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# Synthesis of carbon nanomaterials and their effect on the *in-vitro* growth of *Ehretia laevis* Roxb.

Admuthe N. B. and Nalwade A. R.
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## ABSTRACT

The present study was undertaken to study the synthesis of carbon nanomaterials and their effect on the *in-vitro* growth of *Ehretia laevis* Roxb. Carbon nanomaterials were synthesized from carbon soot. The water soluble carbon nanomaterials were produced by sonication. The nodal sector explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations 0.5-5.0 mg/l of Benzyl Amino Purine (BAP). The obtimum result of BAP (2.5mg/l) induced mean of 5.1 shoots, 2.34cm shoot length and 10.6 leaves/explants. Murashige and Skoog (MS) medium 2.5mg/l BAP was supplemented with increasing concentrations 20, 40, 60, 80, 100 and 120 µg/ ml of water soluble carbon nanomaterials. MS medium without carbon nanomaterials served as control. *In vitro* grown shoots were inoculated on the MS medium for the study. Inoculation of culture tubes was carried out under aseptic conditions in the laminar airflow cabinet and incubated at 25±2$^0$C temperature and 3000 lux light intensity in the culture room. After 4 weeks results were obtained. The results of the present investigation showed that at lower concentration of carbon nanomaterials have stimulatory effect, while above 60µg/ml the detrimental effect was observed.

**Key words**: Carbon nanomaterials, explants, sonication, soot, shoots, *Ehretia laevis*.

## INTRODUCTION

Synthesis of water-soluble carbon nanotubes is an important topic because such materials have potential applications in biology and material science. Carbon nanotube (CNTs) in agriculture is a newly up-coming area of research. Carbon nanomaterials were obtained from carbon soot. Carbon soot from mustard oil was modified to make water soluble carbon nanomaterials of desired size and morphology. Synthesized carbon nanomaterials were made water soluble. Plant tissue culture is the technique of growing plant cells, tissues and organs in an artificial nutrient medium, under aseptic conditions. Tissue culture is also referred as *in vitro* growth, which literally means growth in the glass containers like test tubes, bottles etc. *Ehretia laevis* Roxb. commonly known as Ajanvruksh, Datrang belongs to family Boraginaceae is widely used as medicinal plant. *Ehretia laevis* Roxb. is high valued medicinal plant and becoming rare in the state of Maharashtra. Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend on the these plants for the production of pharmaceutical compounds (Chand *et al.*, 1997). WHO estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. The developed nations are also looking for eco-friendly treatment of various diseases through plant based source.

The present investigation was carried out to study the synthesis of carbon nanomaterials and their effect on the *in-vitro* growth of *Ehretia laevis* Roxb.

## MATERIALS AND METHODS

This study was undertaken in the Tissue Culture Laboratory, Department of Botany, Annasaheb Awate Arts, Commerce and Hutatma Babu Genu Science College, Manchar, Dist.-Pune, Maharashtra, India.

**Collection of Explant Material**

The flowering twigs of *Ehretia laevis* Roxb. were collected from Pune, Maharashtra, India. The taxonomic identification was accomplished with the help of the Flora of Presidency of Bombay (Cook, 1958) and Flora of Maharashtra State (Singh, *et al.,* 2000) for identification. The herbarium was prepared (Jain and Rao,1976) and it was identified and authenticated at Botanical Survey of India, Pune, Maharashtra, India (Voucher number is BSI / WRC / TECH / 2013 / 156).

Carbon nanomaterials were synthesized by Dubey *et al* (2005) method from soot. The raw carbon soot was produced using stainless steel pot from the top of the flame by burning mustard oil using cotton wick in insufficient air. The dried sample was used for the further study. Water soluble carbon nanomaterials were produced by keeping 2 g carbon soot in 100 ml concentrated nitric acid for 24 h. Excess of nitric acid was removed and black mass washed with distilled water several times till it was neutral. Repeated adding of water and evaporation under boiling water bath removed all traces of nitric acid. The black mass was dried and subjected to analysis. Carbon nanomaterials thus formed became water soluble after sonication.

**Surface sterilization of explants and non- living articles**

Nodal segments of *Ehretia laevis* Roxb. were collected from wild plants. The shoot segments were washed under running tap water for 30 min, after that immersed in liquid detergent and washed thoroughly with distilled water. The surface sterilization of explants was done by dipping them in freshly prepared 0.1% $HgCl_2$ solution for 10 minutes and finally rinsing three times with sterile distilled water to remove all traces of $HgCl_2$. The sterilization procedure of non living articles such as nutrient medium, glass goods, distilled water, instruments was carried out by autoclaving under stream at a pressure of 15 $lb/in^2$ and a temperature of 120 $^0$ C for 15 minutes.

**Preparation of culture medium and inoculation**

The most popular and responsive Murashige and Skoog (1962) medium was used for the present investigation. Chemicals used for the investigation were obtained from Sigma Chem. USA. MS medium was supplemented with different concentrations of growth regulators and carbon naomaterials. The pH of the medium was adjusted to 5.8 using 0.1N HCl or 0.1N NaOH before autoclaving. 20 ml medium was dispensed in each culture tube. The medium was sterilized at 15 $lb/in^2$ for 15 min at 120$^c$ temperature.

**Culture condition**

After the surface sterilization, explants were inoculated on the MS medium. Each culture tube was inoculated with the one explants. All cultures were incubated in the culture room for 16 hrs light/ 8 hrs dark photoperiod under the light intensity of 3000-5000 lux and 25 ± 2°C temperature.

**Shoot initiation**

Nodal segment explants of *Ehretia laevis* Roxb. were cultured *in vitro* on MS medium supplemented with increasing concentrations of BAP (0.0 to 5.0 mg/l) Optimum concentration of BAP for shoot initiation was recorded.

**Preparation of medium augmented with carbon nanomaterials for the *in-vitro* shoots growth**

Murashige and Skoog (MS) medium containing 2.5mg/l BAP (optimum concentration for shoot initiation) was prepared for the study of shoot growth. MS medium was supplemented with increasing concentrations (20, 40, 60, 80, 100 and 120 μg/ml) of water soluble carbon nanomaterials. MS medium without carbon nanomaterials served as control. Ten culture tubes of each concentration were prepared. *In vitro* grown shoots were inoculated on the MS medium supplemented with increasing concentration of 20, 40, 60, 80, 100 and 120 µg/ ml of water soluble carbon nanomaterials in culture tubes for the study of shoot growth. Optimum concentration was recorded.

**Statistical analysis**

The data were means of three replicates with standard deviation as the measures of variability. One-way ANOVA test was performed to determine significant differences due to various treatments. Fisher's LSD (Least significant difference) was used as post hoc test to as certain significant differences among treatments at p = 0.05. Statistical analysis and graphical data presentations were carried out by using Sigma stat (ver.3.5).

## RESULTS AND DISCUSSION

When nodal segment explants were cultured on MS medium supplemented with BAP (2.5 mg/l), produced maximum number of shoots (5.1shoot/explants), highest shoot length (2.34cm) and highest number of leaves/explants (10.6) were recorded after 4 weeks. Similar results are reported by Faisal *et al.* (2007) in *Tylophora indica* (Burm f.) Merrill plants, Owk *et al.* (2011) in *Withania somnifera* (L) Dunal and also Arya et *al.* (2013) in *Dalbergia sissoo* Roxb.

**Effect of carbon nanomaterials on the *in-vitro* shoot growth of *Ehretia laevis* Roxb.**

The results of *in vitro* shoot growth are presented in the Table. These results show the comparative study of effect of different concentrations of water soluble carbon nanomaterials. It was observed that number of shoots per explants was linearly decreased with increasing concentration of carbon nanomaterials, while shoot length was increased linearly up to 60 µg/ ml carbon nenomaterials and then it was decreased. .The highest shoot length ((3.46cm) was recorded at 60 µg/ ml. The number of leaves per explants increased with increasing concentration of carbon nanomaterials and highest number of leaves (12.7) was observed at 60 µg/ ml carbon nenomaterials.

**Table: Effect carbon nanomaterials with optimum conc. of Cytokinin BAP on shoot growth from *in vitro* shoot explants of *Ehretia laevis* Roxb. No. of shoot/ explants, shoot length (cm), No. of leaves/ explants**

| MS+2.5 mg/l BAP+ CNT | No. of Shoot / explants | Shoot length (cm) | No. of leaves /explants |
|---|---|---|---|
| 00 | 5.1±0.20 | 2.46±0.10 | 10.8±0.43 |
| 20 µg/ml | 5.0±0.25 | 2.88±0.14 | 11.6±0.58 |
| 40 µg/ml | 4.3±0.30 | 3.01±0.21 | 11.4±0.80 |
| 60 µg/ml | 4.2±0.13 | 3.46±0.10 | 12.7±0.38 |
| 80 µg/ml | 3.6±0.22 | 2.92±0.18 | 11.6±0.70 |
| 100 µg/ml | 3.3±0.20 | 2.92±0.18 | 10.4±0.62 |
| 120 µg/ml | 3.8±0.15 | 2.83±0.11 | 10.2±0.41 |
| SEM± | 0.17 | 0.12 | 0.47 |
| F-value | 30.54 | 11.41 | 6.56 |
| P-value | 0.01 | 0.01 | 0.01 |
| $LSD_{0.05}$ | 0.37 | 0.26 | 1.02 |

Data are means of three replicates ± standard deviation. Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test.

The effects of functionalized single walled carbon nanotubes (SWCNTs) and non-functionalized SWCNTs on root elongation of 6 different crop species (cabbage (*Brassica oleracea*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), onion (*Allium cepa*) and tomato (*Solanum lycopersicum*) were studied to understand their toxicity to crops (Canas *et al.*, 2008). CNTs enhanced root elongation in onion and cucumber and nanotubes sheets were formed by both fCNTs and CNTs on cucumber root surface due to their interaction with root surface; however none entered into the roots. Cabbage and carrot were not affected by either form of nanotubes. Root elongation in lettuce was inhibited with fCNTs and tomato was found to be most sensitive for CNTs with significant root length reduction.

However a very recent work reported on the effects of multiwalled carbon nanotubes (MWCNTs) on the seed germination and growth of tomato plants showed a positive response upon interaction with nanotubes (Khodakovskaya *et al.*, 2009). Their results showed an increased water uptake by seeds in the presence of MWCNTs which enhanced the germination process.

Water soluble carbon nanotubes (WSCNTs) show enhancement of the growth rate of common gram (*Cicer arietinum*) plants. Treating plants with up to 60 μg/ml of water soluble CNT shows an increased growth rate in every part of the plant including the roots, shoots and also in branching (Tripathi *et al.*, 2011).

Khodakovskaya *et al.* (2012) reported that MWCNTs can affect plant phenotype and the composition of soil microbiota. Tomato plants grown in soil supplemented with carbon nanotubes produce two times more flowers and fruit compared to plants grown in control soil.

Nalwade and Neharkar (2013) studied the effect of carbon nanotubes on the seedling growth and yield of Bt cotton Var. ACH-177-2 and found that number of bolls per plant increased 2.8 times and boll size 1.85 times of control at 100 g/ml and yield was increased five times than that of the control.

Factory-synthesized multi-walled-CNTs (MWCNTs) of quality-controlled specifications were seen to enhance the germinative growth of maize seedlings at low concentrations but depress it at higher concentrations. Growth enhancement principally arose through improved water delivery by the MWCNT. Polarized EDXRF spectrometry showed that MWCNTs affect mineral nutrient supply to the seedling through the action of the mutually opposing forces of inflow with water and retention in the medium by the ion-CNT transient-dipole interaction (Tiwari *et al.*, 2014).

Nalwade and Bonawate (2014) observed that seed germination of onion was enhanced at 80 μg/ml of carbon nanomaterials. Shoot and root growth as well as dry matter production were increased manifold at 80 μg/ml concentration of carbon nanomaterials. The stimulation of seed germination and enhancement of seedling growth in onion (*A. cepa* L.) Var. Phule Suvarna at 80 μg/ml.

Accumulation of reactive oxygen species (ROS) was induced by MWCNTs resulting in increased oxidative stress and decreased cell proliferation, which eventually led to complete cell death (Tan *et al.*, 2009).

Our results are in accordance with the reports cited above. However, the response of plants to nanomaterials varies with the type of plant species, their growth stages and the nature of nanomaterials since the studies showed contradictory effects of the same nanomaterial in different plants at different developmental stages.

The results of the present investigation showed that at lower concentration of carbon nanomaterials have stimulatory effect, while above 60 μg/ml the detrimental effect was observed. These detrimental effects may be due to production of reactive oxygen species (ROS).

## CONCLUSION

The present study deals with synthesis of carbon nanomaterials and their effect on the *in-vitro* growth of *Ehretia laevis* Roxb. The obtimum result of BAP 2.5mg/l induced mean of 5.1 shoots, 2.34cm shoot length and 10.6leaves/explants. Murashige and Skoog (MS) medium 2.5mg/l BAP was supplemented with increasing concentrations 20, 40, 60, 80, 100 and 120 μg/ ml of water soluble carbon nanomaterials. MS medium without carbon nanomaterials served as control. After 4 weeks optimum results were MS+2.5mg/l BAP+60 μg/ ml CNT shows 4.2 shoots/explants, 3.46cm shoot length and 12.7 leaves/explants. were obtained.

From the results it can safely concluded that lower concentration of carbon nanomaterials has a stimulatory effect on shoot length and number of leaves, while higher concentration (above 60 μg/ml) has inhibitory effect.

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