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Research Article

Impact of ZnO nanoparticles on growth and biochemical characteristics of *Macrotyloma uniflorum* (LAM.) Verdcourt

Balasundari Ponpandi [1], Aarthi Jeganathan [1], Sradha Sajeev [1], Anju Rani George [1], Kavimani Thangasamy [1], Madhu Priya Govindhan Anbazhagan [1] and Geetha Natesan [2]*

[1] Research Scholar, Department of Botany, Bharathiar University, Coimbatore-641 046, Tamil Nadu, India

[2] Professor, Department of Botany, Bharathiar University, Coimbatore-641 046, Tamil Nadu, India

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*For Correspondence:

Geetha Natesan, Professor, Department of Botany, Bharathiar University, Coimbatore-641 046, Tamil Nadu, India

Abstract

The present work reports the green synthesis of zinc oxide nanoparticles (ZnO NPs) using green tea leaves and its effect on germination, morphology and some biochemical attributes of *Macrotyloma uniflorum* (kollu or horse gram). The phytosynthesized ZnO NPs were characterized by UV-VIS, FTIR, Zeta sizer and SEM. After surface sterilization, the overnight soaked seeds of horse gram were treated with different concentrations of green synthesized ZnO NPs (10 – 50 mg/L) and allowed to germinate at room temperature with regular spraying of respective nano-formulations for 15 days. On fifth day of growth, germination percentage of seeds was determined. Shoot and root lengths were measured on 15th day of seedling growth. At the end of the experiment, the whole plants were harvested and used for estimation of some primary metabolites and secondary metabolites using the standard procedures. The data obtained in the experiment were statistically analysed. UV-VIS and FTIR analyses confirm that the polyphenol contents of green tea leaves are involved in the biosynthesis of ZnO NPs. Stronger stability and semi-spherical morphology of green synthesized ZnO NPs were confirmed by zeta potential value and SEM, respectively. Higher levels of germination percentage, shoot and root lengths, primary metabolites (total chlorophyll, protein, carbohydrates) and secondary metabolites (total phenols, flavonoids, tannins and terpenoids) were observed in 10 and 20 mg/L ZnO NPs treated plants compared to control. Thus, the results demonstrate that the application of ZnO NPs at a lesser concentration may increase seed germination, morphological and biochemical attributes of horse gram seedlings.

Keywords: *Macrotyloma uniflorum*, horse gram, green synthesis, ZnO NPs, Zeta Sizer, biochemical attributes

1. INTRODUCTION

Nanotechnology is an up-and-coming field of science, whose potential is seen in almost every aspect of life. The field of nanotechnology uses various engineered nanoparticles (NPs) and includes various fields of science under one umbrella, including biology, physics, chemistry, and engineering. The areas starting from drug release to wastewater cleanup, soil remediation, and controlled fertilizers come under the purview of nanotechnology. Nanotechnology deals with the synthesis of nanoparticles with controlled size, shape and delivery of materials at the nanometer scale length[1]. Nanoparticles have received much consideration because of their novel properties, which differ from those of bulk materials [2,3].

Chemical fertilizers increase human and animal food toxicity and environmental pollution and also, they harmfully affect chemical and physical properties of soil, ground water, fauna and ecosystem [4]. Thus, it is important to use an alternative soil fertility improvement technique to get high plant growth and yield and at the same time make certain ecological sustainability. The use of a variety of NPs is increasing day by day in the field of agriculture, where NPs have found used as fertilizers and stress reducing agents [5]. During the past decades, various engineered NPs are included in the different fields of agriculture in the form of nano fertilizer, nano pesticides and nano devices in order to increase the effectiveness and sustainability of agricultural practices [6,7].

Zinc (Zn) is an indispensable micronutrient for both plants and animals owing to its involvement in several enzyme activities and metabolic functions. Zinc deficiency in plants leads to poor yield levels, while, in humans, it contributes to malnutrition and several ailments [8,9]. Also, it poses serious threats to soil and water ecosystem, if it is present more than permissible limit. The permissible quantity of Zn in the soil is 70 to 400 mg/kg [10]. Zn is absorbed in the form of divalent cations and it is important for various vital physiological

processes of plants such as synthesis of protein, maintenance of membrane integrity and production of energy [11], biosynthesis of different plant growth hormones [12], formation of chlorophyll pigment, regulation of starch biosynthesis and root development and activation of enzymes like dehydrogenases, phosphohydrolases, peptidases and proteases [13].

Nanoparticles based nanofertilizers have exhibited significant effects on various aspects of agriculture including seed germination, plant growth, development, enhanced yield [14], nutritional enrichment [15], disease [16] and plant stress management [17]. Nanofertilzers are found effective to crops by releasing the nutrients on-demand with targeted and controlled release [18,19]. Nanofertilizers are prepared from organic and inorganic nanomaterials by physical or chemical methods using various inorganic metal oxides such as AgO (silver oxide), MgO (magnesium oxide), ZnO, (zinc oxide) and TiO_2 (titanium oxide) and organic nanomaterials like lipids, polymers and carbon nanotubes. In addition to this, biodegradable, biocompatible and eco-friendly, natural biopolymer chitosan is used to synthesize polymeric NPs based fertilizer [20].

Zinc oxide nanoparticles (ZnO NPs) are extensively synthesized and used nanomaterials worldwide with a diameter of 1–100 nm. Due to their small size, ZnO NPs can be absorbed by the stomata and cuticle of root hair cells and inflowing into various plant tissues and promoting plant growth and yield [21]. Compared to traditional ZnO fertilizers, ZnO NPs have characteristics such as small size, large specific surface area, easy dissolution and diffusion and allowing plants to quickly absorb the Zn released by ZnO NPs. For instance, the application of ZnO NPs has been found to appreciably enhance growth and yield of various plant species [22,23,24].

Conventional nanoparticle synthesis methods frequently involve utilizing harmful chemicals and high-energy processes, increasing environmental concerns and producing poisonous by-products. Green nanotechnology is emerging an eco-friendly alternative by using microorganisms, plants and agricultural waste as sources for nanoparticle synthesis [25,26]. ZnO NPs are used in different scientific and industrial applications because of their wide band gap and large excitation binding energy [27]. Among the physical and chemical methods of synthesis of ZnO NPs, green synthesis using biological materials appear to be highly flexible due to their simplicity, biocompatible and eco-friendly approaches [28]. For the green synthesis of nanoparticles, plants are found to be the most suitable organisms. In addition, plants consist of various primary metabolites (protein, chlorophyll, carbohydrates etc.) and secondary metabolites (phenols, flavonoids, tannins, terpenoids, alkaloids etc.). Different functional groups present in these metabolites can react with inorganic metal ions and convert them into metal nanoparticles with nano ranged size [29]. Phenolic compounds present in the green tea leaves (*Camellia sinensis* (L.) Kuntze) show higher antioxidant property and they are found to be very good reducers of metal ions [30]. Plants mediated ZnO NPs synthesis has been reported in various plant species. For example, *Calatrophis giganta* [31], *Acalypha indica* [32], *Parthenium hysterophorus* [33], *Hibiscus rosasinensis* [34], *Azardirachta indica* [35], *Punica granatum* [36], *Murraya koengii* [37], *Atalantia monophylla* [38] and *Piper betel* [39].

Horse gram (*Macrotyloma uniflorum* (Lam.) Verdcourt (Syn., *Dolichos uniflorus* Lam., *Dolichos biflorus* auct. Non L.) [40] belonging to Fabaceae, is protein-rich and found acquiescent for cultivation in dry conditions and marginal soil fertility [41]. In Tamil it is known as kollu. In ayurvedic medicine, whole seeds of horse gram is consumed for the treatment of renal stones, piles, oedema, etc. [42]. The seeds have been reported to be a important source of iron, molybdenum [43] and protein [44]. Various studies have reported their anti-hypercholesterolemic [45], anti-microbial [46, 47], anti-helmintic [48,49], anti-diabetic [50], anti-inflammatory [51], anti-oxidant [52, 53] and anti-urolithiatic [54, 55, 56] properties. Based on literature, it is understood that so far, no studies have been addressed to study the effects of green synthesized ZnO NPs on various growth parameters of *M. uniflorum*. Hence, the study was conducted to determine the germination capacity, some morphological and biochemical attributes of *M. uniflorum* after treatment with various concentrations of phytosynthesized ZnO NPs.

2. MATERIALS AND METHODS

2.1 Chemicals

Zinc acetate dihydrate ($Zn(CH_3COO)_2 . 2H_2O$, 99%) was procured from Sigma Aldrich and used for the synthesis of ZnO NPs. Green tea leaves were purchased from a commercial shop and seeds of horse gram variety Paiyur 2 were purchased from TNAU, Coimbatore and used for the present study. Analytical grade reagents and double distilled water were used for the entire study.

2.2 Preparation of aqueous green leaf extract

The aqueous green tea leaf extract was prepared by boiling 10 g green tea leaves using 100 mL distilled water at 60°C for about 20 minutes, until the colour of the aqueous solution changes from watery to light yellow. Then the extract was cooled at room temperature and filtered using Whatman No.1 filter paper and the filtrate was used for making ZnO NPs.

2.3 Synthesis of ZnO NPs

ZnO NPs were synthesized using green tea leaves by following the method of Shah *et al.* [57] with some minor changes. In brief, 45 mL of 0.1M of zinc acetate solution was mixed with 5 mL of green tea leaf extract to prepare ZnO NPs and the mixture was kept under dark condition at room temperature for overnight. After incubation period, the colour changed solution *i.e* pale yellow to dark brown was filtered using Whatman No. 1filter paper and the filtrate collected on the filter paper was scraped gently and used for further experiments.

2.4 Characterization of synthesized ZnO NPs

The green synthesized ZnO NPs were spectroscopically characterized by UV-Visible spectrophotometer (Shimadzu), Fourier Transform Infrared (Perkin Elmer), Zeta sizer (Malvern analytical, Chennai India) and and

Field emission scanning electron microscope (JSM 7610F, JOEL, USA).

2.5 Treatment of horse gram seeds with ZnO NPs

Overnight soaked seeds of horse gram were surface sterilized with 0.1% sodium hypochlorite solution for 10 min. After sterilization, the seeds were thoroughly rinsed with distilled water to remove excess of chlorine and were placed in petriplate containing absorbent cotton. 10 seeds were sown per petriplate. Different concentrations of green synthesized ZnO NPs *i.e* 10 – 50 mg/L were taken and dissolved in 100 mL distilled water and then they were added to each petriplate separately. The plates were kept at room temperature along with control (without ZnO NPs) and regularly sprayed with respective nano-formulations for15 days.

2.6 Percentage of seed germination

The seed germination percentage was determined based on total number of germinated seeds on fifth day of seed germination (ISTA, 1996). It was calculated by means of the following formula:

$$\text{Germination percentage (GP \%)} = (Gf/n) \times 100$$

Where, Gf is the total number of germinated seeds at the last part of experiment and n is the total number of seed used in the test.

2.7 Measurement of shoot and root lengths

Shoot and root lengths for all the treated plants were measured using scale after 15 days of growth.

2.8 Effects of ZnO NPs on biochemical attributes of horse gram

After 15 days of treatment with ZnO NPs, plants were harvested, then dried and ground with mortar and pestle. The whole plant powder was stored at 4°C for experimental use.

2.9 Estimation of total chlorophyll, protein, carbohydrate contents

The chlorophyll content in the sample was estimated by Arnon's method[58]. Lowry's method[59] was adopted for the estimation of protein concentration. Total carbohydrate content was estimated by anthrone method [60].

2.10 Quantitative phytochemical analysis

The four important secondary metabolites such as flavonoids, tannins, terpenoids and phenols were quantitatively determined. Total phenolic content of the sample was estimated as described by Singleton and Rossi[61] and modified by Gülçin *et al.*[62] Total flavonoid content of the sample was measured following a spectrophotometric method of Dewanto *et al.*[63]. The total terpenoid content of the sample was determined based on an assay described by Ghorai *et al.*[64] with some modifications. The tannins were determined by Folin-Ciocalteu method.

2.11 Statistical analysis

The mean and standard deviation data obtained from measurement of various treatments in three replicates were statistically analyzed using ANOVA (analysis of variance) and Tukey's multiple comparison tests to examine the significant difference among various concentrations nanoparticles treatment. P values of <0.05 and lower were considered significant.

3. RESULTS AND DISCUSSION

3.1 Green synthesis of ZnO NPs

When green tea leaf boiled extract incubated with 0.1 M Zn acetate, the color changed from pale yellow to light brown initially and after overnight incubation under dark condition, it completely turned into deep brown color (Fig.1). Various phytocompounds present in the green tea leaves act as a reducing as well as stabilizing agents during ZnO NPs synthesis and the color change from pale yellow to dark brown was completed well after overnight incubation. No more color change was observed after this incubation. This indicates that the polyphenol contents of green tea leaves are concerned in the green synthesis and stability of ZnO NPs, particularly a water-soluble phytocompound epigallocatechin gallate acts as both a reducing and stabilizing agent [65]. Due to the excitation of surface plasmon resonance in ZnO NPs, the color change occurred [66].

(a) (b)

Figure 1: (a) shows ZnO synthesis within few hours, (b) shows ZnO synthesis after overnight

3.2 Characterization of green synthesized ZnO NPs

3.2.1 UV-VIS spectrophotometer analysis

Optical properties of ZnO nanoparticles were characterized using UV–Vis spectrophotometer. The UV–Vis absorption curve of ZnO nanoparticles is shown in fig. 2. Zinc oxide formation was confirmed as the absorption peak (lambda max) was found near 380 nm and similar result was shown by Karimzadeh *et al.*[67].

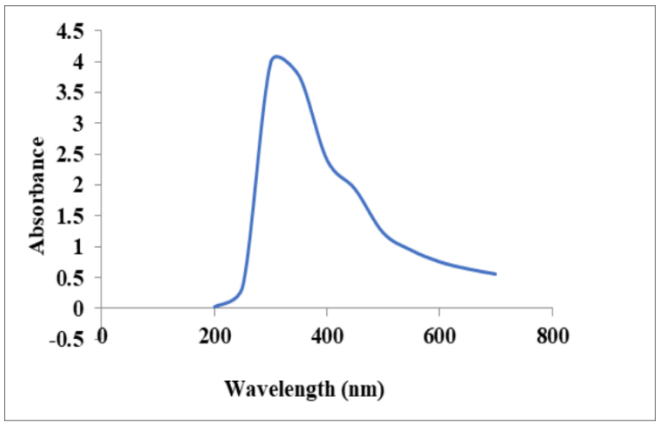

Figure 2: UV-VIS spectrum of green synthesized ZnO NPs

3.2.2 Fourier Transform Infrared (FTIR) analysis

FTIR analysis of the synthesized ZnO NPs was carried out at room temperature and a frequency range between 600-4000 cm^{-1}. The FTIR spectrum (Fig.3) shows the composition of the various bioactive molecules of green tea leaf and their distribution on the surface of the ZnO NPs. There is a broad stretch between 1000 - 4000 cm^{-1}. The broad stretch indicates the presence of various phytochemicals unique to green tea leaf such as alkyl amine, amides, aromatic ketones, phosphine, carboxyl acid, halogen compound and so on which aid in the stabilization of ZnO NPs. The obtained result was in agreement with the results of Lingegowda *et al.* [68], Lazar *et al.* [69], Hemmalakshmi *et al.* [70].

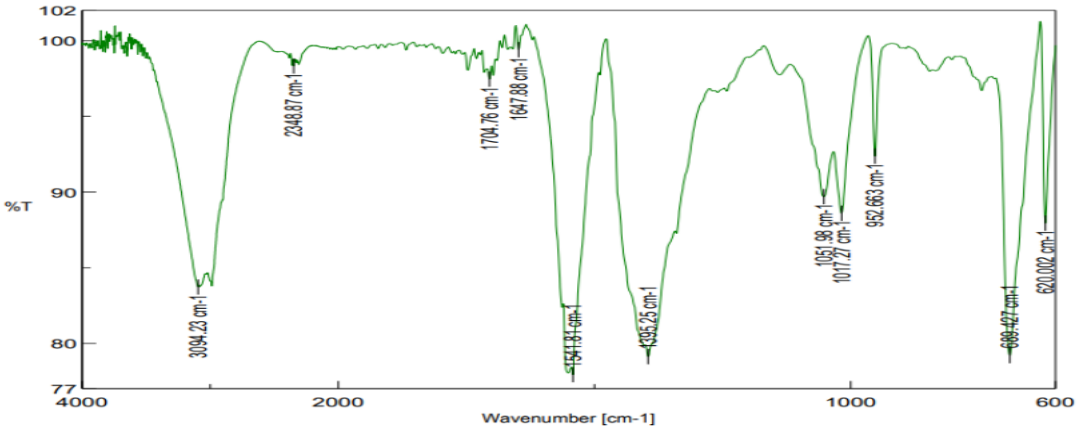

Figure 3: FTIR spectrum of green synthesized ZnO NPs

3.2.3 Zeta Potential

The Zeta potential of the green synthesized zinc oxide nanoparticles was determined in water as dispersant. The zeta potential of green synthesized ZnO NPs was found to be 8.32 mV as shown in Fig. 4. Zeta potential analysis was carried out to find out the surface charge of biosynthesized ZnO NPs [71]. The result of zeta potential indicates the capping phytocompounds present on the biosynthesized ZnO NPs were mostly comprised of negatively charged groups [72]. The detected negative charge of the ZnO NPs revealed the electrostatic repulsion between the synthesized nanoparticles [73]. A large positive or negative value of zeta potential indicates improved physical colloidal stability due to the electrostatic repulsions between the individual particles. On the other hand, particles with a smaller magnitude of zeta potential may lead to aggregation due to the action of Van der Waals forces [74].

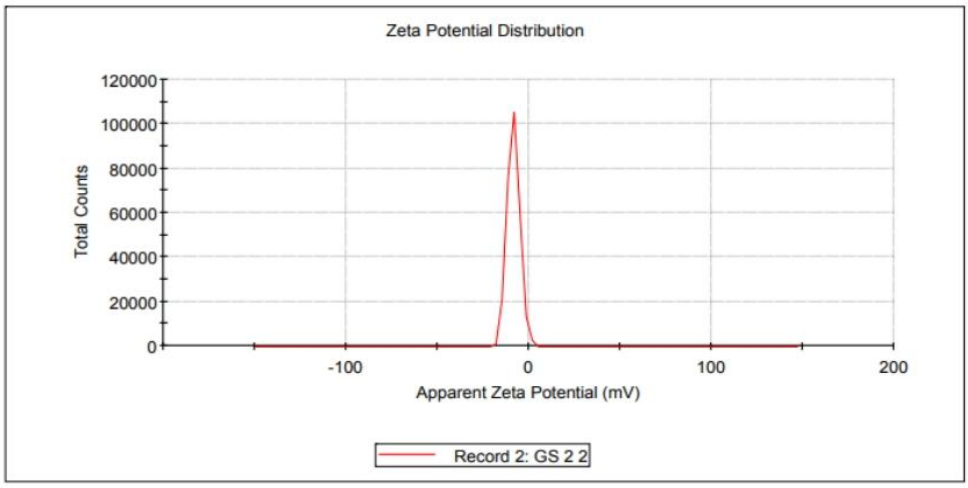

Figure 4: Zeta potential of green-synthesised ZnO NPs

3.2.4 SEM analysis

The topography of the green synthesized ZnO NPs was analyzed by SEM [75]. The image has shown individual semispherical as well as aggregated form of ZnO NPs (Fig.5). Similar result was reported by Wang *et al.* [76] during coffee leaf mediated ZnO NPs synthesis.

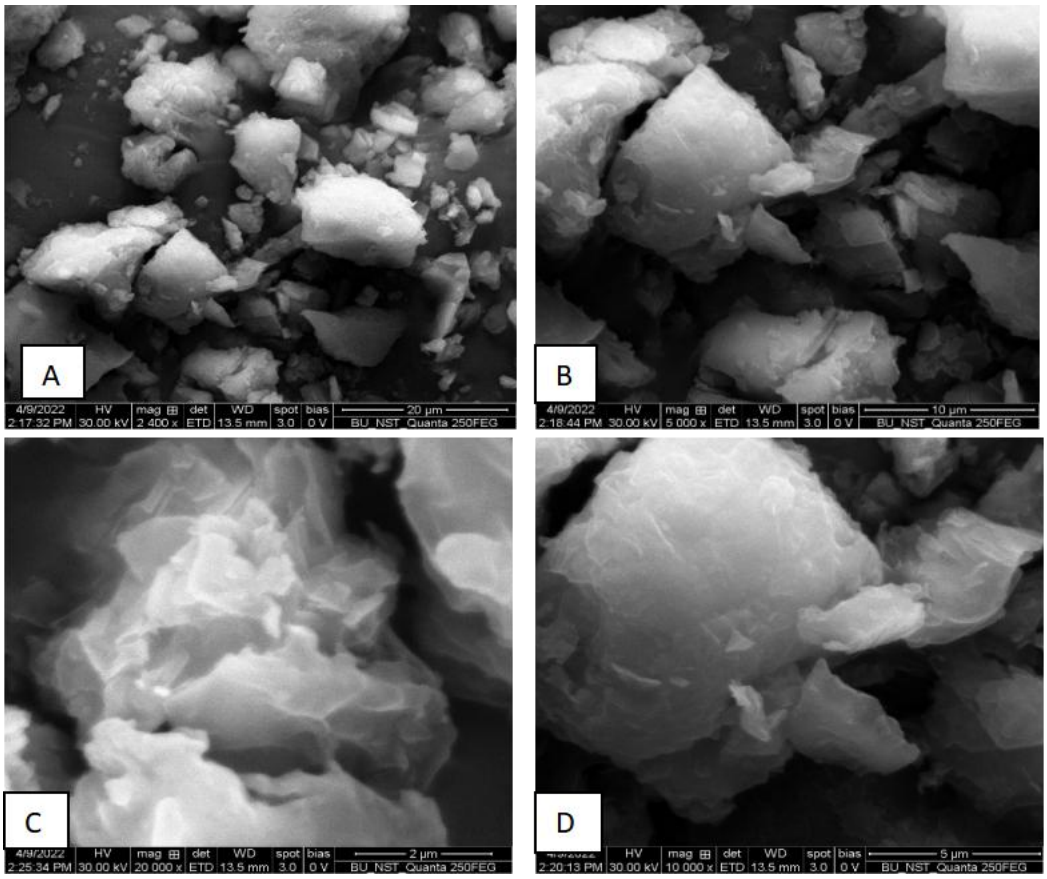

Figure 5: SEM micrographs of green-synthesised ZnO NPs synthesized

3.3 Effects of ZnO NPs on germination, morphological and biochemical attributes of *M. uniflorum*

3.3.1 Seed germination percentage

Table 1 shows the data regarding seed germination percent of *M. uniflorum* treated with different concentrations of ZnO NPs on fifth day of observation. ZnO NPs had significant effect on seed germination percentage (Fig.6 A-D). 10 and 20 mg/L ZnO NPs treated seeds showed the highest germination percentage (100 and 95%, respectively) compared to control seeds (61%). The germination percentage was dropped considerably with the increase of ZnO NPs. The lowest germination percentage was observed in 50 mg/L ZnO NPs treated seeds (40%). There are many reports that the application of nanomaterials at lower doses can enhance seed germination and plant growth [77,78,79,80]. The enhanced plant growth and development is due to the interaction between plant cells and nanoparticles. This interaction leads to changes in various metabolic pathways [22]. Small pores are produced when seeds are treated with nanoparticles which in turn boost the water absorption capacity and germination ability of the seeds [81,82].

Table 1: Germination percentage of *M. uniflorum* treated with different concentrations of ZnO NPs

S.No	Concentration (mg/L)	Germination percentage (%)
1	Control	61±1.00[c]
2	10	100±0.00[a]
3	20	100±0.00[a]
4	30	69.6±1.50[b]
5	40	51±1.00[d]
6	50	39.6±1.52[e]

Different letters within one column indicate significant difference between treatments

($p<0.01$) on DMRT analysis. Values consisted of Mean±SD

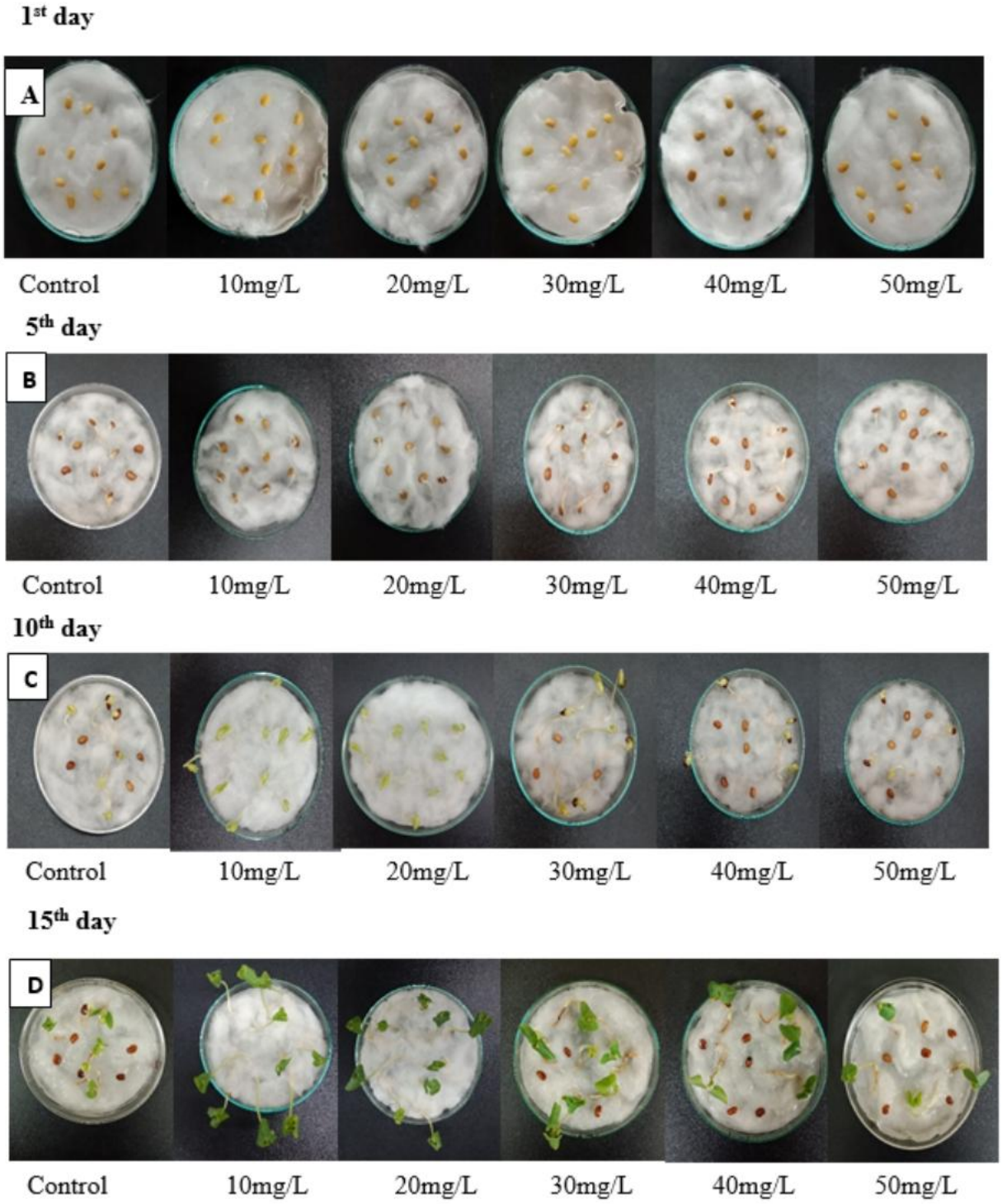

Figure 6: A- day One; B-Fifth day observation of seedlings of horse gram treated with different concentrations of ZnO NPs; C- Tenth day observation of seedlings of horse gram treated with different concentrations of ZnO NPs; D-Fifteenth day observation of seedlings of horse gram treated with different concentrations of ZnO NPs.

3.3.2 Shoot and root length of ZnO NPs treated plants

Data pertaining to shoot and root lengths horse gram plants treated with different concentrations of ZnO NPs is presented in the Table 2. Increased concentrations of ZnO NPs had noteworthy effect on shoot and root lengths of germinated seedlings of horse gram (Fig.7). With the increase of concentration of NPs, both shoot and root lengths were reduced. The lowest shoot and root lengths were observed in seedlings treated with 50mg/L ZnO NPs (2.26±0.25 for shoot; 3.23±0.25 for root). The highest shoot and root lengths were observed in seedlings treated with 10 mg/L (10.03±0.25 for shoot; 8.46±0.15 for root) followed by 20 mg/L ZnO NPs (9.8.13±0.12 for shoot; 7.02±0.10 for root).

Figure 7: Fifteenth-day-old seedlings of horse gram treated with different concentrations of ZnO NPs used for measurement of shoot and root lengths

Table 2: Shoot and root length of horse gram treated with different concentrations of ZnO NPs

Treatment (mg/L)	Shoot Length (cm)	Root Length (cm)
Control	4.03±0.55[d]	2.30±0.30[d]
10	8.46±0.15[b]	10.03±0.25[a]
20	10.03±0.25[a]	10.63±0.15[a]
30	7.53±0.15[c]	4.70±0.20[b]
40	4.3±0.30[d]	3.66±1.76[c]
50	2.26±0.25[e]	3.23±0.25[c]

Different letters within one column indicate significant difference between treatments

($p<0.01$) on DMRT analysis. Values consisted of Mean±SD.

3.4 Effects of ZnO NPs on biochemical attributes of horse gram

3.4.1 Estimation of total chlorophyll content

Table 3 presents the data regarding chlorophyll contents of horse gram treated with different doses of ZnO NPs. Various concentrations of ZnO NPs had considerable effect on chlorophyll a, chlorophyll b and total chlorophyll contents of germinated seedlings. Compared to control, these contents were gradually increased with the increase of ZnO NPs concentrations. The data revealed that maximum total chlorophyll contents were in plants treated with 10 and 20 mg/L ZnO NPs (17.30±0.005 and 15.85±0.004, respectively). The contents were found reduced gradually from the dose of 30 mg/L ZnO NPs. The amount of chlorophyll a and b in control leaf was less when compared to green

synthesized ZnO NPs treated plants. Earlier studies stated that ZnO NPs at lower dose significantly augmented chlorophyll content in *Triticum aestivum* [83], *Cenchrus americanus* [84], *Cicer arietinum* [82], *Cyamopsis tetragonoloba* [85] and *Persicaria hydropiper* [86]. Enhanced chlorophyll contents are due to zinc which acts as a structural and catalytic component of proteins and enzymes and as a co-factor for the characteristic development of pigment biosynthesis [87].

Table 3: Total chlorophyll contents of horse gram treated with different concentrations of ZnO NPs

S.No	Concentration (mg/mL)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
1	Control	3.07±0.002^{f}	1.56±0.003^{f}	4.64±0.005^{f}
2	10	14.01±0.003^{a}	3.29±0.001^{c}	17.30±0.005^{a}
3	20	11.06±0.002^{b}	4.79±0.002^{a}	15.85±0.004^{b}
4	30	8.86±0.003^{c}	3.56±0.003^{b}	12.43±0.006^{c}
5	40	5.90±0.002^{d}	2.60±0.002^{e}	8.50±0.004^{d}
6	50	5.41±0.012^{e}	2.71±0.003^{d}	8.13±0.015^{e}

Different letters within one column indicate significant difference between treatments ($p<0.01$) on DMRT analysis. Values consisted of Mean±SD.

3.4.2 Determination of total protein

The result presented in the fig. 8 shows the total protein content of germinated seedlings of horse gram treated with different concentrations of ZnO NPs. The plants treated with 10 and 20 mg/L ZnO NPs had significantly higher level of protein compared to control. Protein content was found to be reduced gradually when doses increased from 30 mg/L. These findings were in accordance with Raliya and Tarafdar [85], Tarafdar *et al.* [84] and Mukherjee *et al.* [80] who showed ZnO NPs at lower dose significantly enhanced the total protein content in cluster bean, pearl millet and green pea, respectively. Zinc is essential as structural and catalytic constituent of protein and enzymes for normal growth and development of plants [88].

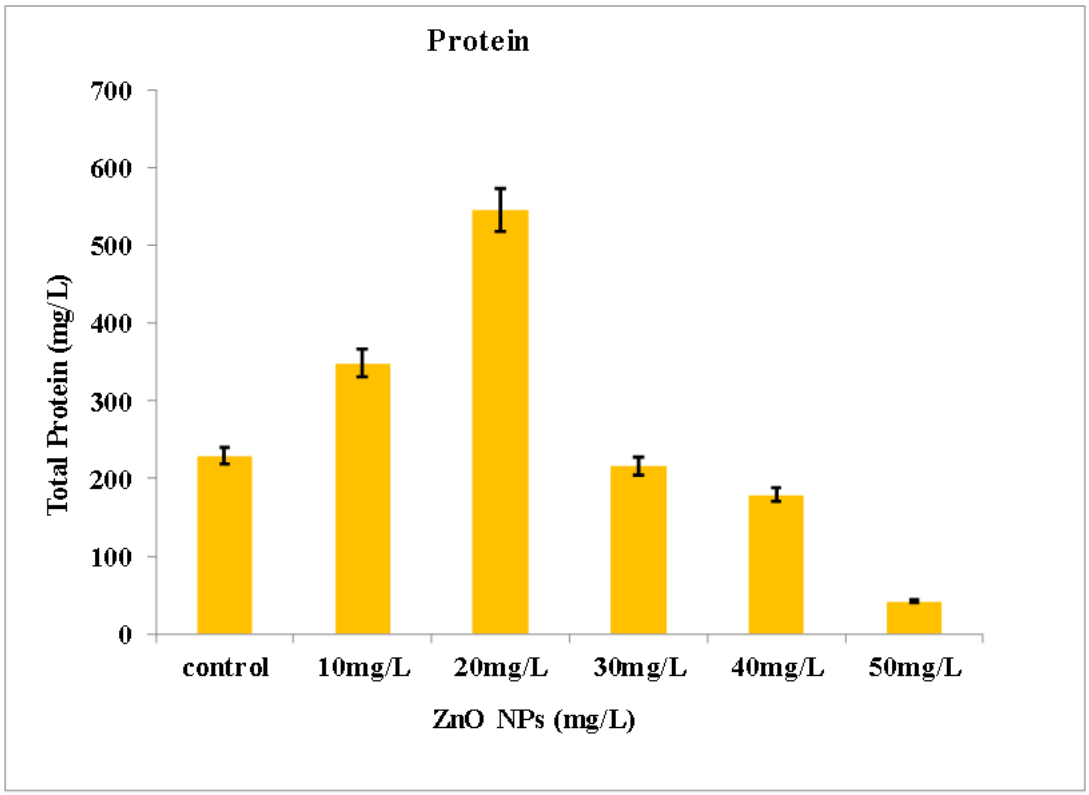

Figure 8: Total protein content of *M. uniflorum* treated with different concentrations of ZnO NPs

3.4.3 Determination of total carbohydrate

Fig. 9 presents the mean data regarding the effect of ZnO NPs on total carbohydrate content of germinated seedlings of *M. uniflorum*. The results show that ZnO NPs has a significant rising effect on carbohydrate levels in all the germinated seedlings. The data indicated that maximum carbohydrate contents were in plants treated with 10 and 20 mg/L ZnO NPs 360.0±14.93 and 240.3±1.15, respectively). Similar results were shown in ZnO NPs on growth of *sesamum indicum* L. [89]. A higher level of total carbohydrate was noted at a lower dose of ZnO nanoparticles treated with *Sesamum indicum* [89] and *Cicer arietinum* seedlings [82].

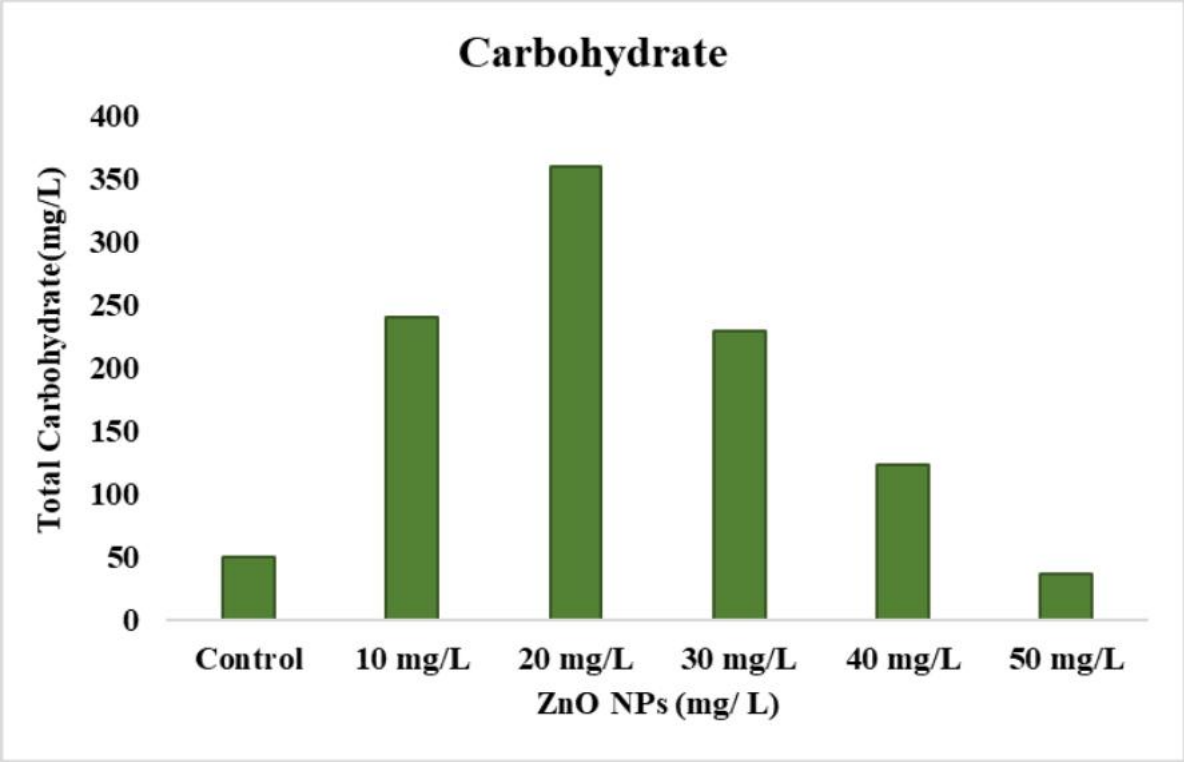

Figure 9: Total carbohydrate content of *M. uniflorum* treated with different concentrations of ZnO NPs.

3.4.4 Quantitative phytochemical analysis

Table 4 presents the mean data regarding the effect of ZnO NPs on four secondary metabolites *i.e* total phenolic, flavonoid, terpenoid and tannin contents of germinated seedlings of *M. uniflorum*. The data revealed that maximum level these contents were in plants treated with 10 mg/L ZnO NPs. Generally, the application of NPs in lower concentrations has been found to enhance plant growth and secondary metabolite production[90].

Table 4: Quantitative analysis of secondary metabolites of *M. uniflorum* treated with different concentrations of ZnO NPs

S.No	ZnO NPs (mg/L)	Phenols (mg GAE/g extract)	Flavonoids (mg RUE/g extract)	Terpenoids (mg LIN/g extract)	Tannins (mg TAN/g Extract)
1	Control	19.88±0.13^{f}	15.38±0.06^{f}	6.68±0.11^{f}	15.59±0.02^{f}
2	10	44.17±0.03^{b}	45.54±0.03^{b}	18.92±0.09^{b}	56.38±0.03^{b}
3	20	61.42±0.05^{a}	57.53±0.03^{a}	24.11±0.06^{a}	65.91±0.03^{a}
4	30	32.67±0.04^{c}	31.68±0.20^{c}	16.01±0.09^{c}	35.80±0.04^{c}
5	40	29.34±0.07^{d}	17.21±0.03^{d}	12.48±0.42^{d}	28.29±0.03^{d}
6	50	20.52±0.04^{e}	16.52±0.01^{e}	11.20±0.04^{e}	18.44±0.06^{e}

Different letters within one column indicate significant difference between treatments ($p<0.01$) on DMRT analysis. Values consisted of Mean±SD

4. CONCLUSION

This study presents the effect of green synthesized ZnO NPs using green leaves at different concentrations on *Macrotyloma uniflorum* (kollu) seed germination, shoot and root lengths and some primary and secondary metabolites. Maximum levels of germination percentage, shoot and root lengths, primary metabolites such as total chlorophyll, protein, carbohydrates and secondary metabolites such as total phenols, flavonoids, tannins and terpenoids were noted in 10.0 mg/L ZnO NPs treated plants compared to control. With the increase of concentrations ZnO NPs, all the parameters were found to be decreased. These findings indicate that the application of ZnO NPs at a lower concentration may increase germination, morphological and biochemical attributes of *M. uniflorum*. Further investigations are required to understand the mechanisms at a molecular level for ZnO NPs to be used as nanofertilizers or growth stimulants for this plant.

Ethics statement: As the seed materials of *Macrotyloma uniflorum* (Lam.) Verdcourt variety Paiyur 2 were purchased from TNAU, Coimbatore 641 046, Tamil Nadu, India, ethical permission is not required.

Author contributions: Balasundari Ponpandi and Aarthi Jeganathan carried out the experiments. Both Sradha Sajeev and Anju Rani George written and edited the whole manuscript, Kavimani Thangasamy and Madhu Priya Govindhan Anbazhagan assisted in making figures and other related work. Geetha Natesan designed the experiments and supervised.

Declaration of competing interest: The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of data and materials: The authors confirm that the data supporting the findings of this study are available within the articles and its supplementary material. Raw data that support the findings of this study are available from the corresponding author, upon reasonable request.

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