 | 1.3±0.2 | 1.4±0.3 |
| **CD (5%)** | 0.34 | 0.39 | 0.40 | 0.48 | 0.46 |

*Data are mean of three replications; Data after ± indicate standard error values.

**Table 4.4: Shoot elongation of TS-520 after 15, 18, 21, 24 and 27 days of treatment with different concentrations of copper solution**

| Concentration of $CuSO_4$ (mM) | Shoot length (in cm) | | | | |
|---|---|---|---|---|---|
| | **15d** | **18d** | **21d** | **24d** | **27d** |
| **Control** | 9.2±0.5 | 10.4±0.4 | 11.2±0.5 | 13.3±0.7 | 14.2±0.9 |
| **0.5** | 8.9±0.3 | 9.4±0.3 | 10.6±0.3 | 12.3±0.5 | 13.5±0.7 |
| **1.0** | 8.1±0.4 | 8.9±0.2 | 9.8±0.5 | 11.3±0.6 | 11.7±0.5 |
| **1.5** | 7.6±0.4 | 8.2±0.3 | 8.8±0.2 | 9.7±0.5 | 11.2±0.4 |
| **2.0** | 6.9±0.3 | 7.2±0.2 | 8.7±0.3 | 9.2±0.4 | 10.1±0.5 |
| **2.5** | 6.2±0.2 | 6.6±0.5 | 7.8±0.2 | 8.8±0.4 | 9.5±0.4 |
| **3.0** | 5.8±0.3 | 6.3±0.4 | 7.3±0.4 | 8.3±0.5 | 8.9±0.3 |
| **3.5** | 5.0±0.3 | 6.2±0.4 | 7.1±0.3 | 8.0±0.6 | 8.5±0.5 |
| **4.0** | 4.6±0.3 | 5.8±0.4 | 6.7±0.4 | 7.2±0.4 | 8.1±0.2 |
| **4.5** | 4.1±0.2 | 5.5±0.2 | 6.4±0.5 | 6.9±0.6 | 7.7±0.3 |
| **5.0** | 3.6±0.2 | 4.9±0.3 | 5.2±0.2 | 6.3±0.6 | 6.5±0.4 |
| **5.5** | 3.0±0.3 | 4.2±0.5 | 5.2±0.2 | 5.4±0.3 | 6.2±0.4 |
| **6.0** | 2.7±0.1 | 3.6±0.4 | 4.8±0.4 | 5.3±0.2 | 5.3±0.4 |
| **6.5** | 2.1±0.2 | 3.6±0.2 | 4.2±0.2 | 4.7±0.1 | 5.1±0.4 |
| **7.0** | 1.8±0.3 | 3.1±0.3 | 3.8±0.2 | 4.2±0.3 | 4.2±0.3 |
| **7.5** | 1.5±0.2 | 2.7±0.3 | 3.2±0.3 | 4.2±0.2 | 3.5±0.3 |
| **8.0** | 1.3±0.3 | 1.4±0.3 | 1.6±0.2 | 1.8±0.2 | 2.9±0.4 |
| **CD (5%)** | 0.29 | 0.22 | 0.28 | 0.33 | 0.50 |

*Data are mean of three replications; Data after ± indicate standard error values.

### 4.2.3. Effect of different concentration of copper on root elongation

The effect of the different concentrations of $Cu^{2+}$ on root elongation of three cultivars of germinated tea seeds are represented in Tables 4.5, 4.6 and 4.7. In general there was a reduction in root elongation as metal concentrations increased in comparison to the control (Figs. 4.3.1 and 4.3.2). The control seedlings showed 8.0 cm of growth after 27 days, however, the length of the roots in those treated with 8 mM Cu was found to be only 1.2 cm in TS-520 variety. Similar results were obtained with the other cultivars. Minimum growth was recorded in TS-463 which showed very little root growth (0.6 cm) after 27 days of Cu treatment. Minimum effect of copper was evident in TS-462 seedlings which recorded maximum growth (2.3 cm) in 8 mM exposed plants among the three cultivars. Maximum reduction in length of root was observed in TS-463 variety (91.5%) followed by TS-520 (85%) and TS-462 (69.3%) (Fig. 4.5). Morphological observations showed that higher concentrations of $Cu^{2+}$ (>6.5mM) caused several damaging effects such as reduced root hair proliferation, reduction in the number of root hairs, blacking of the root tips, stunted growth, deformed root and shoot structure and substantial reduction in the length of the root and shoots in all tested cultivars (Fig. 4.6).

### 4.2.4. Effect of different concentration of copper on dry weight of shoots and roots

Effect of different concentration of $Cu^{2+}$ on dry weights of shoots and roots of three cultivars of tea seedlings were also tested and the results are summarized in Figures 4.7 and 4.8. Dry weight of shoots and roots decreased with increasing concentration of $Cu^{2+}$. Overall results indicated that TS-462 was most resistant against $Cu^{2+}$ treatment and TS-463 was most susceptible. Dry shoot mass in 8 mM exposed seeds were found to be very low (0.041 mg) when compared to control (0.302 mg) in TS-462 cultivar. In TS-463 and TS-520 cultivars, the shoot mass reduced from 0.278 mg and 0.291 mg (control) to 0.011 mg and 0.029 mg respectively in tea seedlings treated with 8 mM concentration of $Cu^{2+}$ (Fig. 4.7). Dry weight in roots also showed substantial reduction when exposed to high $Cu^{2+}$ concentration. Dry weight of roots reduced to 0.019 mg, 0.011 mg and 0.041 mg in tea seedlings treated with 8 mM concentration of $Cu^{2+}$ in comparison to control


**Fig. 4.3.1:** Tea seedlings (cultivar TS-462) allowed to germinate and grow under increasing concentrations of $CuSO_4$ (0.5-8.0 mM) for 27 days.

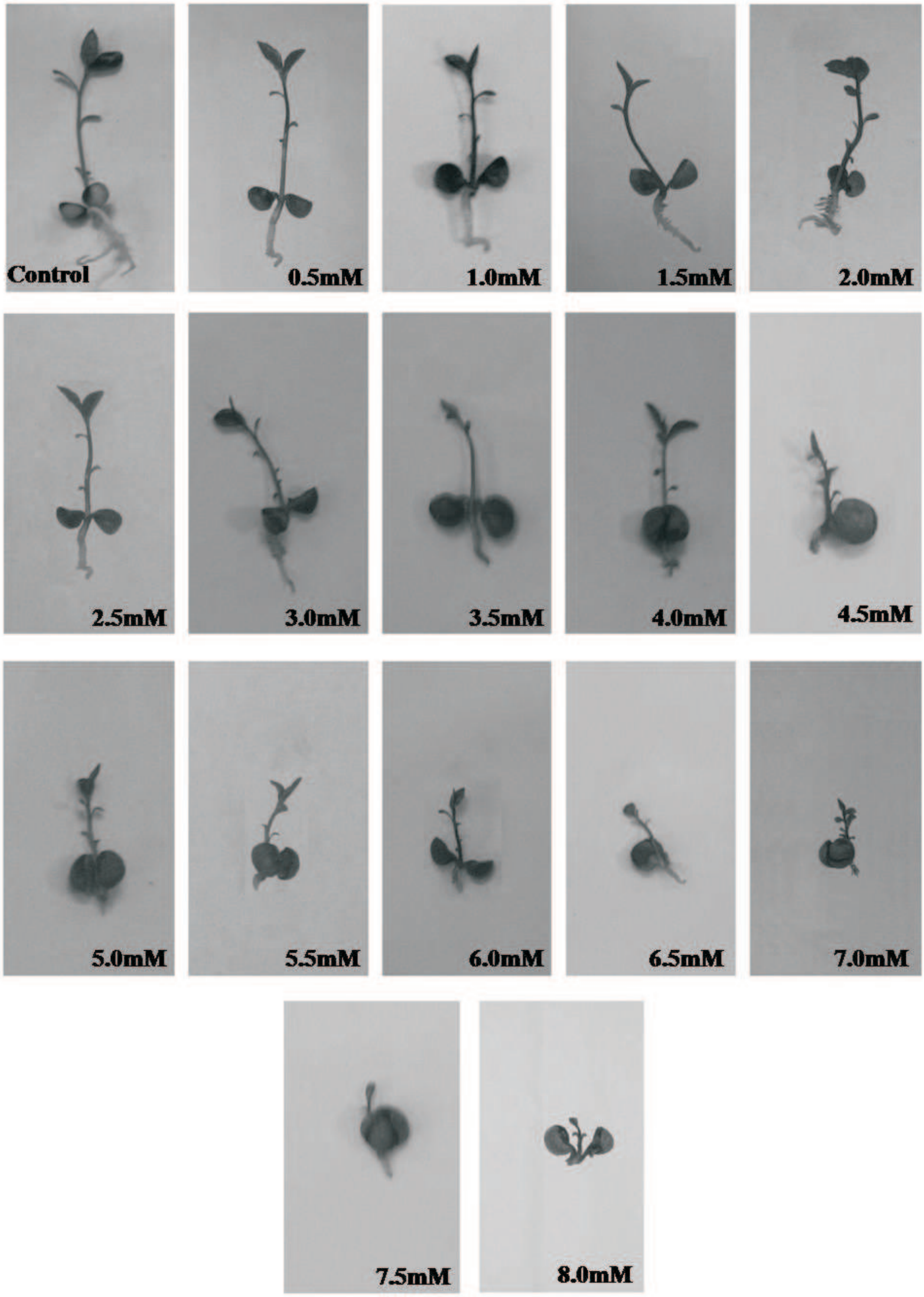


**Fig. 4.3.2:** Tea seedlings (cultivar TS-520) allowed to germinate and grow under increasing concentrations of $CuSO_4$ (0.5-8.0 mM) for 27 days.

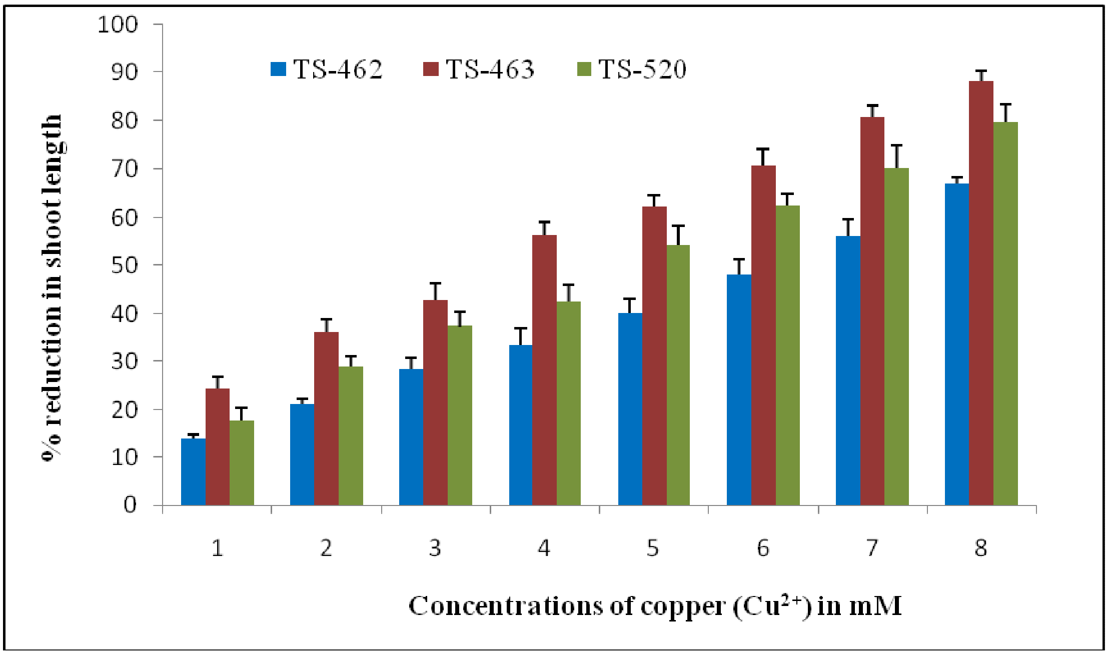


**Fig. 4.4:** Percent reduction in shoot length of tea seedlings of three cultivars of tea (TS-462, TS-463 and TS-520) allowed to germinate and grow under different concentrations of $CuSO_4$ solution for 27 days. Data are mean of three replicates ± SE.

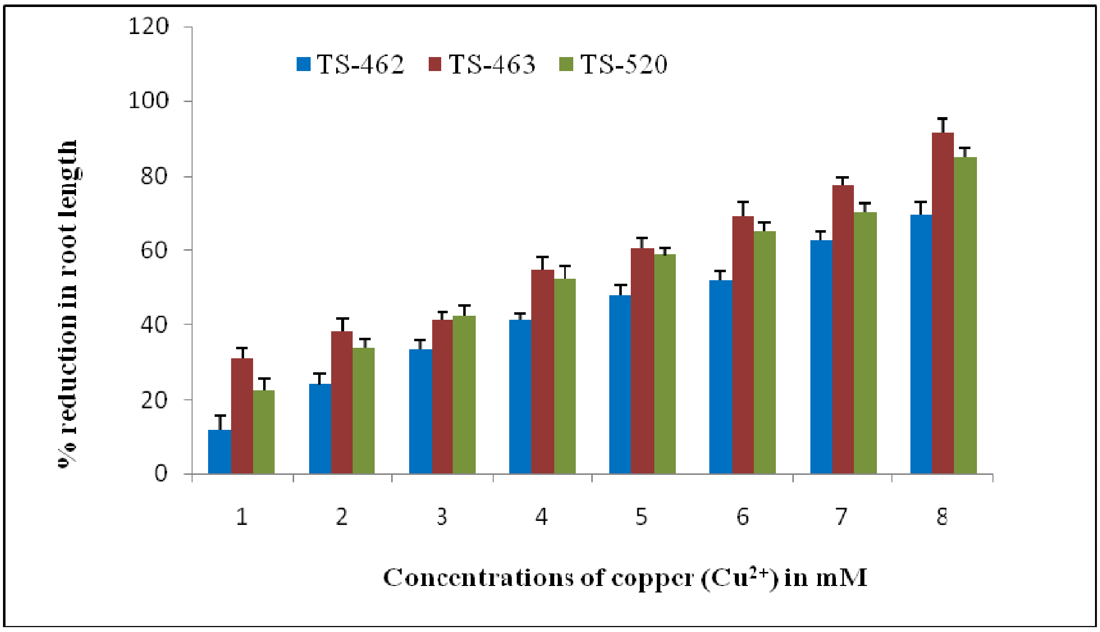


**Fig. 4.5:** Percent reduction in root length of tea seedlings of three cultivars of tea (TS-462, TS-463 and TS-520) allowed to germinate and grow under different concentrations of $CuSO_4$ solution for 27 days. Data are mean of three replicates ± SE.

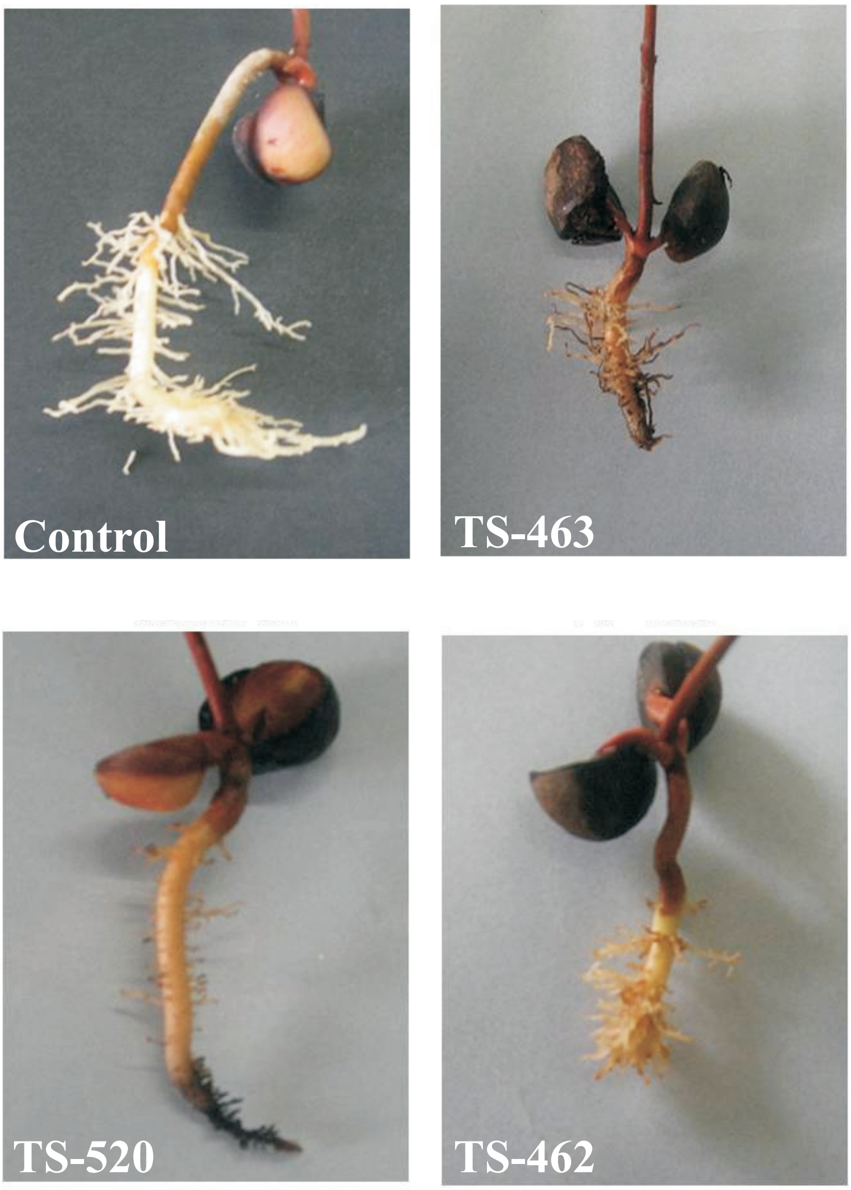


**Fig. 4.6:** Root deformations observed when tea seeds were allowed to germinate in high $Cu^{2+}$ concentrations (6.5mM) for 60 days.

which recorded 0.152 mg, 0.144 mg and 0.162 mg root dry weight of TS-520,TS-463 and TS-462 respectively (Fig. 4.8).

**Table 4.5: Root elongation of TS-462 after 15, 18, 21, 24 and 27 days of treatment with different concentrations of copper solution**

| Concentration of $CuSO_4$ (mM) | Root length (in cm) | | | | |
|---|---|---|---|---|---|
| | **15 d** | **18 d** | **21d** | **24d** | **27d** |
| **Control** | 3.8±0.4 | 4.9±0.2 | 5.8±0.3 | 7.0±0.3 | 7.5±0.4 |
| **0.5** | 3.7±0.3 | 4.5±0.1 | 5.1±0.1 | 6.2±0.4 | 7.0±0.3 |
| **1.0** | 3.5±0.3 | 4.0±0.1 | 4.8±0.2 | 5.9±0.2 | 6.6±0.3 |
| **1.5** | 3.3±0.2 | 3.5±0.1 | 4.5±0.1 | 5.4±0.3 | 5.9±0.4 |
| **2.0** | 2.9±0.1 | 3.3±0.2 | 4.0±0.2 | 4.7±0.3 | 5.7±0.3 |
| **2.5** | 2.8±0.3 | 3.1±0.1 | 3.3±0.2 | 4.0±0.5 | 5.3±0.2 |
| **3.0** | 2.6±0.2 | 2.9±0.3 | 3.1±0.1 | 3.6±0.3 | 5.0±0.3 |
| **3.5** | 2.5±0.3 | 2.7±0.3 | 3.0±0.2 | 3.5±0.2 | 4.7±0.2 |
| **4.0** | 2.4±0.1 | 2.5±0.1 | 2.9±0.4 | 3.3±0.2 | 4.4±0.1 |
| **4.5** | 2.2±0.2 | 2.4±0.2 | 2.7±0.3 | 3.2±0.1 | 4.3±0.2 |
| **5.0** | 1.8±0.2 | 2.1±0.2 | 2.6±0.1 | 3.0±0.3 | 3.9±0.2 |
| **5.5** | 1.5±0.1 | 2.0±0.1 | 2.4±0.2 | 2.9±0.2 | 3.7±0.3 |
| **6.0** | 1.3±0.2 | 1.8±0.1 | 2.2±0.2 | 2.8±0.1 | 3.6±0.1 |
| **6.5** | 1.0±0.3 | 1.3±0.2 | 2.0±0.1 | 2.6±0.1 | 3.2±0.2 |
| **7.0** | 0.9±0.2 | 1.2±0.3 | 1.8±0.1 | 2.3±0.2 | 2.8±0.3 |
| **7.5** | 0.7±0.1 | 1.0±0.1 | 1.6±0.1 | 2.0±0.1 | 2.5±0.2 |
| **8.0** | 0.6±0.2 | 0.9±0.1 | 1.3±0.2 | 1.9±0.3 | 2.3±0.4 |
| **CD (5%)** | 0.11 | 0.14 | 0.16 | 0.17 | 0.19 |

*Data are mean of three replications; Data after ± indicate standard error values.

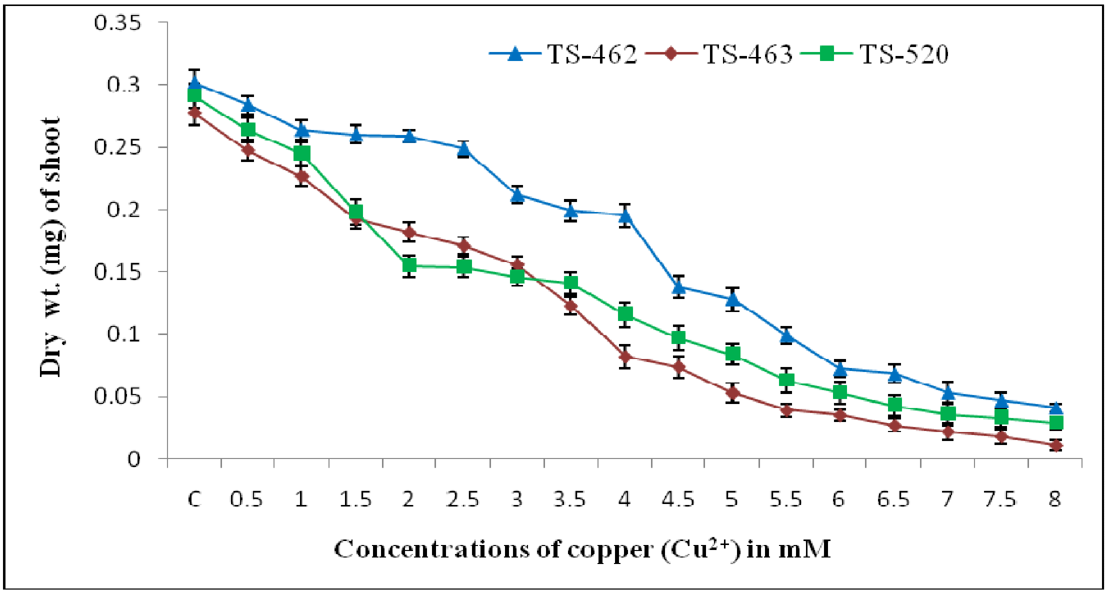


**Fig. 4.7:** Dry weight of shoots of tea seedlings of three cultivars of tea (TS-462, TS-463 and TS-520) germinated at different concentrations of $CuSO_4$ solution after 27 days of treatment. Data are mean of three replicates ± SE. C = Control.

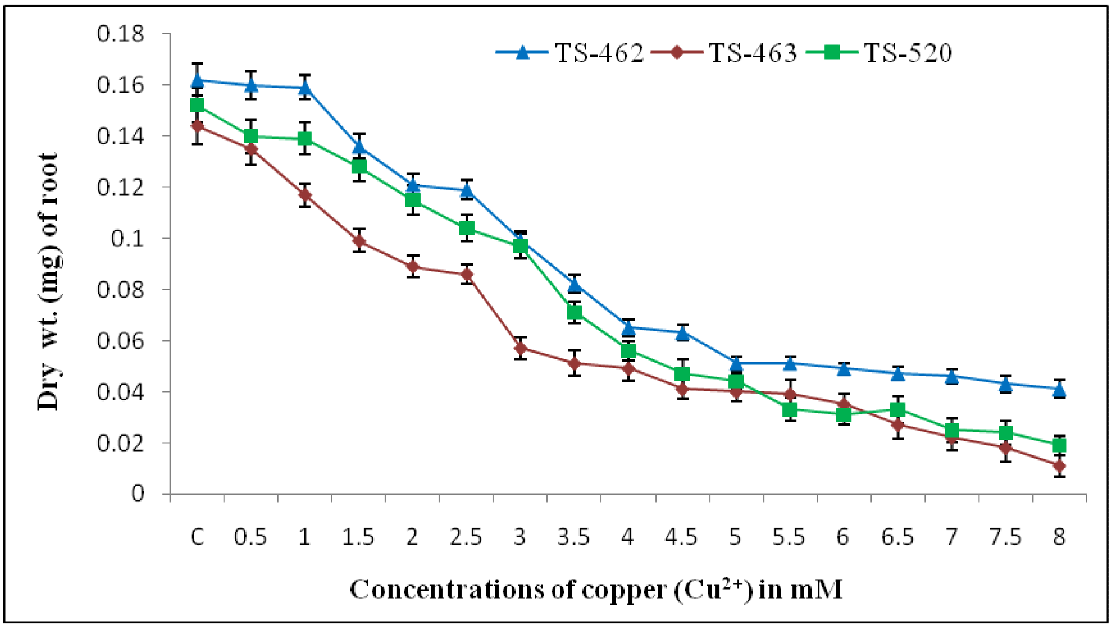


**Fig. 4.8:** Dry weight of roots of tea seedlings of three cultivars of tea (TS-462, TS-463 and TS-520) germinated at different concentrations of $CuSO_4$ solution after 27 days of treatment. Data are mean of three replicates ± SE. C = Control.

**Table 4.6: Root elongation of TS-463 after 15, 18, 21, 24 and 27 days of treatment with different concentrations of copper solution**

| Concentration of $CuSO_4$ (mM) | Root length (in cm) | | | | |
|---|---|---|---|---|---|
| | **15 d** | **18 d** | **21d** | **24d** | **27d** |
| **Control** | 3.1±0.4 | 4.5±0.3 | 5.5±0.4 | 6.6±0.5 | 7.1±0.5 |
| **0.5** | 2.9±0.5 | 3.5±0.3 | 4.8±0.3 | 5.7±0.3 | 6.3±0.4 |
| **1.0** | 2.7±0.4 | 3.1±0.2 | 3.6±0.3 | 4.2±0.4 | 4.9±0.3 |
| **1.5** | 2.5±0.3 | 2.9±0.5 | 3.4±0.4 | 4.0±0.4 | 4.5±0.3 |
| **2.0** | 2.3±0.2 | 2.8±0.4 | 3.2±0.5 | 3.7±0.2 | 4.4±0.4 |
| **2.5** | 2.1±0.4 | 2.1±0.1 | 2.9±0.2 | 3.3±0.3 | 4.1±0.3 |
| **3.0** | 1.9±0.2 | 2.1±0.5 | 2.8±0.3 | 3.1±0.5 | 3.8±0.2 |
| **3.5** | 1.7±0.2 | 1.9±0.1 | 2.7±0.1 | 3.0±0.2 | 3.4±0.3 |
| **4.0** | 1.5±0.3 | 1.8±0.2 | 2.1±0.2 | 2.5±0.2 | 3.2±0.2 |
| **4.5** | 1.3±0.4 | 1.7±0.1 | 2.0±0.5 | 2.4±0.3 | 2.9±0.3 |
| **5.0** | 1.1±0.2 | 1.6±0.2 | 1.8±0.3 | 2.3±0.3 | 2.8±0.3 |
| **5.5** | 1.0±0.4 | 1.4±0.3 | 1.7±0.3 | 2.1±0.2 | 2.5±0.2 |
| **6.0** | 0.9±0.2 | 1.1±0.1 | 1.6±0.2 | 2.0±0.1 | 2.2±0.3 |
| **6.5** | 0.8±0.3 | 1.0±0.2 | 1.3±0.2 | 1.9±0.2 | 2.1±0.2 |
| **7.0** | 0.6±0.2 | 0.9±0.4 | 1.1±0.3 | 1.3±0.1 | 1.6±0.2 |
| **7.5** | 0.4±0.1 | 0.8±0.3 | 0.8±0.2 | 0.9±0.2 | 1.0±0.1 |
| **8.0** | 0.3±0.1 | 0.4±0.1 | 0.5±0.1 | 0.6±0.2 | 0.6±0.2 |
| **CD (5%)** | 0.12 | 0.15 | 0.16 | 0.18 | 0.19 |

*Data are mean of three replications; Data after ± indicate standard error values.

**Table 4.7: Root elongation of TS-520 after 15, 18, 21, 24 and 27 days of treatment with different concentrations of copper solution**

| Concentration of $CuSO_4$ (mM) | Root length (in cm) | | | | |
|---|---|---|---|---|---|
| | **15 d** | **18 d** | **21d** | **24d** | **27d** |
| **Control** | 4.5±0.3 | 6.8±0.3 | 7.2±0.5 | 7.7±0.6 | 8.0±0.6 |
| **0.5** | 4.3±0.2 | 5.2±0.3 | 6.6±0.3 | 6.7±0.4 | 7.3±0.6 |
| **1.0** | 4.1±0.2 | 5.1±0.4 | 5.8±0.3 | 5.7±0.4 | 6.2±0.4 |
| **1.5** | 3.9±0.3 | 4.5±0.2 | 5.2±0.2 | 5.5±0.6 | 5.7±0.5 |
| **2.0** | 3.7±0.4 | 4.2±0.4 | 4.9±0.4 | 5.0±0.5 | 5.3±0.4 |
| **2.5** | 3.5±0.3 | 3.9±0.3 | 4.5±0.5 | 4.7±0.3 | 5.1±0.3 |
| **3.0** | 3.2±0.2 | 3.5±0.3 | 4.1±0.5 | 4.3±0.3 | 4.6±0.2 |
| **3.5** | 2.9±0.3 | 3.1±0.2 | 3.6±0.3 | 3.8±0.4 | 4.1±0.3 |
| **4.0** | 2.7±0.2 | 3.1±0.4 | 3.5±0.4 | 3.6±0.3 | 3.8±0.4 |
| **4.5** | 2.6±0.4 | 2.9±0.4 | 3.2±0.3 | 3.3±0.2 | 3.5±0.2 |
| **5.0** | 2.3±0.1 | 2.8±0.4 | 3.0±0.5 | 3.1±0.3 | 3.3±0.3 |
| **5.5** | 2.2±0.3 | 2.4±0.3 | 2.6±0.2 | 2.7±0.2 | 2.9±0.2 |
| **6.0** | 2.0±0.2 | 2.3±0.4 | 2.4±0.3 | 2.6±0.2 | 2.8±0.4 |
| **6.5** | 1.7±0.3 | 1.9±0.3 | 2.2±0.2 | 2.3±0.3 | 2.5±0.2 |
| **7.0** | 1.4±0.3 | 1.6±0.2 | 2.0±0.4 | 2.1±0.3 | 2.4±0.2 |
| **7.5** | 0.9±0.2 | 1.1±0.3 | 1.4±0.3 | 1.6±0.2 | 1.7±0.3 |
| **8.0** | 0.6±0.2 | 0.7±0.2 | 0.9±0.3 | 1.0±0.2 | 1.2±0.3 |
| **CD (5%)** | 0.11 | 0.13 | 0.14 | 0.15 | 0.18 |

*Data are mean of three replications; Data after ± indicate standard error values.

## 4.3 DISCUSSION

Seed germination is a process of crucial importance and is therefore tightly regulated. It is also a stage in the plant life cycle that is well protected against various stresses (Li *et al.*, 2005). Immediately after imbibition and at the onset of vegetative developmental processes, they become stress-sensitive in general. So, seeds are equipped with sensing mechanism that allow it to obtain the information required to assure that germination will only occur when environmental factors are favourable to complete developmental process. Therefore, seeds are thought to carefully monitor such external parameters as light, temperature and nutrient in order to maintain the protective state until external conditions become favourable for following developmental processes (Karssen, 1982; Pritchard *et al.*, 1993; Bungard *et al.*, 1997). Although such critical regulatory mechanisms are operated in seeds, little information is known about how stress tolerance is modulated at different phases of germination.

In the current study, seed germination percentage reduced considerably in all the tested cultivars under copper stress. In a study on *Miscanthus* species of Taiwan, Hsu and Chou (1992) found that seed germination percentage decreased from 98% (control) to 28% in *M. transmorrisonensis* seeds and from 94.6% (control) to 13.6% in *M. floridulus* seeds that were exposed to 1000 ppm $Cu^{2+}$. Peralta *et al.* (2000) investigated the individual effects of several doses of heavy metals on seed germination and growth of alfalfa plants using solid media. They used 0, 5, 10, 20 and 40 ppm doses for experiment and according to them, the data obtained in general showed a reduction in seed germination as metal concentration in the growing media increased. In another study on wheat and cucumber, it was observed that the inhibitory effect of Cu on germination of wheat seeds was apparent even at 0.5 mM concentration, causing a more than 20% decrease in germination rate (Munzuroglu and Geckil, 2002). Singh *et al.* (2007) studied the seed germination and seedling growth in wheat under the influence of different concentrations of copper (5, 25, 50 and 100 mg $l^{-1}$). They observed that the germination percentage decreased with increase in copper concentration after 14$^{th}$ and 21$^{st}$ day of treatment. In another similar study, Hu *et al.* (2007) found that with the enhancement of copper stress, the germination percentage of wheat seeds decreased gradually, however, nitric oxide could greatly reverse this inhibitory effect. In a study on rice seed germination, Ahsan *et al.* (2007) found that the process of rice seed germination was inhibited by excess copper. Ye *et al.* (2014) also studied the mechanism of copper-induced inhibition of seed germination in rice seeds and found that Cu concentration at 30 μM effectively inhibited germination of rice caryopsis. In a study on seeds of bean (*Phaseolus vulgaris*), Sfaxi-Bousbih *et al.* (2010) observed that, copper stress provoked a reduction in germination rate. Gill *et al.* (2012) studied the physiological response of *Arabidopsis* seeds constitutively over-expressing SOD of *Potentilla atrosanguinea* which is known to ameliorate oxidative stress during germination in response to varied concentrations of copper sulphate (Cu stress). Transgenics showed higher germination percentage and required less "mean time to germination" under Cu-stress. In response to Cu stress, 39 differentially expressed protein spots were detected by 2-D electrophoresis in proteins of germinating wild type (WT) and transgenic seeds, of which 14 spots appeared exclusively in transgenics. The authors concluded that changes in key proteins, *vis-a-vis* alleviation of oxidative stress in transgenic *Arabidopsis* over-expressing SOD possibly alleviated toxicity of Cu-induced stress during seed germination, resulting in higher germination rate and germination percentage.

However, there are also reports that excess copper did not affect the seed germination percentage or rate of seed germination although other damaging effects such as growth inhibition were seen. For instance, the results obtained by Li *et al.* (2005) showed that seedling growth is more sensitive to heavy metals including $Cu^{2+}$ in comparison to seed germination. Mahmood *et al.* (2005) observed that germination of corn (*Zea mays*) was not affected by copper concentrations at 3 to 12 ppm. Similarly, Verma *et al.* (2011) found that copper sulphate solution has insignificant effect on the percent germination of mungbean (*Vigna radiata*).

In the current study, seedling growth showed the damaging effects of copper stress as both shoot and root elongation decreased with increasing copper concentration. Moreover a significant variation was seen among cultivars, TS-462 being the most resistant. In a study on *Miscanthus* species, Hsu and Chou (1992) found that the percent inhibition of overall seedling growth was between 66.1% and 85.4% in germinated seedlings exposed to $Cu^{2+}$ ions at 10 ppm. Peralta *et al.* (2000) observed a concentration dependent inhibition of root growth in alfalfa plants at the dose of 20 and 40 ppm of $Cu^{2+}$. The authors found that excess Cu (II) exerts detrimental effects causing a shoot and root elongation reduction of 70.0% and 54% respectively. Munzuroglu and Geckil (2002) observed that the higher concentrations of Cu caused a complete inhibition of roots at greater than 1.5 mM concentration and coleoptiles at greater than 2.0 mM concentration in wheat. Similarly, Singh *et al.* (2007) observed that plumule elongation and radicle elongation was considerably reduced in wheat exposed to excess (100 mg/L) copper.A study on *Zea mays* showed that shoot length reduced from 6.28 cm in control to 4.25 cm and root length reduced from 3.49 cm in control to 1.25 cm in seedlings exposed to 12 ppm $Cu^{2+}$ (Mahmood *et al.* 2005). Ali *et al.* (2006) observed that root treated with 50 μM copper resulted in 52% and 89% growth inhibition after 20 & 40 days of treatment respectively in *Panax* *ginseng*. Ahsan *et al.* (2007) found that shoot elongation in germinated rice seeds was inhibited by excess copper. Di Salvatore *et al.* (2008) evaluated the toxicity of heavy metals including Cu on lettuce, broccoli, tomato and radish seed and observed that copper ions inhibited radical development in the crops during their seed germination. Zhao *et al.* (2010) studied root elongation in turfgrass (*Festuca arundinacea* and *Lolium perenne*). The seeds of the plants were germinated and treated with different concentrations of Cu supplied as $CuCl_2$ solution {0(control), 30, 60, 90, and 120 mg/L}. Cu treatments significantly inhibited root elongation. The decrease was more pronounced in *F. arundinacea.* After two weeks, roots of *F. arundinacea* and *L. perenne* treated with 30 mg/L $Cu^{2+}$ decreased by 58.5% and 40.2% respectively versus the controls. Manivasagaperumal *et al.* (2011) reported that 100-200 mg/kg concentration of copper reduces the growth of the shoots and roots of *Vigna radiata*. In another study on the same plant, Verma *et al.* (2011) observed the germination, plant growth, protein content and antioxidant enzyme activity of mungbean (*Vigna radiata*) under the influence of different concentrations of copper. The authors observed that plumule and radicle length decreased with increase in copper concentration (50, 200, 500 and 1000 µM copper sulphate solution).

In the current study, morphological changes such as reduced root hair proliferation, reduction in the number of root hairs, blacking of the root tips, stunted growth, deformed root and shoot structure was evident on exposure to excess copper. The shoot and root dry weight also declined making it evident that high concentration of copper caused deleterious effects on tea seed germination and early seedling growth. Among the tested cultivars TS-462 was found to perform better than the other two under copper stress. Zheng *et al.* (2004) worked on the response of three ornamental crops, chrysanthemum, miniature rose and zonal geranium to different solution levels of $Cu^{2+}$ (ranging from 0.4-40 µM). They observed that excessive copper reduced the shoot and root dry weight of all three species. In another study, Sheldon and Menzies (2004) observed that excess copper in Rhodes grass (*Chloris gayana*) seeds caused disruption of the cuticle on the main root, reduction in the number and length of root hairs on the main root and damage to the root meristem. Lateral roots showed excessive branching and deformation. Singh *et al.* (2007) found that the number of lateral roots in germinated wheat seedlings decreased in 50 and 100 mg/L of copper treatment. According to Azooz *et al.* (2012), copper concentration above 10 mM reduced the dry weight of wheat. Ahsan *et al.* (2007) found that increasing copper concentrations from 0.2 mM to 1.5 mM caused a decrease in plant biomass in germinated rice seeds. In another study in turfgrass (*Festuca arundinacea* L. and *Lolium perenne* L.) where the seeds were treated with different concentrations of Cu (0, 30, 60, 90, and 120 mg/L), visible symptoms of copper toxicity were found at higher $Cu^{2+}$concentration. After 7d of Cu treatment, growth was severely retarded, and short browning roots were observed. The dry root biomass of the two turfgrasses also decreased by 37.1% and 62% under copper stress, especially at the higher (120 mg/L) Cu concentration (Zhao *et al.*, 2010). Manivasagaperumal *et al.* (2011) reported the decline of dry weight of *Vigna radiata* with increasing copper concentration of 100-200 mg/kg.

From these observations it can be concluded that excess copper had some effect on germination, growth and dry matter yield of three commonly grown tea biclonal seed stocks. Varietal differences in response towards $Cu^{2+}$ were observed in the shoot elongation study. However, in the roots, the differences were less prominent possibly due to the fact that $Cu^{2+}$ accumulation occurs much more in the roots than in the shoots leading to a greater damage in the roots which minimizes the differential effect within cultivars. Differences among cultivars in response to $Cu^{2+}$ stress have been found in other plants such as *Triticum durum* (Ciscato *et al.*, 1997), *Holcus lanatus* (Hartley-Whitaker *et al.*, 2001) and *Kummerowia stipulacea* (Xiong *et al.*, 2008). Inhibitory action of excess copper in root and shoot elongation and in their damage observed during the present study may be due to reduction in cell division, toxic effect on respiration and protein synthesis (Manivasagaperumal *et al.*, 2011; Kupper *et al.*, 1996; Sonmez *et al.*, 2006). Our results indicate that a detail study on the effect of excess copper on tea plants is warranted.