CENTRE FOR BOREAL RESEARCH
TECHNICAL NOTE #46
PLANT AND SEED TECHNOLOGIES – FEBRUARY 2022

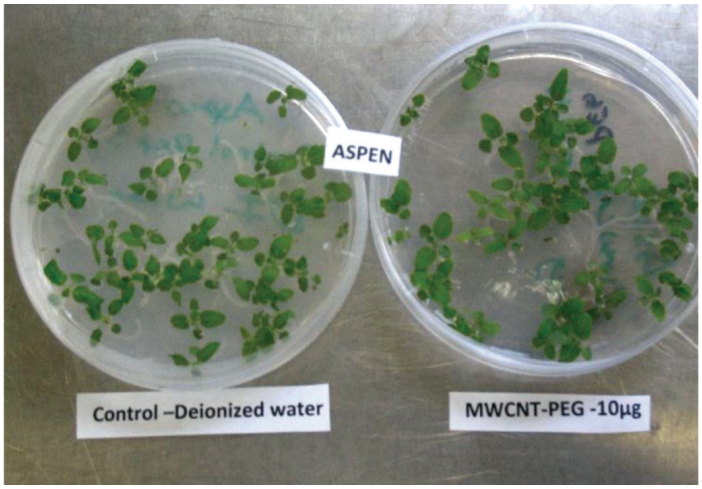
The Use of Nanomaterials to Improve Propagation of Native Boreal Species

INTRODUCTION

Rapid and uniform seed germination and seedling emergence are important determinants of successful stand establishment (Rajjou *et al.* 2012; Chen and Arora, 2013). Seed germination begins with water uptake by the mature dry seed (imbibition) and terminates with the elongation of the embryonic axis, usually the radicle, through the seed envelope, resulting in the protrusion of root and shoot (Rajjou *et al.* 2012). In recent years, carbon-based nanoparticles (NP) (fullerene and carbon nanotubes) have been applied as seed pre-treatment agents to promote seed germination and seedling growth (Mahakham *et al.* 2017). NPs were shown to enter the seed and alter various biological pathways, which modified their physicochemical properties and resulted in different effects on plant growth (Nadiminti *et al.* 2013).

Plant responses to treatments with NP were observed to vary depending on the chemical composition of the NPs, concentration, aggregation state (Liu *et al.* 2009), metabolic abilities of the treated plant species, plant growth environmental conditions (Schultz *et al.* 2014), and exposure time (Mrakovcic *et al.* 2013).

Generally, nanopriming techniques are applied to enhance seed germination and seedling vigour in agronomic crop production systems. Recent work using nanopriming with multi-walled carbon nanotubes (MWCNT) and functionalized with carboxylic acid (MWCNT-COOH) combined with cold stratification techniques, was very successful in improving the germination and seedling vigour of two dormant boreal forest species, green alder and buffaloberry seeds (Ali *et al.*, 2020, Gao *et al* 2011; Mondal *et al* 2011; Morla *et al* 2011; Smirnova *et al* 2011). Findings from these studies indicate various nanomaterials work by enhancing water uptake in seeds and activate enzymatic and hormonal response during seed germination and plant growth.



Nanopriming of aspen seeds. MWCNT-PEG = multiwall carbon nanotube functionalized with polyethylene glycol. Control = seeds imbibed with de-ionized water

These experiments also indicate that the beneficial effects of nanomaterials on seed germination, plant growth and development are dependent on the types of nano-particles used, concentration, plant species and specific experimental conditions.

Though nanotechnology has been used extensively on agricultural crops, there remains the need to investigate further the potential of nanopriming with carbon nanoparticles to improve the germination and seedling vigour of key native boreal species.

OBJECTIVE

The primary objective of this project is to evaluate the effects of multi-walled carbon nanotubes in improving seed germination, seedling vigour and growth in select native boreal plant species ideally suited for forest reclamation following oil and gas mining.

METHODS

Two native boreal species aspen (*Populus tremuloides*) and fireweed (*Epilobium angustifolium*) were used for this study because their seeds are very small, they are not amenable to mechanical seeding and have low field establishment from direct seeding.

Seed Priming: 25 seeds per species were surface sterilized (4% sodium hypochlorite), then primed (4 hrs) using 0 ug (control, DI water), 1 ng, 10 ug, 20 ug and 40 ug/mL of either hydrated C60 fullerences, multi-walled carbon nanotubes (MWCNT) or oxidized multi-walled carbon nanotubes (OMWCNT). Seeds were then dried back to original weight at room temperature and stored in normal laboratory condition for 15 days.

Seed Vigour/Germination tests: After 15 days, seeds were surface sterilized, then transferred to petri plates containing filter paper moistened with 10 mL of DI water or nanoparticle solutions (see concentrations above). Germination rate (radical emerge), seedling emergence (radicle emerge and plumule unfold from testa), and growth rate were recorded every 24 hours for 15 days to determine effects of nanoparticles on rate of germination, seedling emergence, and growth rate. Seed vigour tests (tetrazolium and membrane integrity tests) were done 24 hours after rehydration/imbibition and on ungerminated seeds.

RESULTS

Imbibing aspen and fireweed seeds in the presence of 0, 1, 10, 20, and 40 ×g of various carbon nanoparticles (graphene, multi-walled carbon nanotubes (MWCNT), and functionalized MWCNT (hydroxide (OH), or polyethylene glycol (PEG) was observed to be effective in increasing germination (Fig.1A-D) and seedling vigour (Fig.2A-D) in fireweed. Concentrations of 1 or 10 ×g/mL were found to be most effective in improving fireweed growth and germination performance (Fig. 1-2). In aspen, multi-walled carbon nanotube functionalized with polyethylene glycol (MWCNT-PEG) was found to be most effective in enhancing germination (Fig.3A-D), and seedling vigour (Fig. 4A-C). The concentration of 40 ×g/mL gave the best response in aspen seeds imbibed with MWCNT-PEG (Fig 3-4).

Seed nanopriming with a low concentration of carbon nanotubes was an effective technique to enhance seed vigour, germination, or biomass accumulation in fireweed and aspen. These findings have potential applications in the development of novel seed treatment techniques and plant products to improve the germination rate, growth, and establishment of these species on disturbed boreal forest sites. The data obtained indicates that there is the potential to use carbon-based nanoparticles at low concentrations (1-10 ×g) to enhance the establishment of fireweed on disturbed boreal forest sites with varying adverse environmental conditions. For aspen, MWCNT-PEG at 40 ×g/mL should be used, while for fireweed, 1 or 10 ×g/mL of either MWCNT, MWCNT-OH, MWCNT-PEG or graphene should be used.

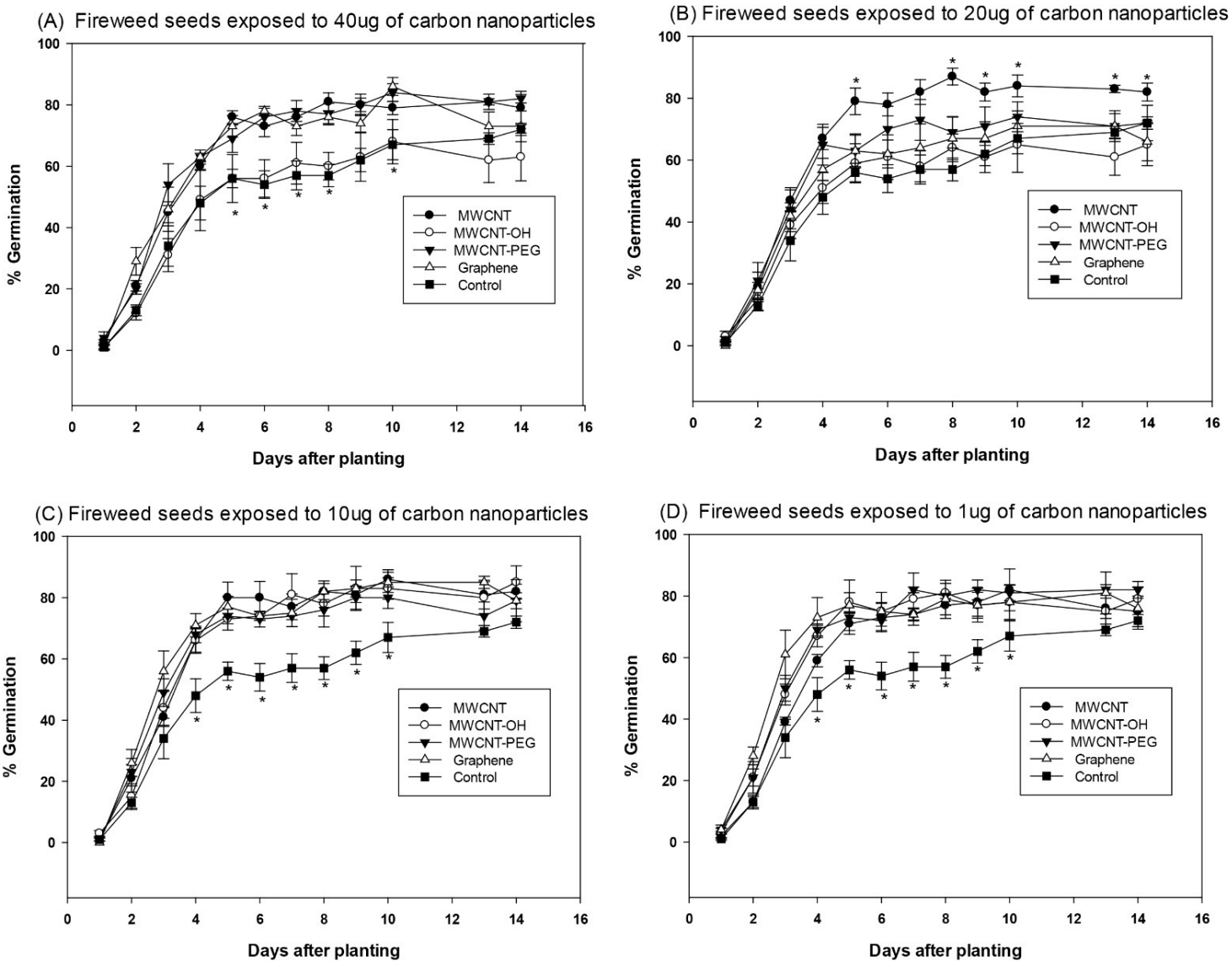


Figure 1A-D. The germination rate of fireweed seeds primed in various concentrations of carbon nanoparticles. Values represent means 4 SE of 4 replicates, each consisting of 50 seeds. MWCNT = multi-wall carbon nanotube, MWCNT-OH = multiwall carbon nanotube functionalized with a hydroxyl group, MWCNT-PEG = multiwall carbon nanotube functionalized with polyethylene glycol. Control = seeds imbibed with de-ionized water. * represent significant difference at Ð= 0.05.

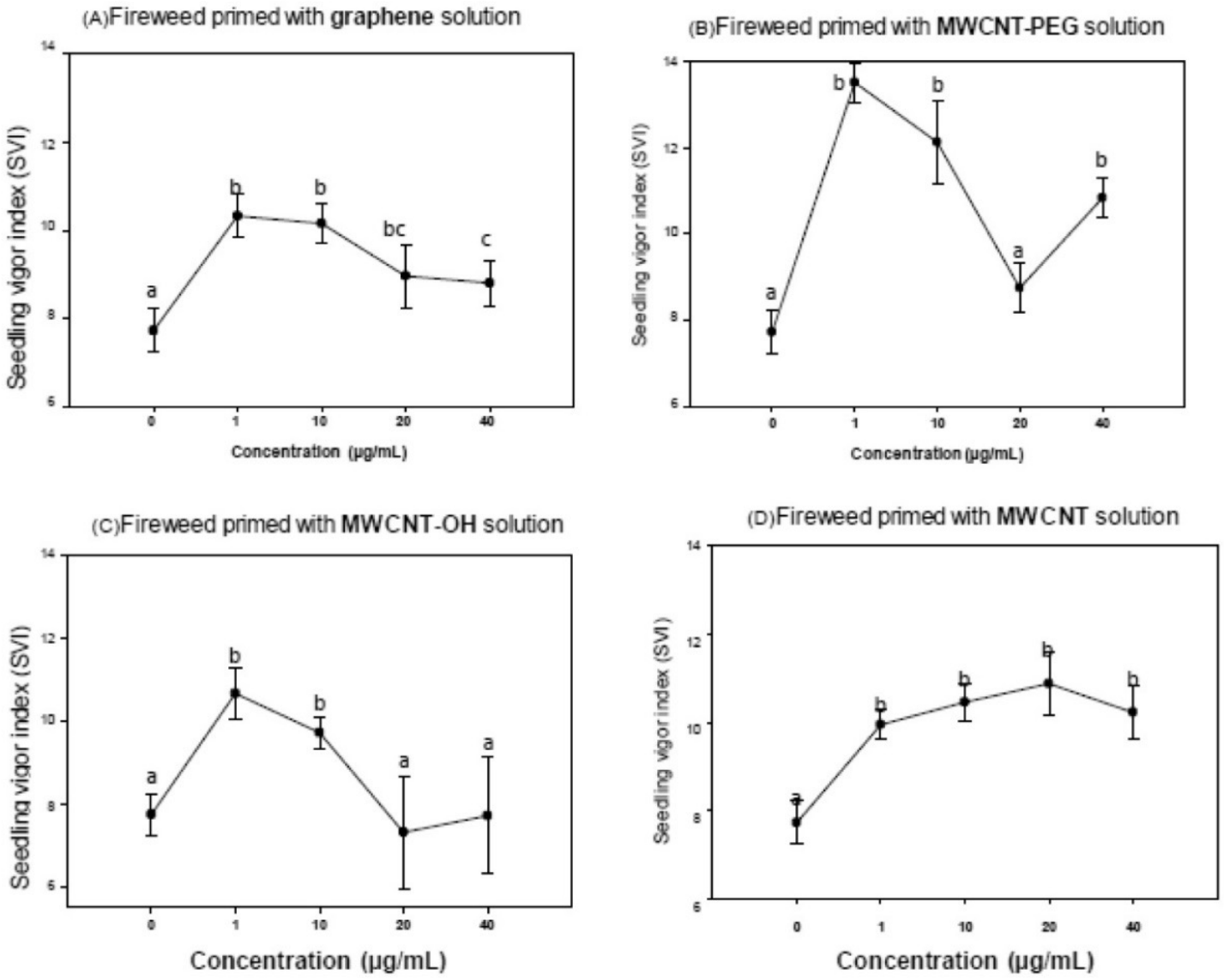


Figure 2A-D. Seedling vigour index of fireweed seeds primed with various concentrations of carbon nanoparticles. Values represent means 4 SE of 4 replicates, each consisting of 50 seeds. Treatments with overlapping error bars and denoted by the same letters are not significantly different at LSD = 0.05. MWCNT = multi-wall carbon nanotube, MWCNT-OH = multiwall carbon nanotube functionalized with a hydroxyl group, MWCNT-PEG = multiwall carbon nanotube functionalized with polyethylene glycol. Control = seeds imbibed with de-ionized water

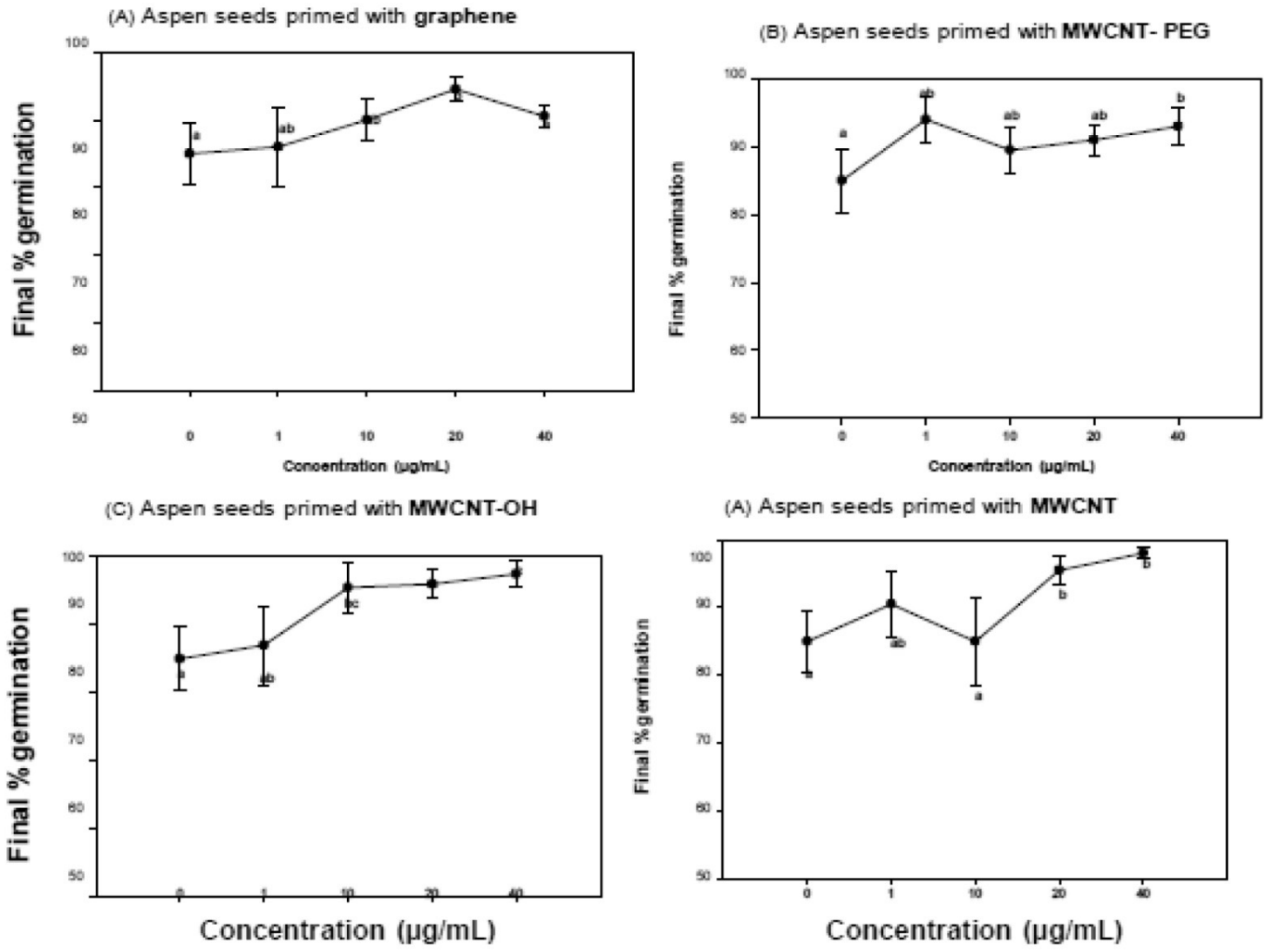


Figure 3A-D. Percent germination of aspen seeds primed with various concentrations of carbon nanoparticles. Values represent means 4 SE of 4 replicates, each consisting of 50 seeds. Treatments with overlapping error bars and denoted by the same letters are not significantly different at LSD = 0.05. MWCNT = multi-wall carbon nanotube, MWCNT- OH = multiwall carbon nanotube functionalized with a hydroxyl group, MWCNT-PEG = multiwall carbon nanotube functionalized with polyethylene glycol. Control = seeds imbibed with de-ionized water

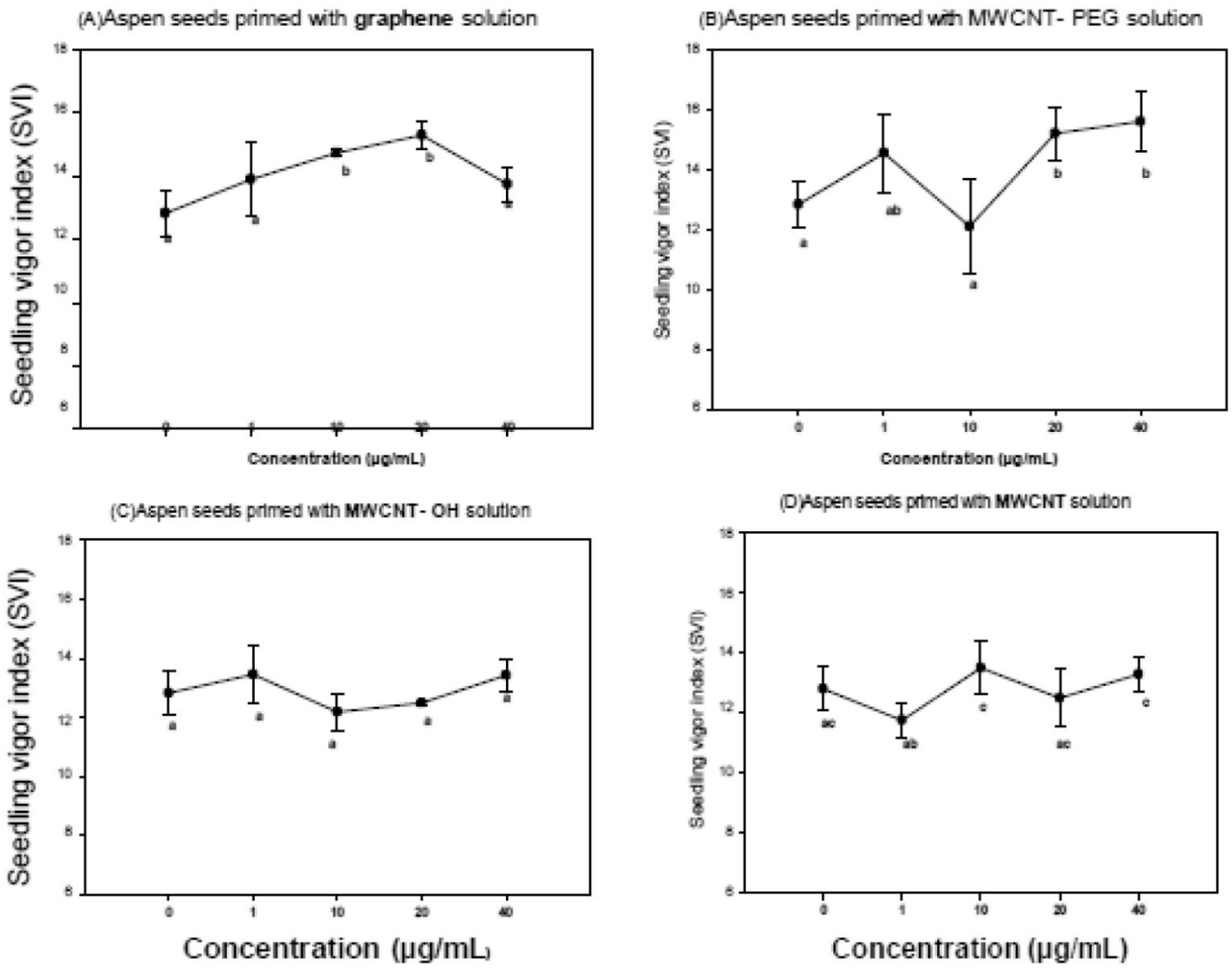


Figure 4A-D. Seedling vigor index of aspen seeds primed with various concentrations of carbon nanoparticles. Values represent means 4 SE of 4 replicates, each consisting of 50 seeds. Treatments with overlapping error bars and denoted by the same letters are not significantly different at LSD = 0.05. MWCNT = multi-wall carbon nanotube, MWCNT- OH = multiwall carbon nanotube functionalized with a hydroxyl group, MWCNT-PEG = multiwall carbon nanotube functionalized with polyethylene glycol. Control = seeds imbibed with de-ionized water

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