Eco-Friendly Nitrogen Doped Carbon Dots from Sauropus Androgynus Leaf Extract: A Sustainable Tool for Mung Bean Germination, Bioimaging and Tissue Culture Enhancement

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Cytotoxicity Evaluation Studies

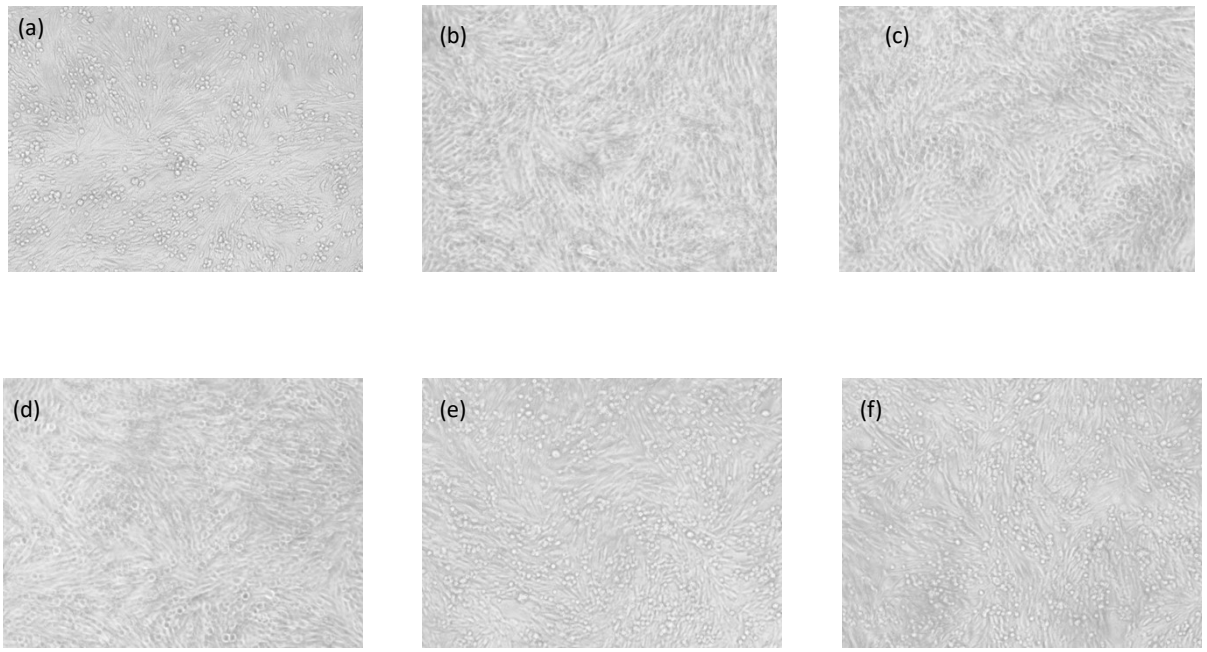

Fig S.1 MTT assay plates showing proliferation of CHO-K1 cells after 48 hours incubation in various concentrations of CDs (a) control (b) 62.5µg/mL (c) 125 µg/mL (d) 250 µg/mL (e) 500 µg/mL (f) 1000 µg/mL

Tissue culture studies

Methodology

Preparation of glassware and instruments (Cleaning and sterilization)

Glassware used in the study was cleaned with soap solution followed by thorough washing in tap water. After rinsing with distilled water the glassware was kept inverted on a clean draining tray and allowed to dry. The glassware was then sterilized by drying at 160^0C for 2 hours in a temperature controlled hot-air oven before use. Sterilization of Petri-dishes, forceps, scalpels, etc. was done by autoclaving at 121°C, 15 lbs for 20 min. These were separately wrapped with aluminum foil and then with brown paper, packed in autoclavable bag and autoclaved.

To maintain aseptic conditions inside the Laminar airflow chamber, the workbench was wiped using 70% ethanol before and after working. Tools such as forceps, scalpels, inoculation loops, Petri-dishes etc. used for inoculation were again sterilized by dipping in alcohol and flaming before and during use in the Laminar air flow.

Preparation of Tissue culture media

For the tissue culture studies, Readymade Murashige and Skoog media (1962) (Himedia) was used (Appendix). The Plant Growth Regulators (PGRs) were prepared as stock solutions of concentration 1 mg/mL and stored at 4 ºC. Following addition of media constituents and desired concentrations of required PGRs, the medium was made-up to the final volume and the pH of the medium adjusted by addition of 1 N NaOH/ 1 N HCl. Subsequently, 6% (w/v) of agar powder added in the media as gelling agent and melted by heating on a water bath. The molten media was thoroughly mixed and dispensed in aliquots of 15 mL in the case of culture tubes (20x150 mm) and 40 mL for culture bottles. Tubes were then plugged with cotton plugs (non-absorbent cotton wrapped in surgical gauze) and culture bottles were capped with polypropylene closures before autoclaving at 121^0C/1.5 Kg/cm^{-2} for 18 min.

In cases where solid culture media was required in containers (100 ml), the autoclaved medium was cooled to bearable temperature ahead of solidification and dispensed (30 mL) into sterile containers under aseptic conditions of Laminar air-flow hood. The medium in container was allowed to solidify, closed with the lids and stored for future utilization.

Table S1: Seed germination *in vitro* on MS basal media with varying concentrations of CDs (0.5-3 mg/mL)

Treatement	Day of bud emergence *in vitro*	Shoot Length (cm)	P value	Root length (cm)	P value	Seedling fresh biomass (g)	P value
A0 - Control	3	1.73 ± 0.68		1.2 ± 0.36		0.150 ± 0.05	
A1 With	3	0.87 ±	0.1017	0.7 ±	0.0816	0.076 ±	0.0811

0.5 mg/mL NCD		0.25		0.1		0.024	
A2 With 1 mg/mL NCD	3	1.07 ± 0.15	0.1732	0.63 ± 0.12	0.0605	0.086 ± 0.02	0.1064
A3 With 2 mg/mL NCD	3	1 ± 0.27	0.1570	0.67 ± 0.15	0.0777	0.080 ± 0.03	0.1048
A4 With 3 mg/mL NCD	2	3.67 ± 0.76	0.0307	2.63 ± 0.42	0.0108	0.234 ± 0.007	0.0457

Table S2: Shoot Multiplication with apical shoot explants in MS media with 0.5 mg/L BAP and varying concentrations of CDs (0.5-3 mg/mL)

Treatment	Average Number of shoots	P value	Total Length of shoots	P value	Average Shoot Biomass	P value
A0	2 ± 0		1.467 ± 0.252		0.133 ± 0.021	
A1-With 0.5 mg/mL CD	3 ± 0.58	0.3739	1.667 ± .153	0.3046	0.1497 ± 0.022	0.3898
A2- With 1 mg/mL CD	4 ± 0.58	0.0161	1.5 ± 0.30	0.8899	0.142 ±0.033	0.7123

A3- With 2 mg/mL CD	3± 0.58	0.1161	2 ± 0.173	0.0390	0.192 ±0.005	0.009
A4- With 3 mg/mL CD	4 ± 1	0.1583	1.83 ±0.153	0.0263	0.182 ± 0.011	0.0239

Table S. 3: Callus culture multiplication and biomass increase from an initial biomass of 0.05 g, MS media with 0.5 mg/ml 2,4-d and varying concentrations of CDs

Treatment	Average Weight of callus (g) 7th day	**P value**	**Average Final Fresh Weight of callus (g) (15 th day)**	**P value**
A0	0.244 ± 0.045		0.259 ± 0.056	
A1 With 0.5 mg/mL NCD	0.803 ± 0.068	0.003	0.834 ± 0.069	0.0004

A2 with 1 mg/mL NCD	0.192 ± 0.040	0.2128	0.222 ± 0.033	0.3847
A3 with 2 mg/mL NCD	0.253 ± 0.048	0.813	0.281 ± 0.032	0.5866
A4 with 3mg/mL NCD	0.261 ± 0.038	0.6383	0.306 ± 0.049	0.3820

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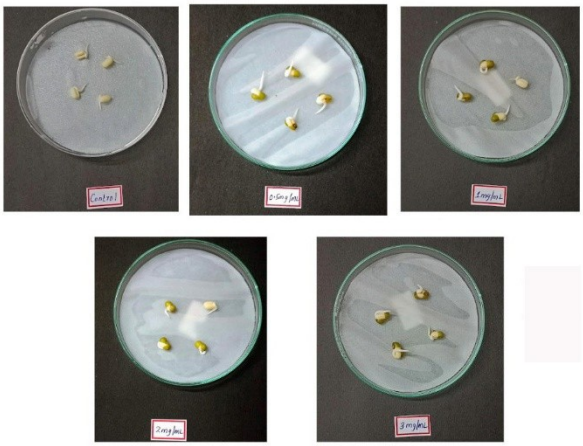
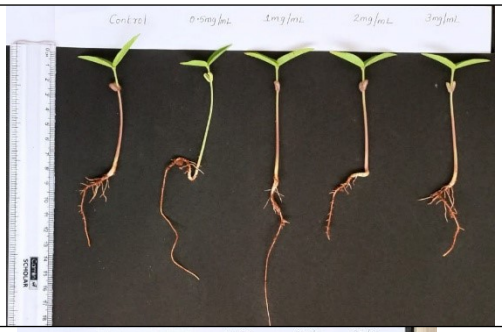
Day 1	
Day 3	 
Day 7	 

Day 14	 

Figure S.2 Images showing mung bean seedling growth on Day 1, Day 3,Day 7 and Day 14 after priming with NCDs at different concentrations