ABSTRACT

Tea, an evergreen woody perennial, is an important plantation crop of north east India. Tea industry is the second largest in India in terms of employment and drives the economy of the entire region where it is grown. Being a monoculture crop with a productive lifetime of 25 years, serious damages by pests and diseases are common with significant impact on productivity and quality. Prevention of crop loss requires spraying of huge amount of pesticides, several of which are copper based compounds. Accumulation of copper in the soil through the years can cause toxicity problems for the plants. The objective of this work was to study the effect of excess copper on tea in terms of copper accumulation in plant tissue, inhibition of seed germination and early seedling growth and physiobiochemical changes caused by copper excess.

Before beginning the experimental work, a detailed survey of tea estates of sub-Himalayan West Bengal and Barak Valley region of Assam located in the agro-climatic zone of northeast India was undertaken to learn the status of copper-based fungicides which was reported to be applied in tea. Results revealed that all tea gardens applied copper oxychloride or copper hydroxide on a regular basis and some used multiple fungicides all of which contained copper.

Changes in seed germination percentage and early seedling growth in presence of varying concentrations of excess copper was studied using tea seeds of three cultivars, TS-462, TS-463 and TS-520. Sixteen different concentrations (0.5 mM – 8.0 mM) with 0.5 mM increments of copper sulphate solutions were used for application in duplicate sets. TS-463 showed maximum inhibition of germination and growth while TS-462 was least affected. Several morphological changes such as reduced root hair proliferation, reduction in the number of root hairs, blackening 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 were noticed.

Copper induced changes in physiobiochemical parameters was studied under hydroponic system using three month old tea seedlings of TS-462 and TS-520 cultivars. Results were noted on the 4^{th}, 7^{th} and 10^{th} day

after start of treatment with increasing concentrations of copper (50 to 700 μM). Control plants were exposed to nutrient solution only without excess copper. Tea plants were found to accumulate moderate amounts of copper but this copper was mainly retained in the roots and a small amount was transported to the leaves. As copper is a redox active metal, it generates reactive oxygen species (ROS) directly through Haber-Weiss reactions. Several stress related changes primarily due to ROS generation, which are part of the plant's inner capacity to scavenge ROS and minimize the detrimental effects through detoxification mechanisms, were evident. Lipid peroxidation, which was measured in terms of TBARS accumulated, was found to depend on time and concentration. Increased lipid peroxidation in leaves showed the involvement of ROS. This was further proved when dose dependent increase in superoxide anion (O_2^-) production was observed in treated tea seedling cultivars in comparison to control. TS-462 was found to be less prone to ROS damage than TS-520.

A strong indication of injury to plants was evident as chlorophyll a, chlorophyll b and carotenoid content was found to decrease significantly with increasing Cu concentrations and exposure times in both the cultivars of tea.Carbohydrate content in the leaves also decreased progressively in a similar way. The more sensitive cultivar (TS-520) recorded a significantly higher decrease in carbohydrate and pigments content. The quantity of proteins, which are the most abundant cellular component oxidized by ROS was found to increase marginally in the leaves with increasing copper concentrations when the dose was low but decreased considerably at higher doses.

The level of proline, which is regarded as an important molecule in redox signaling and also a scavenger of ROS, increased steadily with increasing concentration of copper in the nutrient solution. However, at high exposure concentrations (> 500 μM), there was a marginal decrease in both cultivars. Both phenolics and o-dihydroxyphenolics content increased in the leaves with increase in concentration of copper in comparison to control. Non-protein thiol content showed an increase with time upto 7th day after which it declined at all exposure concentrations in both root and leaf when compared to control.

Activity of enzymes involved in ROS detoxification showed significant alterations in a time and dose dependent manner in leaves and roots of tea seedlings exposed to excess copper. Superoxide dismutase (SOD) activity increased with increasing Cu concentrations during the first seven days however, with the longer exposure time and above 400 µM Cu concentrations, the activity either levelled off (TS-462) or returned almost to the original level (TS-520). Peroxidase (POD) activity increased at lower exposure concentrations but declined at concentrations higher than 400 µM. Ascorbate peroxidase (APX) activity recorded a four-fold increase in the more sensitive cultivar (TS-520) but the activity declined at concentrations higher than 400 µM. A sharp increase in APX activity was noticed at the 10th day of exposure at concentrations 400 µM to 600 µM in the more tolerant cultivar, TS-462 but declined at 700 µM. Catalase (CAT) activity remained mainly unaltered in response to oxidative damage induced by Cu. A single SOD isozyme was evident to be present in native PAGE gels; highest band intensity was recorded by 500µM copper exposed plants. Two new isozymes POD 3 (R_f 0.42) and POD 4 (R_f 0.51) which was not present in control plants were induced in the leaves of tea exposed to 500µM of Cu. APX activity staining in native PAGE gels revealed two isozymes and both were regulated by fluctuating copper levels. APX 2 increased at 500µM concentrations and decreased at higher levels but APX 1 increased at lower concentrations but the levels did not fall at higher concentrations. CAT activity staining revealed a single band at all treatments; the intensities were slightly increased in plants exposed to higher copper doses.

The immunolocalization and distribution of peroxidases in roots of copper exposed plants were studied by immunogold lebeling followed by silver enhancement. Gold labeling was denser in the cortex and epidermis of treated plants in comparison to control plants. Tansmission electron microscopy revealed that copper was associated with the cell wall and precipitated in vacuole of tea roots.

From the results of the present study it seems most likely that Cu induces oxidative damage in tea leaves leading to the formation of ROS. This in turn caused an induction of enzymes involved in the scavenging of superoxide radical and H_2O_2. But when the ROS production was very high, it exceeded the endogenous capacity of the plant to scavenge the ROS which

upset the regulated balance between the scavenging system and the generating system leading to the inactivation of defense enzymes. In addition, our results showed a significant difference in the two Cu stressed cultivars of tea where the more sensitive cultivar seems to lose its antioxidative capacity at Cu concentrations higher than 400 µM while the more tolerant cultivar being able to withstand a maximum of 600 µM of Cu^{2+} ions. Further studies on the changes that occur at the genetic level causing the disruption of redox poise of the plant are warranted.