The effect of green synthesized gold nanoparticles on rice germination and roots*

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Abstract
In this paper, gold nanoparticles were synthesized by means of a green approach with *Tiliacora triandra* leaf extracts under different conditions. No additional reducing or capping agents were employed. The gold nanoparticles were characterized using UV–visible spectrophotometry, transmission electron microscope, x-ray diffraction and Fourier transform infrared spectroscopy. Gold nanoparticles synthesized at temperature of 80 °C were further used to treat rice (*Oryza sativa*) grains at different concentrations (0, 10, 100, 500, 1000, 2000 mg l⁻¹) for one week. While germination percentages were high (95–98.38%), a slight decrease in root and shoot lengths relative to the control was observed. Phytotoxicity results indicated that the plant synthesized gold nanoparticles were of minimal toxicity to rice seedlings. Increases in cell death, hydrogen peroxide formation and lipid peroxidation in roots and shoots were noted. However, these increases were not statistically significant. The overall results confirmed that *Tiliacora triandra* synthesized gold nanoparticles are biocompatible and can be potentially used as nanocarriers in agriculture.

Keywords: gold nanoparticles, green synthesis, plant extract, *Tiliacora triandra*

Classification numbers: 2.10, 4.02

1. Introduction

Advances in nanotechnology are opening new doors for the production and utilization of engineered nanoparticles. Silver and gold nanoparticles seem to be in the top tier of importance, judging by the sheer amount of publications on them. Gold nanoparticles are known to be of interest to researchers due to their tunable surface plasmon resonance (SPR), among other things. This unique SPR has been made use of in biomedical applications such as drug delivery, tissue/tumor imaging and in photothermal therapy [1]. The use of gold nanoparticles in the biomedical sciences compels stakeholders to seek out methods of synthesis that guarantee the biological safety of the end-products. Green synthesis fits the bill. It has the edge over conventional chemical and physical methods in that, it is cost effective, environmentally friendly, not energy intensive and strives to be toxic chemical free. By extension, the development and encouragement of greener approaches go a long way to address safety concern which is a hot-button issue as with any burgeoning scientific endeavor. In green synthesis, biological systems such as microbes, fungi and plant extracts are exploited to synthesize metal nanoparticles. Metal nanoparticles are a favorite because they are easily
synthesized. Moreover, nanoparticles produced by plants have the advantage of being stable. Plant mediated synthesis with the likes of Couroupita guinensi, Citrus maxima, Terminalia arjuna, lemongrass and tamarind have been reported [2, 3].

While numerous plants have been used to synthesize gold nanoparticles, there are no available reports on its synthesis using Tiliacora triandra (T. triandra). T. triandra is a flowering plant native to Southeast Asia. It is used in the cuisines of Northeast Thailand, Laos, Vietnam and Cambodia. The leaves of this plant contain antioxidants, beta-carotene, condensed tannins, triterpenes, flavonoids and saponins [4]. This plant has been used in Thai local communities for a long time as an antipyretic and analgesic. While we have established the importance of pursuing green methods of synthesis, it will be foolhardy to turn a blind eye to the possible ramifications of nanomaterials/nanoparticles on biological systems and the environment. Numerous experiments have been carried out with respect to illuminating the scientific community’s understanding of this subject. For example, plant response to nanomaterials at different stages of development has been reported as both favorable and unfavorable [5, 6]. Uptake, translocation, accumulation and the consequent effect of these, are based on reasons such as the size, type, chemical composition, concentration, duration of exposure and the species of plant under consideration [7]. To the best of our knowledge, none of these experiments has entertained the notion of the effect of green synthesized nanoparticles on economically important plants like rice and wheat. In line with the quest to gain more comprehension in this regards, we explored the outcome of green synthesized gold nanoparticle interaction with rice seed germination and root growth.

2. Materials and methods

2.1. Preparation of T. triandra leaf extract

Fresh T. triandra leaves were washed several times with distilled water. 5g of the leaves were chopped in bits, blended, filtered and the leaf extract made up to a 100ml with distilled water. The extract served as both a reducing and a stabilizing agent in the reaction.

2.2. Biosynthesis of gold nanoparticles using T. triandra extract

Gold (III) chloride trihydrate was purchased from Sigma Aldrich, USA. All other reagents used were of analytical grade with no need for further purification. All glassware used in the synthesis process was cleaned with freshly prepared aqua regia (HNO₃/HCl, 3:1, v/v), rinsed thoroughly with distilled water, and later dried prior to use. Distilled water was used throughout the experiments. To synthesize gold nanoparticles, 10ml of the aqueous leaf broth was added to 190ml of 1 mM aqueous gold chloride trihydrate solution. To study the effect of temperature on particle morphology, synthesis preparation occurred at temperatures of 25 °C, 65 °C and 80 °C. The concentration of the aqueous solution of gold chloride trihydrate was kept constant under all conditions of preparation. The formation of nanoparticles was accompanied by an observable change in color from dark yellow to various tinges of deep brown with a hint of purple. The resulting gold nanoparticle solution was purified by repeated centrifugations at 12000 rpm for duration of 60 min ensued by re-dispersion of pellet in distilled water at least three times. These nanoparticles were subsequently oven-dried overnight.

2.3. Characterization of gold nanoparticles

Gold nanoparticles prepared under different conditions, as previously indicated, were characterized by using UV–Vis spectroscopy, transmission electron microscope (TEM), x-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). Using a Cary 300 UV–Vis spectrophotometer, the optical properties of the gold colloids were analyzed in the wavelength range of 400–700 nm at a resolution of 1 nm. TEM images for study of morphology were captured with a Zeiss electron microscope (EM 902, West Germany). XRD analysis was performed using a Bruker D2 x-ray diffractometer with Cu-Kα radiations (λ = 1.5406 Å). Samples used in this analysis had been previously dried and powdered. FTIR analysis of the synthesized gold nanoparticles occurred in the range 40–4000 cm⁻¹ following the freeze-drying of the samples. These measurements were carried out on a Bruker Tensor 27 FTIR.

2.4. Rice grain and gold nanoparticle treatment

Rice grains of the variety KDML 105 were surface sterilized for 15 min in 2.5% of sodium hypochlorite. It was then washed three times with distilled water to get rid of all traces of the disinfectant, prior to being soaked in gold nanoparticles suspensions of varying concentrations (0, 10, 100, 500, and 1000 mg l⁻¹). Gold nanoparticles used in treatment were those that had been synthesized at a temperature of 80 °C. The suspensions were prepared by dispersing appropriate amounts of the nanoparticles in distilled water with the help of an ultrasonic bath for 45 min. Rice grains in the respective concentrations were soaked for 4 h in an incubator at ambient temperature. At the end of soaking period, the seeds from each of the suspensions were transferred into labeled petri dishes containing Whatman filter paper no. 1. Three replicates, each containing 20 grains, were made for each treatment. To each of these petri dishes, 4 ml of distilled water (control) or the corresponding concentration of nanoparticle suspension was added. These petri dishes were moved into an incubator with temperatures set at 25 °C, and germination allowed for a period of 7 d. After a week of exposure, the germination percentage, root/ shoot lengths and fresh weights measurements were made. Only grains with coleoptile longer than 2 mm were considered germinated in this study.

2.5. Seedling relative water content

The relative water content of the germinated seedlings was determined at the end of the germination period [8]. Seedlings from each treatment were initially weighed to obtain the fresh weight. They were then floated for 24 h in the dark at
temperatures of 4 °C. At the end of this phase, the excess water on the seedlings was blotted with tissue paper just before weighing to obtain turgor weight. Dry weight of the seedlings was obtained by oven-drying at temperatures of 80 °C overnight. Relative water content (RWC) was calculated using the formula:

\[ \text{RWC} = \frac{\text{Fw} - \text{Dw}}{\text{Tw} - \text{Dw}} \times 100, \]

where Fw is fresh weight, Dw is dry weight and Tw is turgor weight of the seedling.

2.6. Cell death evaluation

Plant root cell death was evaluated following treatment with gold nanoparticle suspensions based on the method by [9]. The rice roots were incubated in 5 ml 0.25% v/v of Evans blue solution for 15 min. To remove excess and unbound dye, the roots were washed for 30 min. Into 7 ml of N, N-dimethylformamide, severed root tips measuring 3 cm were soaked for 50 min at room temperature. Using N, N-dimethylformamide as blank, and the absorbance of released Evans blue was measured at 600 nm. Cell death was expressed as the absorbance of treated roots in relation to the control.

2.7. Determination of the effect of seed soaking and incubation on root elongation

Three treatment setups were designed to determine the effects of seed soaking, the incubation process and phytotoxicity on root elongation. A concentration of 2000 mg l\(^{-1}\) of the nano-gold suspension was used in all three setups. In the first treatment, all grains were soaked in the nano-gold suspension for 7 d was preceded by soaking in distilled water for 7 d. In the second treatment, incubation in the 2 h, rinsed in distilled water three times, and incubated in distilled water on the seedlings was blotted with tissue paper just before weighing to obtain turgor weight. Dry weight of the seedlings was obtained by oven-drying at temperatures of 80 °C overnight. Relative water content (RWC) was calculated using the formula:

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2.8. Estimation of hydrogen peroxide and lipid peroxidation

Hydrogen peroxide levels in the roots and shoots of gold nanoparticle exposed seedlings were estimated [11]. Root and shoot samples (0.5 g) homogenized in liquid nitrogen were immersed in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). Each of the homogenates was centrifuged at 6000 rpm for 15 min, and 2 ml of the respective supernatants were added to 2 ml of 10 mM K-phosphate buffer (pH 7.0). Into this mixture, 4 ml of 0.1 M KI was added. The intensity of the yellow color of the supernatant was measured at 410 nm, 0.1% of TCA was used as blank. A standard curve of hydrogen peroxide was plotted.

Lipid peroxidation levels were determined [12]. Root (0.1 g) and shoot (0.2 g) tissues from both the control and treated seedlings were homogenized in 10 ml of 5% (w/v) TCA. These homogenized samples were centrifuged for 10 min at 4000 g, and its supernatant (3 ml) mixed with 3 ml of 0.67% (w/v) thiobarbituric acid (TBA). The mixtures were then boiled at 90 °C for 30 min and cooling followed. Absorbance of the supernatant were read at 532 nm (Abs\(_{532}\)) and 600 nm (Abs\(_{600}\)) using a UV–Vis spectrophotometer. To determine the amount of 3,4-methylenedioxymethylamphetamine (MDA) in tissue samples, the following formula was used:

\[ \text{MDA} (\mu \text{mol g}^{-1}) = \frac{\Delta \text{Abs}}{\varepsilon \cdot b \cdot \text{Fw}} \times 1000, \]

where \(\Delta \text{Abs} = \text{Abs}_{532} - \text{Abs}_{600}\). \(\varepsilon\) is extinction coefficient (\(\varepsilon = 155 \text{mM}^{-1}\text{cm}^{-1}\)), \(b\) is light path (\(b = 1 \text{cm}\)), Fw is fresh weight of plant (in gram).

2.9. Data analysis

All treatments were carried out in replicates of three. Statistical analysis was done using one-way ANOVA and Tukey’s multiple range tests \((p < 0.05)\) with SPSS 18.0 (SPSS Inc., Chicago, USA).

3. Results and discussion

3.1. Optical and morphological properties of bio-reduced gold nanoparticles

Figure 1 shows the UV–visible spectra of plant mediated gold colloids upon completion of reaction. Reaction completion is signaled by a change in color. The change in color is attributed to a mechanism known as SPR [3]. The color changes and observed SPR band shifts are characteristic of the SPR of the different sizes and shapes of gold nanoparticles in the colloids [13].
Figure 1 shows the UV–visible spectra of gold colloids synthesized at different temperatures. The SPR bands of gold colloids synthesized at the highest temperature (80 °C) was comparatively broader than those synthesized at lower temperatures. Broader SPR bands have been linked to increased particle sizes [14]. Furthermore, the width of the absorption spectra SPR is related to size distribution and implicated nanoparticle dispersity [15, 16]. The broadened peak observed in the present context is proof of synthesized gold nanoparticle polydispersity [17] consistent with TEM results. Crystallite size measurements are not in accordance with the observation of broader SPR bands being linked to increased particle sizes. However, TEM measurements of size seem to be in line with this position. The particle size distribution graph of TEM measurements indicated that the majority of particles synthesized at temperatures of 80 °C, 65 °C and 25 °C had sizes of

Figure 2. Size distribution histogram of gold nanoparticles synthesized at (a) 80 °C, (b) 65 °C and (c) 25 °C.

Figure 3. TEM images of gold nanoparticles of varying shapes synthesized using *T. triandra* leaf extracts at temperatures of (a) 25 °C, (b) 65 °C and (c) 80 °C.
60 nm, 20 nm and 10 nm, respectively (figure 2). Moreover, the majority of particles tended to be more spherical with increasing temperatures (figure 3). The selected area electron diffraction (SAED) analysis revealed the crystalline nature of the nanoparticles in the form of four bright circular rings with lattice spacing corresponding to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes of the face centered cubic lattice of gold nanoparticles (figure 4). This ring pattern is associated with polycrystalline materials and is evidence of nanoparticle formation [18].

3.2. Analysis of bio-reduced gold nanoparticles

XRD analyses were performed to verify the crystalline nature of the gold nanoparticles. Figure 5 shows the XRD pattern of these particles synthesized at different temperatures. The diffraction peaks at 38°, 44°, 64° and 77° correspond to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes of crystalline gold, respectively, indicating a face centered cubic (fcc) structure (JCPDS: 04-0784). The peak corresponding to (1 1 1) is the most intense plane. This suggests that it is most predominant orientation, in addition to the synthesized nanoparticles being crystalline in nature [19]. Using the Scherrer equation, the average crystallite size of ~39.3 nm did not match with those obtained from the TEM image (~59.9 nm) of gold colloids synthesized at temperatures of 80 °C. The difference in particle size averages is the result of instrumentation and particle structure. While TEM estimates particle size, the Scherrer equation estimates crystallite region. A particle may consist of a single or several domains, the equivalent of a crystallite or crystallites; agreements in measurement are only feasible when each particle consists of a single crystallite.

*T. triandra* leaves as previously mentioned contain antioxidants beta-carotene, condensed tannins, triterpenes, flavonoids and saponins. FTIR analyses were conducted to identify the biomolecules responsible for bio-reduction and stabilization. The spectrum of the leaf extract revealed a number of intense bands at 3279, 2363, 1643, 1551 and 1039 cm\(^{-1}\) (figure 6).

The intense and broad band observed at 3279 cm\(^{-1}\) is characteristic of the O–H stretching vibration which can be found in the flavonoids and terpenes [3, 20]. The band at 1643 cm\(^{-1}\) can be assigned to the amide I band which springs from the carbonyl stretch vibrations of the proteins and/or N–H bond of the primary amines [19, 21]. This is no surprise since *T. triandra* is known to contain protein [22]. The bands at 1551 and 1039 cm\(^{-1}\) correspond to asymmetric stretching of C–N groups, respectively. The C–N stretch may equally be an alphatic amine or phenol. The weak band at 2916 cm\(^{-1}\) corresponds to asymmetric stretching of C–H groups [23] or amine II [24]. The absorption band at 1250 (1234) cm\(^{-1}\) is characteristic of the amide III bands of polypeptides or protein [25]. The FTIR spectrum (figure 6) following bio-reduction showed the bands at 1551 and 2363 cm\(^{-1}\) to be suppressed. This suggested that the nitro functional group (1551 cm\(^{-1}\)) along with H\(_2\)O*/C–O group or C = NH\(^{+}\) (ionic amines) of *T. triandra* might have played a leading role in bio-reduction [26–28]. This in no way infers that other functional groups from the plant’s phytochemical constituents did not aid in
bands represent shifts from 522 to 535, 1039 to 1031, 1551–1234, 1524, 1653, 1734, 2921 and 3310 cm$^{-1}$, respectively. The IR bands observed at 1524, 1653, 1734, 2921 and 3310 cm$^{-1}$ represent the stretching modes for N–O, C–H and O=C/N, O, C3310 represents the stretching modes for N–O, C–H and O=C/N, respectively. The proteins by means of free amines or cysteine residues in saponins may have been adsorbed onto particle surfaces adding to stabilization.

The resulting FTIR spectra of gold nanoparticles colloids showed bands at 535, 1031, 1234, 1524, 1653, 1734, 2921 and 3310 cm$^{-1}$ along with other small bands. The aforementioned bands represent shifts from 522 to 535, 1039 to 1031, 1551 to 1524, 1643 to 1653, 1759 to 1734, 2916 to 2921, and from 3279 to 3310 cm$^{-1}$. The bands at 535 and 1031 cm$^{-1}$ were identified as alkyl halides and aliphatic amines (C–N), respectively. The IR bands observed at 1524, 1653, 1734, 2921 and 3310 represents the stretching modes for N–O, C = C/N–H, C–O, C–H and O–H bonds, respectively. Shifts in band are associated to the binding of functional groups contained in plant aqueous extract to nanoparticle surfaces [32].

A possible mechanism of gold nanoparticle formation likely involved the carbonyl group/ionic amines along with a host of other functional groups provided by bioactive agents, initially binding to gold ions (Au$^{3+}$) to form complexes. Reduction occurred to yield seed particles with oxidation states of zero (Au$^0$). Upon aggregation of some of these seed particles, nucleation sites were formed. Gold metal ions eventually built up around these seeds in a process known as secondary growth [33].

### 3.3. Phytotoxicity response of rice seedlings to biosynthesized gold nanoparticles

Though minimal by comparison, reduction in root and shoot lengths were observed. The control seedlings had the highest average root length of 6.2 cm (figure 7(a)). The average seedling root length (5.6 cm) at concentration of 500 mg l$^{-1}$ was the least. A non-threshold relationship was noted for plant root response to nano-gold particle doses. *Brassica juncea* seedlings treated with increasing concentrations of citrate synthesized gold nanoparticles experienced a similar decline in overall growth [34].

Going by the explanation of the authors of this article, reduction in root length with respect to the control is the result of scarce metabolic resources dedicated for normal growth and development being re-routed to combat toxicity elicited by the likes of gold nanoparticles. The average shoot length of the control equally had the highest average of 3.41 cm (figure 7(b)). The average shoot lengths for treatments ranged from 3.0 to 3.4 cm. The effect of concentration on the germination percentage was minimal; it ranged between 93.09 to 98.38% (figure 7(c)). The acidic nature of the nano-gold particle suspensions did not adversely affect germination due to the protective and semi permeable nature of rice seed coats [35]. In a study carried out by [36], citrate synthesized gold nanoparticles were reported to have mild effect on germinating rice seedlings as is the case here. Toxicity of the nanoparticles used in this experiment might have been lessened due to the nature of the particle’s surface stemming from the manner of synthesis [37]. The potential of surface modification to alter the outcome of toxicity was confirmed in a study by [38]. The researchers involved in this study found out that citric acid-coated CeO$_2$ nanoparticles increased root lengths of radish seedlings by 23%, relative to the distilled water control ($p \leq 0.0034$). At the same concentration (100 mg l$^{-1}$), bare CeO$_2$ nanoparticles reduced root elongation by 26%, compared to the distilled water control. Gold nanoparticles have been reported to boost seed germination and metabolic activity by increasing seed coat permeability [39]. The germination and metabolic activity increment was the result of easier access to water and oxygen. *Glorosia superba* seeds treated with gold nanoparticles synthesized from *Terminalia arjuna* fruit extracts, spherical in shape with a size range of 20–50 nm, benefited from this potential [39]. A likely reason why rice grains from the current study did not profit from the seed germination boosted by gold nanoparticles might be due to the nature of the nanoparticles and the seed coat. In this study, however, differences in root and shoot lengths did not account for a statistical difference.

Relative water content was used as a measure of the seedlings’ water status. Upon exposure of the seedlings to the different concentrations, a noticeable increase in the relative water content, albeit not significant, was observed. Relative water content values for all treatments were greater than that of control (figure 8). The relative water content value for the 500 mg l$^{-1}$ treatment was the highest (79.90%). Similarly, the
3.4 Effect of seed soaking and incubation on root elongation

The average root length of rice grains soaked in 2000 mg l⁻¹ of gold nanoparticle suspension before incubation was the highest at 6.62 cm (figure 10(a)). This is probably the case because of the minimal trigger created by the shorter exposure time to the gold nanoparticles during the soaking period. This trigger was sufficient to boost germination upon the resumption of incubation in distilled water. Rice grains in the treatment in which seeds were soaked in distilled water before exposure to gold nanoparticles (treatment II) flourished the least; root length average was 5.29 cm. The sudden change from soaking in distilled water to incubation in a suspension of gold nanoparticles might have left little room for adaptation. The root reduction was therefore resulted. The average root length for grains in the treatment with both soaking and incubation in nano-gold suspension (treatment III) was 6.22 cm. From all indications, longer exposure to treatments worked in favor of the exposed grains; plant tolerance limits were pushed as a result of acclimation to living in its current environment.

On the basis of the results obtained from this experiment, seed incubation affects root elongation more negatively than soaking does. *T. triandra*-synthesized gold nanoparticles were found to be of minimal toxicity on rice plants owing to their null effect on the plants. The extent of toxicity was confirmed by a physiological test to evaluate cell mortality in roots. Based on this test, cell mortality in roots was highest in treatment II and least in treatment III (figure 10(b)). All perceived cell viability differences were statistically insignificant. The growth stage of a plant, the physicochemical properties of nanoparticles and the nature of the exposure medium are properties demonstrating the phytotoxicity of nanoparticles [40]. As opposed to agar media which are semi-solid and resemble soil, the medium of exposure used in this experiment minimally hinders nanoparticle bioavailability. In spite of this factor being in its favor, signs of phytotoxicity were not observed.

3.5 Uptake of gold nanoparticles by seedling roots

The roots of seedlings exposed to 2000 mg l⁻¹ of gold nanoparticles during seed soaking and 7 d incubation were further studied to verify uptake of the nanoparticles; a TEM was used in the process. Microscopic observations show what appear to be nanoparticles contained in the cells of plant roots (figure 11). None of these particles were spotted in the intercellular region; perhaps the possible pathway might be the plants primary choice of translocating these particles. There is the possibility that gold nanoparticles suspended in distilled water can be adsorbed on root surfaces and find their way into root cells passively through cell wall pores [41]. This possibility is reinforced by the fact that plant cell wall pores have the sizes between 5 to 20 nm. Moreover, nanoparticle suspension contained particles with a diameter within cell wall pore size range. In [42] the uptake of gold nanoparticles by rice plants was also observed.

It is well-known that a plant’s ability to effectively take up nutrients/metals is connected to the pH of the medium in which it finds itself. The age of the plant is a contributing factor to root ion uptake. In this regard, young plants show greater physiological activity and are thereby favored [43]. The micronutrient availability of the likes of zinc and copper elements declines with increasing alkalinity. The aforementioned elements like gold, are considered transition metals. As expected, they share a range of chemical properties which likely influences how plants might take up and translocate them. Metal transporters have been reported to take up gold in the ion form [44]. Many of these metal transporters (cation transporters) are already known to be involved in metal uptake, particularly heavy metals. While plant transporters capable of...
taking up gold remain unknown, iron regulated transporters (IRT) are suspected of being able to take up non-essential gold metal ions. This is because these transporters (e.g. IRT1 and IRT2) show activity following exposure to gold and can take up a wide range of metal cations. Tomato plants which have been reported to take up gold nanoparticles, express the proteins LeIR1 and LeIR2 specifically in their roots [45, 46]. This adds validity to the likelihood of these transporters being implicated in gold/gold nanoparticle uptake. Moreover, variants of these transporters known as OsIR1 and OsIR2 are expressed primarily in rice roots [47].

The nano-gold suspension (2000 mg l$^{-1}$) used in this experiment had a pH of 3.92. This acidic pH probably resulted from the release of gold ions furnished by gold nanoparticles owing to their size and enormous specific surface area [48]. Uptake of gold nanoparticles by rice seedlings in the present study was most likely enabled by pH of the medium which favors bioavailability and the age of the plant. The severity of toxicity might have been mitigated, amongst other mechanisms, by storage of the nanoparticles or metal ions in the vacuole [43]. This is substantiated by the presence of the nanoparticles in membrane-bound compartments (figures 11(b) and (c)).

Several plant uptake studies involving gold nanoparticles have been conducted with varying outcomes. Wheat plants exposed to citrate and tannate coated gold nanoparticles did not bioaccumulate these nanomaterials in their tissue [49]. Tobacco plants used in the same experiment bioaccumulated these particles. To explain this occurrence, the authors established a relationship between bioaccumulation, nanoparticle aggregation and the nature of plant root exudates. Failure in wheat uptake of gold nanoparticle was the result of nanoparticle aggregation instigated by its root exudates. It was reported otherwise in the experiments with alfalfa plants exposed to gold nanoparticles in the size range 5–100 nm [44]. However, these plants were found to contain gold nanoparticles in their tissue upon exposure to ionic gold. Such differences in outcome are expected due to the well-known fact that the uptake of engineered nanoparticles in plants depends on their composition, shape, size and the anatomy of the plant in question [10].
3.6. Effect on H$_2$O$_2$ production and lipid peroxidation

Hydrogen peroxide levels were estimated in root and shoot tissue of rice seedlings that had been exposed to gold nanoparticles (figure 12). This is because it is widely accepted that nanoparticle toxicity is caused by excessive production of reactive oxygen species [50]. It was found that hydrogen peroxide levels in the control of both root and shoot tissues were least. Tissues from the 1000 mg l$^{-1}$ treatment in both cases had the highest amount of hydrogen peroxide. No particular trend with respect to the concentration and the amount of hydrogen peroxide in root and shoot tissue was observed. Hydrogen peroxide amounts in root tissues ranged from $3.60 \times 10^{-15}$ µg ml$^{-1}$ to $2.68 \times 10^{-6}$ µg ml$^{-1}$. Those in shoot tissues ranged from $1.94 \times 10^{-18}$ µg ml$^{-1}$ to $4.91 \times 10^{-16}$ µg ml$^{-1}$. Judging from these values, change was not significant.

The by-product of lipid peroxidation which is the degradation of lipids in cell membranes by reactive oxygen species is MDA [51]. Lipid peroxidation measurements showed no significant change in the MDA levels in roots and shoots of seedlings exposed to all treatments (figure 13). A parallel increase between the MDA content of seedling root tissues and gold nanoparticle concentrations was observed. No clear trend was observed for its shoot counterparts. Change as in the previous case, was not significant.

Figure 12. H$_2$O$_2$ content in treated seedling roots (a) and shoots (b). Absorbance at 412 nm.

Figure 13. MDA content in treated seedlings roots (a) and shoots (b).

Citrate-stabilized gold nanoparticles have been reported to increase H$_2$O$_2$ and MDA content in B. juncea seedlings following exposure [34]. While an increase in these compounds were noticed in the current experiment, it all seemed to be within tolerable limits of the plant’s ability to handle; a plausible reason why seedling growth was not adversely affected. Heavy metal ion release from nanoparticles is crucial to the phytotoxicity of metal-based nanoparticles [40]. Perhaps, the release of such gold ions from gold nanoparticles failed to cause significant rise in reactive oxygen species because of the counter effects of reducing substances of plant origin. Low oxidative stress levels in the current experiment might very well be linked to the antioxidant activity of T. triandra leaf extracts [52]. It is highly probable that the functional groups of chemical compound(s) responsible for the plant’s antioxidant activity might have been attached to the surface of the nanoparticles in the process of synthesis. Hence, the insignificant increases in oxidative stress levels in plant tissue following interaction with the nanoparticles. Perhaps, the levels of hydrogen peroxide and MDA did not spike due to a combination of the aforementioned factors, and the scavenging activities of catalase.
4. Conclusion

Briefly, we investigated a green method to synthesize gold nanoparticles using plant leaf extracts of *T. triandra*. Gold nanoparticles obtained under the different conditions of synthesis varied in size and shape. The plant-mediated synthesis method employed in this experiment is simple, cost-effective and eco-friendly. The exposure of these nanoparticles to rice seedlings at high concentrations did not lead to a significant reduction in root growth, increase in hydrogen peroxide levels and MDA amounts. This is probably linked to the bio-compatible nature of these green synthesized nanoparticles.

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