Biochemical responses of maize seedlings exposed to SnNPs

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With the increased use of nanotechnology in almost all aspects of life, there is an increasing chance of risk of plants exposure to different nanoparticles (NPs). However, the studies on its potentially harmful effects in the cultivated crops are not well studied yet. Therefore, the authors aimed to study the effects of tin (Sn) NPs on the growth and physiological aspects of *Zea mays*. Thus, they exposed the maize seedlings to different concentrations of SnNPs for 2 weeks, and results revealed that the SnNPs at the studied concentration were not able to affect the seedling growth at a significant level. However, it induced the oxidative stress which was confirmed by increased guaiacol peroxidase, polyphenol oxidase and catalase activity. They also discussed that exposure duration, aggregation and concentrations were contributing factors in NPs mediated metal toxicity.

1. Introduction: Nanotechnology is focused on the creation and manipulation of particles and materials in nanodimension (up to 100 nm). Recently nanoparticles (NPs) have attracted higher attention because of their potential applications in various areas [1, 2]. They display unusual physical and chemical properties that are distinct from bulk materials [2–4] because of high surface energy induced by high surface area to volume ratio [5, 6]. Owing to the exponential use of NP in diverse fields, NPs are expected to contaminate the aquatic and terrestrial environments. Various regulatory agencies of different countries want to ensure that the products manufactured in industries do not adversely affect human health and environment. The deleterious effects of nanoscale substances, e.g. NP depends on the size, shape or chemical and physical states. So, the safety data of all the nanomaterials is a necessary requirement [7].

Tin NPs (SnNPs) are one of the most studied metal NPs which are investigated extensively and are widely used in various applications. Sn metal and its alloys are used as interconnect materials in the electronics manufacturing process [8, 9]. Considering the potential lowering of reflow temperature and the trend of reduction of feature dimension, Sn-based solder NPs are being investigated for interconnect application [5, 10]. It has been shown that the diffusion of copper (Cu) into Sn during the soldering reaction between Cu NPs and SnNPs occurs below the melting point of Sn [11]. Other than soldering applications, the changes in the electrical resistance after accepting electrons from the donor compounds make SnNPs excellent sensors for ammonia gas [12]. Changes in microstructures and phase transformations of SnNPs during electrochemical reaction revealed excellent cyclability during the reversible sodium insertion/sodium extraction cycles. This showed the potential of SnNPs as an electrode material for rechargeable batteries [13, 14], for advanced heat transfer and thermal energy storage [15].

The large-scale production of SnNPs for commercial use will inevitably lead to their release to the environment, and the probable risk posed by these NPs to plants needs to be accessed. The literature search indicated that ill effects and risk of SnNPs on biological systems are not yet well-identified. Most of the previous toxicity studies were based on the evaluation of the potential toxicity of SnO_2 NPs on bacteria [16–20], animal cell lines [21], soil microbes [22], yeast [23] and tomato [24]. The studies with various bacteria have shown that the smaller size NPs are more toxic and interaction between the charge on the surface of the cell and particles play a critical role in mechanism for NPs toxicity [16]. Moreover, the antibacterial activity can be increased by doping with the other metal and non-metal compounds [17]. On the other hand, study with the cell lines to assess the cytotoxicity of biologically synthesised SnO_2 NPs showed that reactive oxygen species (ROS) generated after the exposure are the major stress factors [21]. In terms of plants, with our best efforts, we could find only one research on the interaction of SnO_2 NPs with tomato plants [24]. According to the results, the exposure of SnO_2 NPs reduced the root and shoot growth in terms of dry weight. The translocation of Sn was less as compared with other NPs, and higher accumulation of Sn metal occurred in the roots [24]. This may be due to incomplete dissolution of oxide NPs, and lesser mineralisation in soil and plant. These results suggested that the effects of pure SnNPs on the biological system need to be evaluated separately. The effects and impact of the NP largely depend on elemental composition, size and stability of NPs. In this Letter, maize was chosen as an experimental plant to characterise the effect of SnNPs exposure. The experiment was conducted to determine the effect of different concentrations of Sn particles on growth and stress-associated marker enzymes such as guaiacol peroxidase (GPX), polyphenol oxidase (PPO) and catalase (CAT) [25, 26].

2. Experimental methods

2.1. NPs characterisation: Commercial SnNPs (80 nm) produced by using an electrical explosion method were used in the present Letter. NPs were characterised using scanning electron microscope (SEM) using high-resolution transmission electron microscope (HRTEM) using 10 and 200 keV beam energies. SEM study was carried out by taking the NPs on carbon tape. For HRTEM, 200 mg of SnNPs were suspended in 50 ml ethyl alcohol and sonicated for 2 min. A drop of NP-alcohol suspension was placed on a lacey carbon film coated 200 mesh Cu grids for HRTEM investigation with a point resolution of 0.23 nm and a lattice resolution of 0.14 nm. The HRTEM micrographs were further analysed using fast Fourier transform (FFT) and inverse FFT. Crystal structure

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and crystallite size of the NPs were determined by X-ray diffraction (XRD) using the 1.5418 Å wavelength of CuKα at a scan rate of 1°/min and by electron diffraction (ED) with the convergent angle of 1.5–2.0 mrad in the HRTEM. Elemental analysis and crystal structure of NPs were determined by XRD, ED and energy dispersive X-ray spectroscopy (EDX) using silicon drift detectors.

2.2. Plant growth conditions: Owing to its high-economic importance in agriculture and foods, maize plant was taken as a model plant to test the effect of different concentrations of nanoSn. Maize seeds were sterilised in a 5% sodium hypochlorite solution for 10 min and 70% ethanol for 5 min. Then, it was rinsed with sterile distilled water several times to remove the metal traces [27]. The sterilised seeds were grown in tissue culture bottles with 100 ml sterilised Murashige and Skoog (MS) medium without sucrose [28]. SnNPs were added in different concentrations (25, 35 and 50 mg/100 ml) immediately before the pourable temperature of media. *The in vitro* study was carried out in plant tissue culture laboratory under controlled conditions of (2500 lx) 8 h dark period, 60–70% relative humidity. The whole experiment was conducted in three replicates ($n = 3$) with three seeds per jam bottle.

2.3. Sample collection: After 2 weeks of seedlings growth, samples were taken out of the container, gently by picking the plant from media and the shoot and root lengths were measured by using a ruler. Fresh and dry weights of the seedlings were measured. For dry weight, samples were dried 80°C. For the enzymatic analysis, plant samples were collected and immediately frozen into liquid nitrogen and stored in −80°C until further analysis.

2.4. Analysis of antioxidant enzymes: All samples were prepared for enzyme analyses by homogenising the frozen tissue material, and extraction was done by the modified method as reported previously [29]. For the seedlings tissue, 100 mg was lyophilised in liquid nitrogen and homogenised with 1 ml of potassium phosphate buffer (100 mM potassium phosphate buffer pH 7.4, containing 1 mM ethylenediaminetetraacetic acid, 1 mM phenylmethyl sulphonyl fluoride and 2% polyvinyl poly pyrrolidine) in a cold mortar. The homogenate was centrifuged at 14,000 rpm for 20 min at 4°C, and the supernatant was used for further enzyme activity analysis. Protein estimation was done by Bradford method [30].

The activity of representative enzymes that was chosen as a stress marker was estimated by standard methods. GPX activity was performed by the method as described previously [31, 32]. The enzyme activity was estimated based on the oxidation of guaiacol by GPX enzyme and the rate of formation of oxidised guaiacol was recorded spectrophotometrically at 470 nm at every 30 s for 5 min. For this, 100 µl of the extract was added to 2.5 ml of a reaction mixture containing 100 mM potassium phosphate buffer (pH 7), 50 µl of guaiacol (20 mM) as a substrate and 50 µl of hydrogen peroxide (H_2O_2) (12.3 mM). The rate of formation of oxidised guaiacol was measured spectrophotometrically at 470 nm every 30 s for 5 min. The enzyme activity was calculated using a molar extinction coefficient (e) of 26.6 mM^{-1} cm^{-1}. GPX activity was expressed as l M guaiacol oxidation min^{-1} mg $protein^{-1}$. The estimation of PPO activity estimation was performed by pre-described method [27, 28]. Catechol was used as an enzyme substrate and PPO activity was expressed as l M product formed per min/mg protein using a molar coefficient value of 1300 M^{-1} cm^{-1}. About 50 µl enzyme extract was added in 2.5 ml of working solution contained 100 mM potassium phosphate buffer (pH 6), 1 M catechol and increased absorbance was recorded at 420 nm after every 30 s for 4 min. CAT activity was determined by a modified method [29, 32]. CAT reaction was initiated by adding the enzyme extract in total 2.5 ml reaction mixture contained 100 mM potassium phosphate buffer (pH 7), 100 µl of 20 mM H_2O_2 and a decrease of H_2O_2 concentration was monitored at 240 nm for 3 min. CAT activity was calculated using a 0.036 mM^{-1} cm^{-1} molar co-enzyme activity was expressed as decomposition of 1 µM of H_2O_2 min^{-1}.

2.5. Statistical analysis: The data were analysed by one-way analysis of variance and the graph was plotted on excel. Multiple comparisons between treatments were performed to identify the significant differences, were considered significant when $p < 0.05$. Multiple comparisons was done by Duncan multiple range tests using SPSS software.

3. Results

3.1. SnNPs characterisation: SEM micrographs in Figs. 1*a* and *b* under lower and higher magnifications show the aggregated spherical SnNPs which are present in various sizes from 40 to 240 nm. HRTEM micrograph in Fig. 1*c* shows that the received SnNPs are spherical and varied in different sizes. Estimation of the sizes using >200 NPs in HRTEM micrographs revealed the diameter of SnNPs between 50 and 150 nm with an average size of 80 nm. NPs appeared to be individual while touching the other NPs at their outer surfaces. The core of the NPs was denser than another part because of darker appearance than the periphery. SEM and TEM studies indicated that NPs were agglomerated because of high surface energy [11]. ED in Fig. 1*d* of the periphery shows the crystalline nature of the NPs. EDX analysis shows that the SnNPs consist of 3–10 at% oxygen.

Melting point measurement of SnNPs at 232°C by differential scanning calorimetry (DSC) study in Fig. 2 and EDX study confirmed that there was only Sn in NPs. The endothermic band around 100°C is due to the evaluation of absorbed water from the surface of SnNPs. However, the presence of oxygen shows that the portions, especially surface, of the SnNP become oxidised due to exposure to air.

Sharp and intense peaks in XRD in Fig. 3 suggest the presence of long-range crystallinity in NPs. All the peaks in X-ray diffractogram were assigned according to the regular (200), (101), (220), (211), (301), (112), (400), (321), (420), (311) and (312) peaks for the crystalline planes of Sn metal [33]. It shows the existence of all the regular peaks of Sn metal reported in the JCPDF Card No.

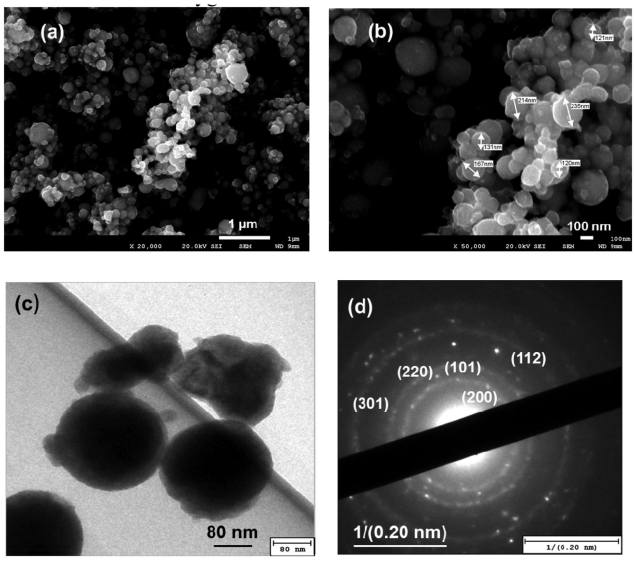

Fig. 1 *SEM micrographs showing SnNPs in*
a Low magnifications (20,000×)
b High magnification (50,000×)
c HRTEM micrographs showing SnNPs
d ED of the SnNPs in (c)

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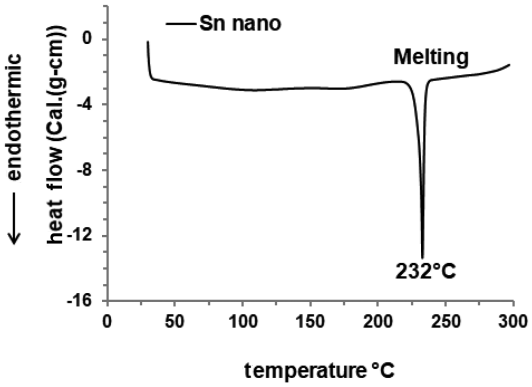

Fig. 2 *DSC study showing the melting point of SnNPs by a sharp endothermic peak centred at 232°C*

040673 [34]. Here, (200), (101) and (211) were the sharp and high intensity peaks in the XRD.

3.2. Effects of SnNPs on the growth of seedlings: The effects of different concentrations of SnNPs on the growth of maize seedlings was evaluated as fresh and dry weight, root and shoot lengths of control and treated seedlings. At the used doses, SnNPs did not cause any significant effects on the growth of roots and stems (data not shown). The reduction of growth was observed after the second week of exposure, but the difference was among the treatment and control was not statistically significant.

3.3. Effects on the activities of some antioxidant enzymes: During any kind of stress in plants, induction of a group of enzymes plays a very important role in defence strategy by plants at the cellular level. Among these enzymatic systems, guaiacol peroxidase (GPX), PPO and CAT can transform peroxides radicals into non-reactive species. The activities of these enzymes in roots and shoots of maize seedlings exposed to SnNPs stress are presented in Figs. 4–6. GPX activity was significantly modified in roots and leaves, while it was strongly stimulated in leaves (about 225%) when exposed to 50 mg/100 ml concentration as compared with 35 mg/100 ml (Fig. 4). There was no effect on the PPO activity in the roots and leaves at a lower dose of SnNPs, but 50 mg/ml stimulated the activity in leaves and roots by 176 and 212%, respectively, as compared with the control (Fig. 5). CAT activity was significantly increased in roots and in leaves with the increase in the concentration of SnNP (Fig. 6).

4. Discussion: The results of the present studies are for short-term exposure of SnNPs on maize seedlings. This period of exposure may not able to translate into the significant effect on the growth and biomass of the exposed seedlings. The duration of exposure, the type of growth medium used in the studies can affect the

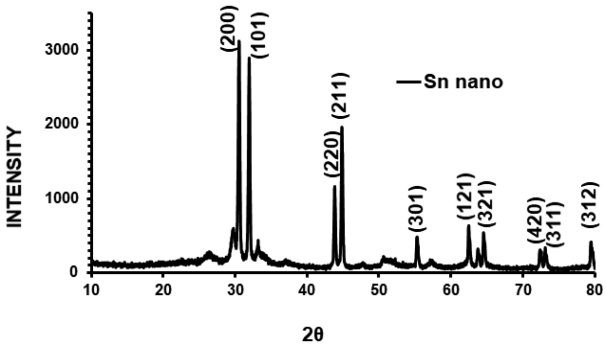

Fig. 3 *XRD of SnNPs*

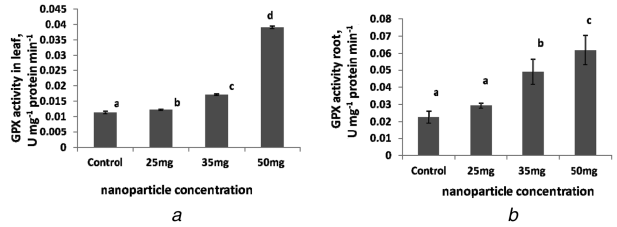

Fig. 4 *Activity of GPX in*
a Leaf of 14 days old maize seedlings grown in MS medium and supplemented with three concentrations of SnNPs
b Roots of 14 days old maize seedlings grown in MS medium and supplemented with three concentrations of SnNPs. Data shown are mean values of at three replications. standard error (SE) are indicated by vertical bars

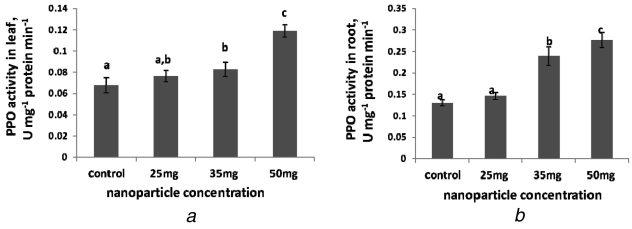

Fig. 5 *Activity of PPO in*
a Leaf of 14 days old maize seedlings grown in MS medium and supplemented with three concentrations of SnNPs
b Roots of 14 days old maize seedlings grown in MS medium and supplemented with three concentrations of SnNPs. Data shown are mean values of at three replications. SE is indicated by vertical bars

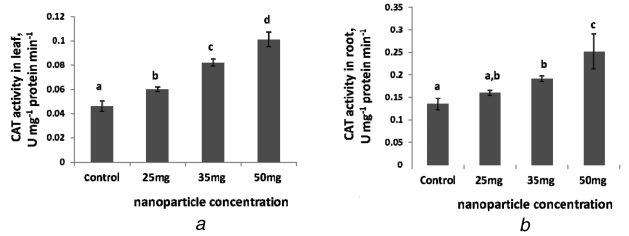

Fig. 6 *Activity of CAT in*
a Leaf of 14-days-old maize seedlings grown in MS medium and supplemented with three concentrations of SnNPs
b Roots of 14-days-old maize seedlings grown in MS medium and supplemented with three concentrations of SnNPs. Data are mean values of at three replications. SE is indicated by vertical bars

behaviour of the metal NPs such as aggregation and translocation [24, 35]. This may be the factor responsible for showing the non-significant impact on plant growth, which is also reported in case of another metal oxide NPs such as cerium (IV) oxide [24] and titanium dioxide [27].

We were interested not only in how SnNPs affect seedlings growth, but also the antioxidant enzymes activity as a marker for stress. Thus, we evaluated the effect of SnNPs on enzymatic activity in maize seedlings. We noted a pronounced increase of GPX activity in leaves of treated seedlings. Several types of researches have shown that toxic concentrations of metals induce GPX activity in crop plants [36, 37], and this can be considered as a maize seedlings reaction to Sn caused oxidative damage at higher concentration. PPO is commonly reported in various stresses, and also metal stress induces its activity [38, 39]. CAT also takes part in an efficient defence mechanism against various stresses in plants. Similar to our results, the CAT activity was increased in the bean that was exposed to Cu metal stress [40, 41]. An increase in the activity of these enzymes is necessary to control the superoxide

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and H_2O_2 content in plants. The balance is maintained by antioxidant systems within the cellular environment. The continuous presence of ROS in stressful environmental conditions has been reported to reducing the photosynthesis capacity by disturbing the balance between energy-producing and energy-utilising processes [42]. These results indicate that SnNPs' exposure, for long periods of time, and in more concentration may exert a negative impact on plant growth by impacting the photosynthesis and other biological processes.

Past studies have shown that roots could be the main route of the plant's exposure to NPs. The findings have also suggested that competitive inhibition of *K* and other cations translocation to stem and leaves, by a metal component of NPs, as an important factor in causing stress in plants [24]. The results of our study have found the relation between SnNPs and maize seedlings; however, no data are available about the mechanism. Therefore, further investigations with different plants and exposure duration seem to be the best options to test the toxicity of these particles.

5. Conclusions: On the basis of the result of the present work, despite there was not a quantifiable difference in growth in maize seedlings, our assumption is that the activation of the stress enzymes might be an important reaction of seedlings toward harmful oxidative effects of Sn. It can be concluded that toxic concentrations of Sn cause oxidative damage, as evidenced by the increased stress marker enzymes. Another possible conclusion is that Sn affects different metabolic processes in different plant and higher concentration may affect the growth parameters. Our study results suggest that exposure of plant with NPs must be studied case by case, as their characteristics are widely dependent on the growth conditions, and time of exposure. Finally, since SnNPs absorbed by the plant are unknown, further studies are needed to study the uptake and translocation on SnNPs.

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