Green Nanotechnologies
to minimize Post-harvest Loss in Perishables

Nanotechnology deals with the manipulation of materials at the atomic level to design processes and fabricate products to enhance agri-food production systems. Nanotechnology has been widely exploited in energy, environment, electronics, health and medicine, but researchers have just started agricultural applications (Subramanian et al., 2015). The global investment in nanotechnology has increased exponentially from just 1 billion USD in 2000 and 2 trillion USD in 2016, which reflects the trend on a growing number of nano-based consumer products. However, there is still a lack of knowledge about the hazards associated with nano-materials, and challenges continue to exist in the development of its regulatory framework.

The "green nanotechnology" concept was introduced by the American Chemical Society – Green Chemistry Institute (ACS – GCI) in 2010 during the *Safer Nanomaterials and Nanomanufacturing*'s (SNNI) Fifth Annual Conference in Portland, Oregon, USA, to address the technical challenges and risks involving in the scale up of nanotechnology (ACS-GCI, 2011). Canada's International Development Research Center (IDRC) funded a research program entitled "Enhanced Preservation of Fruits using Nanotechnology" involving scientists from six countries. The team has been collaborating for the past four years to develop an array of nanotechnologies to address the global challenge of post-harvest loss, estimated globally at 40-50%The team has developed a wide range of technologies such as pre-harvest spray, post-harvest dip, nano-stickers, nano-wraps, nano-sachets, and nano-film involving nanotechnology principles. While these technologies have dramatically reduced post-harvest loss, they need to be critically evaluated on whether the products meet the principles of green chemistry.

Nano-Products developed

Hexanal is used as a core bioactive molecule to preserve perishable fruits. When this naturally occurring compound is sprayed externally on fruits prior to harvest, it can considerably delay the ripening process. We have extensively studied the use of this compound in extending the shelf life of several fruits and vegetables (Tiwari et al., 2010; Sharma et al., 2010; Anusuya et al., 2016; Jincy et al., 2017). The key products developed in our project include:

- Nano-emulsion of hexanal (as pre-harvest spray or post-harvest dip)
- Electro-spun fiber matrix (nano-Wrap or nano-Sticker)
- Cyclodextrin inclusion complex (nano-Sachet)
- Nano-film derived from banana pseudostem
- Hexanal impregnated wax formulation

Principles of Green Chemistry

1. Prevention

The aim here is to prevent or minimize the generation of waste instead of managing the waste that is produced. Based on this principle, the project developed an enhanced freshness formulation (EFF) that carries hexanal, emulsified in water using biodegradable surfactants. No waste is generated in the production process. Moreover, the formulation is stable during storage, reducing the generation of waste. Similar principles applied during the development of the other products (e.g., wrap, sticker, and sachet) that used FDA-approved polymers, materials, or feedstock derived from food processing residuals that are biodegradable.

2. Atom Economy

The 'atom' economy refers to the development of nano-products that maximize the incorporation of all raw materials in the process to produce the final product. Following this principle, EFF is composed of three molecules that transform into a capsule called "micelles," that protects the hexanal (Fig. 1). Electrospun fibers and cyclodextrin are also used to entrap hexanal without producing any byproducts.

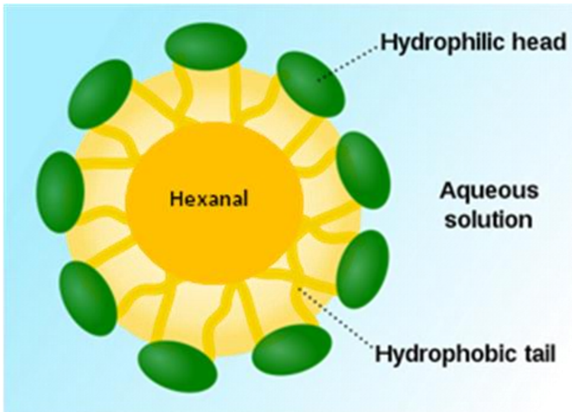


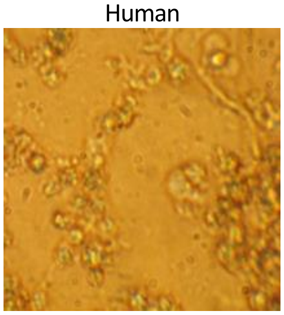
Fig. 1. Micelle of nano-emulsion of hexanal

3. Less Hazardous Chemical Synthesis

Green nanotechnology relies on synthetic methods that are designed to generate substances that produce minimal toxicity. The EFF developed in this project is composed of three FDA-approved molecules that are safe independently or in combination. The emulsification used is well-established and the product is non-toxic to microorganisms, earthworms, natural predators and parasites, honey bees, aquatic organisms, and humans (using cultured cell lines) when tested using protocols developed by the Organization of Economic Cooperation and Development (OECD) (Table 1). A summary of test results has been compiled as a user manual (Gunasekaran et al., 2015).

Table 1. Biosafety of EFF at various trophic levels

Trophic Level	Species Tested	Technique Employed	Results and Inferences
Microbes 	*Pseudomonas fluorescens, Bacillus subtilis, Trichoderma viride, T. harzianum* *Biological activity*	Agar well / paper disk method Dehydrogenase assay	No inhibitory effects No illeffects
Parasitoids 	*Trichogramma chilonis* Ishii, *T. pretiosum, T. japonicum*	Contact toxicity method (Treated on the host eggs)	No toxicity until the F1 generation

Predators 	*Chrysoperla zastrowi arabica* (Esben - Petersen)	Contact toxicity method(sprayed on eggs and grubs of *Chrysoperla*) Food contamination technique(treated *Corcyra* eggs as food)	No mortality detected
Honey Bees 	Indian bees (*Apis cerana indica* F.) Italian bees (*Aphis mellifera* L.)	Contact toxicity method(treated on mango fruits)	Zero mortality
Earthworms 	*Eudrillus eugeniea* (Kinberg)	Contact toxicity method(sprayed on the soil substrate)	No adverse effect including no weight loss
Aquatic animal 	Zebra fish (*Danio rerio* Hamilton)	Poison food technique	No mortality nor abnormalities
Human 	*HeLa* - cervical cancer cells *A549* - adenocarcinomic human alveolar basal epithelial cells *HepG2* - liver tissue cells	Lactate dehydrogenase (LDH) (LDH release into culture medium upon cell death) MTT assay (cellular enzymes reduce tetrazolium dye)	Safe for cell lines (concentration below 2000ppm is found safe)

4. Designing Safer Chemicals

The products are to be designed to achieve their desired function while minimizing toxicity. The homogenization process to produce EFF is simple and safe and it has been widely used in the food industry. The electro-spun fiber matrix is primarily made of FDA-approved polymers in combination with β- cyclodextrin to stabilize hexanal (Fig. 2). In another product, β-cyclodextrin was used as an encapsulating agent to entrap hexanal. After the encapsulation, it is made into a pellet form that can help to preserve fruits. As these products are made of biologically safe products, it qualifies as green nanotechnology.

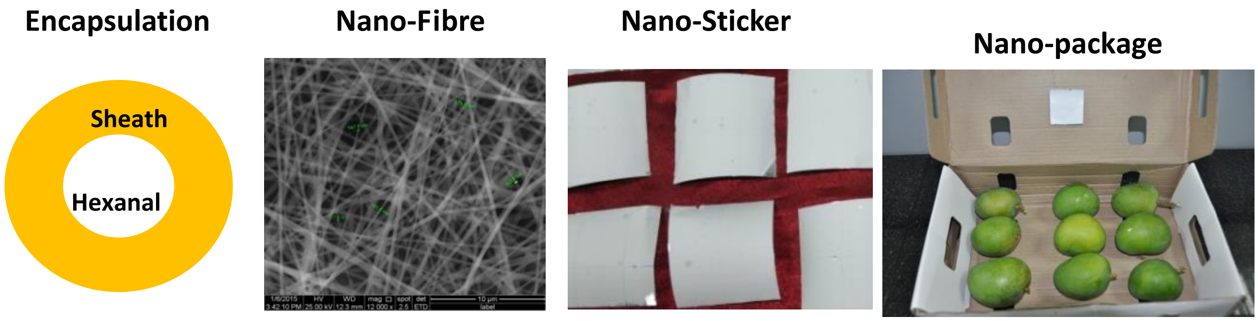


Fig. 2. Nano-matrix (Sticker) for fruit preservation

Fig. 3. Nano-Pellets (Sachet) for fruit preservation

5. Safer Solvents and Auxiliaries

In the EFF formulation, hexanal, ethanol and tween are used at suitable ratios to get a critical micellar concentration. Other than these chemicals, there is no auxiliary chemicals or substances used prior to application Since, no other chemicals are being used in the process, the nano-based EEF products fulfill the requirement of green nanotechnology in terms of the safer solvents and auxiliaries' criteria.

6. Design for Energy Efficiency

Green nanotechnology emphasizes that the energy requirements of chemical processes should be recognized for their environmental and economic impacts and thus should be minimized. Further, the synthetic methods should be conducted at ambient temperature and pressure. In all the cases, the entire process of development of the products is under room temperature. The electrospun fiber matrix (Sticker) and the development of an inclusion complex (Sachet) requires electrospinning and a microwave synthesizer, respectively. However, both machines can provide a high output in a short period of time. Consequently, the energy requirement per unit sticker or sachet is very low.

7. Re-use of Renewable Feedstock

The green nanotechnology should be renewable and economical. The compounds used in product development including hexanal are hydrocarbons and compostable in the ecosystem.

8. Reduce Derivatives

To qualify for green nanotechnology, unnecessary derivatization (use of blocking groups, protection/de-protection, temporary modification of physical/chemical processes) should to be minimized or avoided to ensure that no additional reagents are used that may generate waste. There are no derivatives created during the manufacturing processes. In order address this criterion, the EFF formulations prepared manually or using high pressure homogenizer were monitored by measuring particle size periodically. The data have clearly shown that the nano-emulsion of hexanal formulation is stable for six months (Fig. 4). Since the particle size is maintained during the production and storage, it is presumed that there is no derivatives produced. This fulfills the requirement of green nanotechnology.

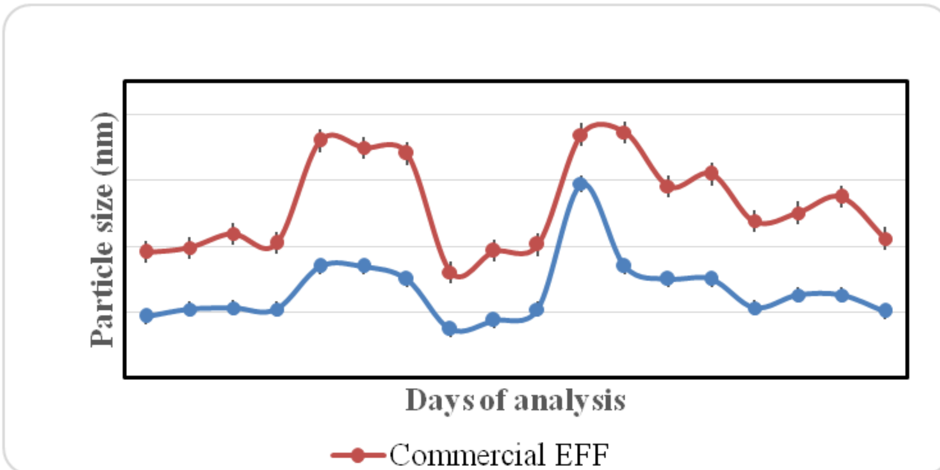


Fig.4. Stability of EFF during storage

9. Catalysis

The nano-based products developed in the project neither involve catalysis nor stoichiometric reactions, but rather physical phenomena.

10. Design for Degradation

The nano-products developed in this project are primarily organic compounds such as hexanal, ethanol, tween, methanol, cyclodextrin, poly (vinyl alcohol), and nano-fibrillated cellulose. In all the cases, the molecules are hydrocarbons and totally biodegradable. Indeed, the EFF-treated soil has shown increased biological activity as indicated by dehydrogenase enzyme measurements. The nano-based delivery systems degrade and yield products that are naturally present in the environment.

11. Real Time Analysis for Pollution

The EFF-sprayed orchards were regularly monitored for the assessment of ecological changes in the ecosystem. No variations in biological diversity between sprayed and unsprayed ecosystems were observed. The study revealed that the richness of insect species in both sprayed and unsprayed ecosystems remained unchanged (Fig. 5). Further, life cycle studies were performed on some of the organisms and parasite eggs, honey bees, and earthworms showed no deterrence to either first or second-generation stages. The protocols used for the study were adopted from the OECD.

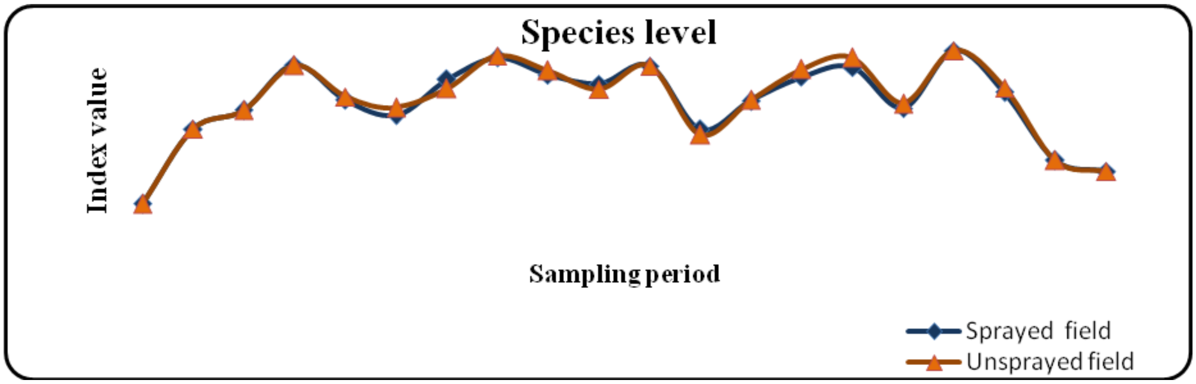


Fig. 5. Species richness in EFF-sprayed and unsprayed ecosystems

12. Inherently Safer Chemistry for Accident Prevention

The EFF carries 10% ethanol. Although ethanol is flammable it does not catch fire at 10% volumes. Generally, most cough syrups and human oral medicines carry 5–7 % ethanol, and they are never considered as flammable materials. However, to prevent any potential accidents, care should be given to proper bottling, handling, and transport. Therefore, nano-formulations carrying ethanol, such as EFF, can be considered as risk-free material during handling and transport.

Checklist on the Status of Green Nanotechnology of Emerging Nano-Products

	Parameter	EFF (All)		Electrospun Fibre Matrix (TNAU & UoG)		Inclusion Complex (TNAU)	Biowax (ITI)	Wrapper Sheet (ITI)
		Spray	Dip	Sticker	Wrap	Sachet	Dip	Package
1	Prevention							
2	Atom economy							
3	Less hazardous chemical syntheses							
4	Designing safer chemicals							
5	Safer solvents and auxiliaries							
6	Design for energy efficiency							
7	Use of renewable feedstocks							
8	Reduce derivatives							
9	Selective catalysis							
10	Design for degradation							
11	Real time analysis of pollution							
12	Inherently safer chemistry for accident prevention							

Note: TNAU: Tamil Nadu Agricultural University, India; UoG : University of Guelph, Canada; and ITI :Industrial Technology Institute, Sri Lanka.

Nano-product fully meets the stipulated guidelines of green nanotechnology

Deviate from green nanotechnology in terms of presence of flammable substances or use of high energy during the manufacturing of nano-products

Summary

The emerging nano-products from the CIFSRF (Canadian International Food Security Fund) project on "*Enhanced Preservation of Fruits using Nanotechnology*" being funded by Global Affairs Canada (GAC) through International Development Research Center (IDRC), have been evaluated for their suitability to qualify under green nanotechnology. The seven products developed from the project were evaluated for their fulfillment of green nanotechnology criteria. This paper revealed that all the seven products matched the major part of the criteria apart from flammability and energy requirement.

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