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Research Article

Enhanced growth, yield and phytoconstituents in turmeric (*Curcuma longa L.*) plants treated with β-D-Glucan nanoparticle under glass house condition

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The effect of foliar application of β-D-glucan nanoparticles (GNP) on the growth profile and yield of *Curcuma longa* was carried under glasshouse conditions. Various growth and yield related parameters, including plant fresh weight, shoot height, number of leaves, protein profile and rhizome yield were positively affected by the GNP treatment. The increase of large and small subunits of Rubisco was identified on the gel. GNP treatment increased the number of leaves per plant. Application of GNP significantly improved the curcumin content in turmeric plants. The results demonstrate the successful use of GNP in enhancing growth, yield and phytoconsituent of *C. longa.*

Key words: Glucan nanoparticle, curcumin, polypeptides, rubisco, turmeric

INTRODUCTION

Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione] is a phenolic substance derived from the rhizome part of the plant, *Curcuma longa L.* and is used as a dietary spice and a natural coloring agent for foods (Li, et al. 2009). In recent years, it has attracted interest because of its antioxidant, anti-inflammatory, hypolipidemic and potential cancer chemopreventive activities (Li, et al. 2009; Zingg, et al. 2013). Curcumin is also a potent scavenger of various reactive oxygen species (ROS) including superoxide anions and hydroxyl radicals (Ruby, et al. 1995). In addition, it has been suggested that curcumin can be used for prevention and treatment of Alzheimer disease (Lim, et al. 2001). Generally, curcumin content is around 3% by weight (Tayyem, et al. 2006). However, the content varies depending on varieties, location and cultivation practices etc (Hayakawa, et al. 2011; Singh, et al. 2014).Therefore, it is necessary to increase the curcumin content of turmeric plants to meet the global requirements. Development of technologies that improve productivity without causing any adverse effect on the environment is the need of the hour.

Nanotechnology is becoming interestingly important for the agricultural sector. Nanomaterials hold great promise as a novel agrochemical regarding their application in production due to their size-dependent qualities, high surface-to-volume ratio and unique optical properties (Ghormade, et al. 2011). At present, metal-based nanomaterials/carbon-based nanomaterials were used in the agricultural sector to induce growth and development in crop plants (Nair, et al. 2010). Improvement of agronomic traits that documented increased leaf and pod dry weight and grain yield of soybean by exposure to nano-ironoxide has been reported (Sheykhbaglou, et al. 2010). Studies have shown that engineered nanoparticles influence the elemental concentrations, phenolic content and radical scavenging ability in plants (Yasur and Rani, 2013). The potential adverse effects of these nanoparticles on biological systems and possible environmental toxicity due to the unpredicted nature of nanoparticles have raised serious questions of their application in crops. Hence, the selection of nanomaterial for application in the field may be critical as materials which are non-toxic, biocompatible and biodegradable are desirable. Nanoparticles prepared from biopolymers or natural sources possess advantages such as availability from replinishable agricultural resources, biocompatibility, biodegradability and ecological safety (Ghormade, et al. 2011).

Biosynthesis of secondary metabolites is affected considerably by biotic and abiotic stimuli (Sathiyabama, et al. 2016; Gorelick and Bernstein, 2014). Potential elicitation of plants for manipulation of the chemical profile, for increased synthesis of secondary metabolites is less explored. (Gao, et al. 2006) reported that nano TiO2 treated spinach showed enhanced mRNA expressions and protein level. A significant correlation

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between leaf area, yield and phytoconstituent of turmeric rhizome has been reported (Singh, et al. 2014). We report here the improvement in plant growth, changes in protein profile, yield coupled with enhanced content of curcumin through foliar application of GNP.

MATERIALS AND METHODS

Biological material

Rhizomes of *Curcuma longa* (*syn C. domestica*) cultivars Erode local (susceptible) was obtained from farmer's field at Erode, Tamilnadu, India. The fungus *P. aphanidermatum* was obtained from ITCC (Indian Type Culture Collection), New Delhi, India and was maintained on Potato Dextrose Agar.

GNP preparation and foliar application of nanoparticles

GNP from β-glucan of *P. aphanidermatum* was prepared as described earlier (Anusuya and Sathiyabama, 2014). Rhizomes were thoroughly washed with running tap water thrice followed by glass distilled water; surface sterilized by immersion in sodium hypochlorite 0.001% (v/v) solution for 15 min followed by several rinses of sterile distilled water. The rhizomes (2-3 rhizomes each with 3 nodes) were planted in earthen pots (27cm diameter; 26 cm height) containing soil and manure and maintained under glass house condition. GNP (0.1%, w/v) were applied to 30 day old plants (5 ml/plant) by foliar spray and at a regular interval of 30 days up to 210 days. Water sprayed plants served as control. For each experiment 30 plants were used and replicated thrice.

Effect of GNP on plant growth

Growth parameters of turmeric plants such as number of leaves per plant, shoot height, plant fresh weight in control and treated plants were monitored at different age levels up to harvest (215 days).

Extraction and SDS-PAGE separation of proteins

Leaves/rhizomes (1 g/2 ml) from control and GNP treated turmeric plants were homogenized with potassium phosphate buffer (0.02 M, pH 7.6) and centrifuged at 10,000 G for 10 min at 4˚C. The clear supernatant was used as a source of protein.

The samples (10 μg) were denatured for 3 min in a boiling water bath with sodium dodecyl sulphate (SDS) containing sample buffer. They were separated along with standard proteins (Bio Rad chem. Co., USA) on 10% (w/v) polyacrylamide gel with 4% stacking gel as reported by Laemmeli (1970). After electrophoresis, the gels were stained with silver nitrate by the procedure of Blum et al., (1987).

Estimation of phenylalanine ammonia lyase (PAL) activity

PAL (EC.4.3.1.5) activity was determined as the rate of conversion of l-phenylalanine to transcinnamic acid at 290 nm (Dickerson et al., 1984). Enzyme activity was expressed as μmol trans-cinnamic acid min^{-1} g $protein^{-1}$.

Effect of GNP on rhizome yield

Rhizome yield was determined at the time of harvest. The average fresh and dry weight of rhizome per plant was denoted in terms of gram. The results expressed as yield increase percentage using the following formula; Yield increase (%)= [treatment yield - Control yield]/Control yield × 100 (Anusuya and Sathiyabama, 2015).

Effect of GNP on curcumin content

Extraction and HPLC analysis of curcumin: Each raw sample was extracted with methanol at 60˚C for seven hours in a Soxhlet apparatus and dried using rotary evaporator (Yamato RE 601). Curcumin content was analyzed by HPLC (Shimadzu 9A model) using a reverse phase C18 column (250 mm × 4.60 mm) with the mobile phase composed of acetonitrile with 0.1% (v/v) acetic acid at a flow rate of 1.0 ml/min and detected at 420 nm. The injection volume was 20 µL and the analysis time was 20 min per sample. The concentration of curcumin in different samples was quantified by comparing the peak area and the peak height with that of authentic curcumin (Sigma Chem. Co. USA).

Statistical analysis: All the data were subjected to one-way analysis of variance to determine the significance of individual diferences at $p<0.01$ and 0.05 levels. All statistical analysis was conducted using SPSS 16 software support.

RESULTS AND DISCUSSION

Evaluation of a variety of nanomaterials (NMs), mostly metal-based (MBNMs) and carbon-based (CBNMs) were reported for their absorption, translocation, accumulation, and importantly, effects on growth and development in an array of crop plants (Nair, et al. 2010; Rico, et al. 2011). Some of these studies have documented non-consequential or negative effects on plant growth and development upon NM exposure, whereas others report positive results. The positive morphological effects included enhanced germination percentage and rate; length of root, shoot and vegetative biomass of seedlings in many crop plants including corn, wheat, ryegrass, alfalfa, soybean, rape, tomato, radish, lettuce, spinach, onion, pumpkin and cucumber (Kole, et al. 2013).

The number of leaves per plant increased with GNP treatment, as compared to the control seedlings (Figure 1a). A maximum increase of 30% in leaf number was recorded in seedlings with GNP at 215 days (Figure 1a). Also, GNP treatment increases the average leaf area of the treated seedlings (data not shown). Leaf number as well as leaf area is regulated by a complex interaction of various genes whose expression is modulated by growth hormones (Gonzalez, et al. 2010; Arora, et al. 2012). It may be possible that application of GNP could alter the synthesis of endogenous hormones, which results in changes in growth profile.

GNP mediated improvement in growth profile of turmeric seedlings was evident with the increase in shoot height per plant. Application of GNP caused an increase in average shoot height, as compared to the untreated plants (Figure 1b). Nearly 10% increase in shoot height was observed in GNP treated plants. Silver nanoparticle treatment was reported to increase the plant height of Borago (Seif, et al. 2011).

Plants treated with GNP also showed increased plant fresh weight (Figure 1c) and is maximum on 215th day. When nanoparticles are applied on leaf surfaces, they enter through the stomatal openings or through the bases of trichomes and then translocated to various tissues (Fernandez and Eichert, 2009; Uzu, et al. 2010; Wang, et al. 2013). Here, we report GNP successfully improves their growth, leading to an increase in the net productivity in terms of rhizome yield. This is in accord with previous report, that increased leaf number and area exerts high accumulation of curcumin (Singh, et al. 2013).

Effect of GNP on protein profile

Application of GNP significantly accelerated the synthesis of total proteins in turmeric plants (Anusuya and Sathiyabama, 2015) and led to modulate the pattern of protein profile. The polypeptides with molecular mass of 55 kDa and 14 kDa (subunits of Rubisco) were found to be enhanced in leaves of treated plants (Figure 2). Gao, et al. (2006) reported the similar kind of results in nanoanatase treated spinach and suggested that the concentration of photosynthetic enzymes Rubisco was enhanced due to their higher efficiency of carbon fixation. Enhanced photosynthetic activity in some crop plants by metal based nanoparticles has been reported (Kole, et al. 2013). The results strongly support that nanoparticle application is involved in photosynthetic enzyme Rubisco subunits. Rubisco is the most important enzyme to fix carbon dioxide from atmosphere which is inefficient during unfavourable climatic condition and leads to limits agricultural productivity. The increase of larger (55 kDa) and smaller (14 kDa) subunits of Rubisco enzyme by GNP treatment might be the reason to enhance growth and yield of turmeric. These results substantiate that application of GNP involved in the photosynthetic enzyme Rubisco subunits. However the mechanism of GNP on Rubisco activation is unclear. Enhancement of many physiological parameters related to plant growth and development were also reported that include enhanced photosynthetic activity by metal based nanoparticle in few crops including soybean, spinach and peanut (Kole, et al. 2013).

It was evident from (Figure 3) that phenylalanine ammonia lyase (PAL), one of the key enzyme of phenolic (curcumin) biosynthesis (Green, et al. 2008) increased sharply in rhizomes after 4 months in turmeric plants treated with GNP. It has been reported that when a plant is exposed to elicitors, metabolic pathways are induced, which modify the concentration of bioactive secondary metabolites (Ebel and Casio, 1994). PAL can be induced by different environmental stress conditions (Tomas-Barberanand Espin, 2001). Jia, et al. (2005) reported that nanoparticles may bind with different cytoplasmic organelles and interfere with the metabolic processes at that site.

Effect of GNP on yield and curcumin content

Treatment of turmeric plants with GNP also brought about a signiicant increase in yield per plant (Figure 4) both in terms of fresh and dry weight. GNP treated plants showed nearly 75% increase in fresh weight and 135% increase in dry weight of rhizomes (Figure 4a and 4b). The increase in rhizome also translated into an increase in curcumin content (Figure 5). Approximately 10-fold increase in curcumin content was observed in treated plants (Figure 5). There was a significant variation in yield coupled with increase in curcumin content due to foliar application of nanoparticles on turmeric plants. The increased PAL activity corresponds with the increased curcumin content. These results indicate that application of nanoparticles had a tremendous effect on growth and development in turmeric. However, the exact mechanism for induction of curcumin is not known. The ability of carbon nanomaterials to penetrate the cell wall and cell membrane of intact plant cells were recently reported (Liu, et al. 2009). Kole, et al. (2013) reported the improvement in biomass yield, and fruit yield along with enhanced content of phytomedicine in bitter melon through seed treatment with carbon based nanoparticle, fullerol. Improvement of agronomic traits that documented increased leaf and pod dry weight and grain yield of soybean by exposure to nano-iron oxide have been reported (Sheykhbaglou, et al. 2010). The benefits of nanomaterial based formulations are the improvement in efficacy due to higher surface area, higher solubility, and induction of systemic activity due to smaller particle size, higher mobility and lower toxicity (Sason, et al. 2007). The results obtained in this study indicate the potential application of GNP (a replenishable resource) to enhance plant growth along with yield in terms of curcumin in turmeric plants to meet the global requirements.

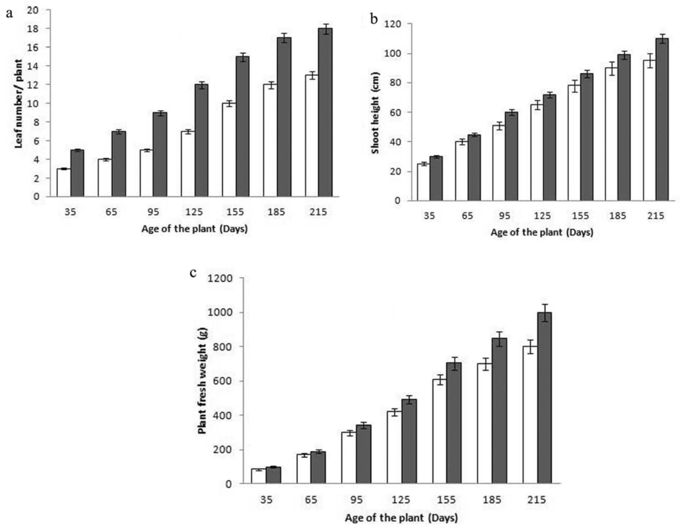

Figure 1. Leaf number/ plant (a), shoot height (b) and plant fresh weight (c) of turmeric plants treated with GNP. All the values are means of three replicates; Data represent Mean ± Standard Error.
Note: ( ) Control, ( ) GNP treated.

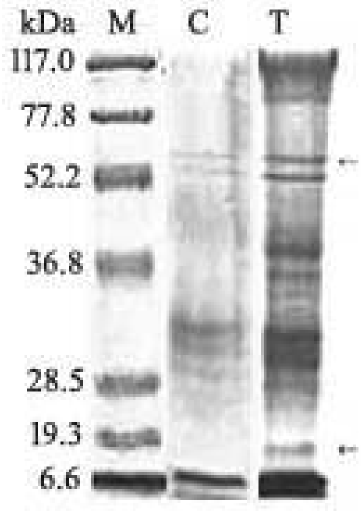

Figure 2. Protein profile of leaves of turmeric plants (7th month): M- marker protein standard (Bio Rad Chem. Co., USA); C-control; T-treated.

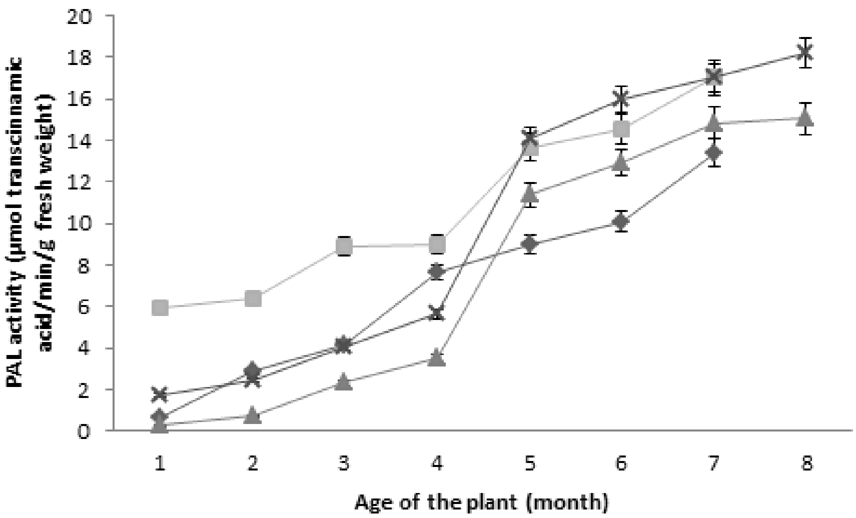

Figure 3. PAL activity in leaves and rhizomes of control and treated turmeric plants. L-Leaf; R-Rhizome; *All the values are means of three replicates; Data represent Mean ± Standard Error. **Note:** (—◆—) Contril-L, (—■—) Treated-L, (—■—) Contril-R, (—▲—) Treated-R.

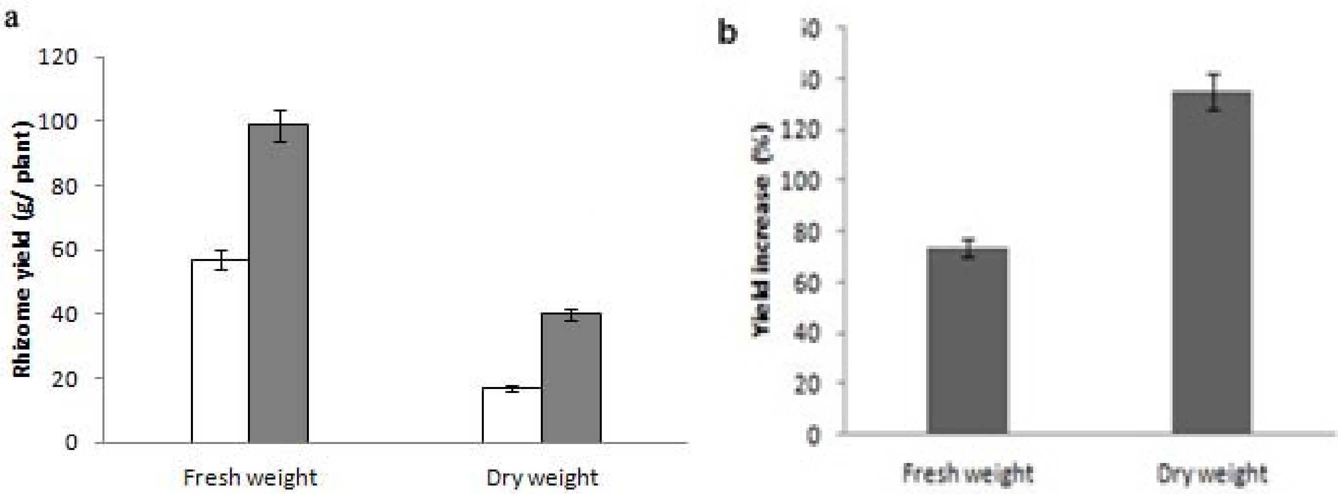

Figure 4. Rhizome yield (a) and curcumin content (b) of turmeric plants.
Note: (☐) Control, (■) GNP treated.

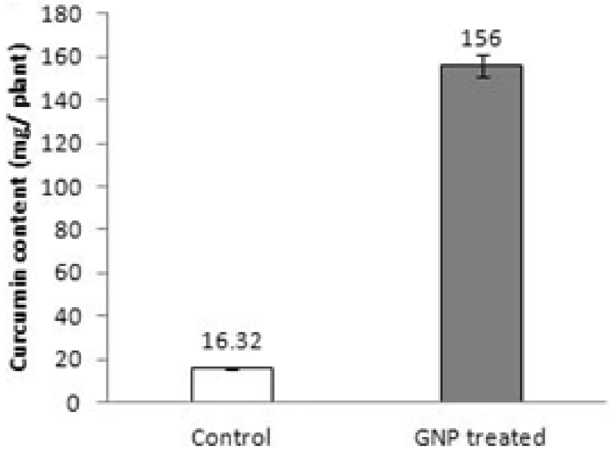

Figure 5. Curcumin content in turmeric plants.
Note: ( ) Control, ( ) GNP treated.

Results of the present study demonstrated the potential of exogenous application of GNP for increased production of curcumin for agro-industrial production. The GNP elicitation increased significantly the production of curcumin in the plant by simultaneous effects on rhizome development and curcumin concentration in the rhizome tissue. The increased bioaccumulation of curcumin in turmeric under GNP elicitation is accompanied by plant growth stimulation and supports the concept of the function of GNP as a plant growth regulator.

CONCLUSION

Application of bio-based nanoparticles in agriculture is still in a nascent stage. In the present study, we demonstrated that GNP induced the growth promotory response in turmeric plants leading to an increase in the net productivity in terms of rhizome yield. There is a correlation between increased Rubisco subunits in treated plants with increased growth and curcumin content. These findings could pave the way for further physiological, genomics, transcriptomics and metabolomics studies underlying genetic causes for promotion of such agro-economic characters.

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