

Eco-friendly Formulation for Fruit Preservation

Context

India stands as the second largest producer of fruits and vegetables in the world next to China. In the past, post-harvest losses of 30-35% have accounted for an annual economic drain equivalent to ~33 billion USD. To address the global challenge of post-harvest losses, Global Affairs Canada and Canada's International Development Research Center have jointly supported a project on "*Enhanced Preservation of Fruits using Nano Technology.*" Scientists from six institutes in Canada, India, Sri Lanka, Kenya, Tanzania, and Trinidad & Tobago have been working together for five years on hexanal-based nano technologies to reduce fruit losses.

Hexanal is naturally found in plants. Its smell is evident to anyone cutting a fresh cucumber or mowing a lawn. Hexanal has been used on many fruit crops and incorporated into several delivery technologies. Indeed, the Tamil Nadu Agricultural University, Coimbatore, India, has recommended that hexanal technology be adopted by the State of Tamil Nadu to promote longer shelf life of several tropical fruits. The technology is being demonstrated on more than 3,000 farms across southern India. **In the US, hexanal has been classified as a Generally Regarded as Safe (GRAS) compound. Here, we outline the biosafety features of hexanal to help policy makers, regulatory bodies, producers, and users during their decision-making processes.)**

Key Messages

- "Hexanal" – a plant derived biomolecule that causes the grassy odour in mowed lawns or cut vegetables
- Plants use the compound to protect themselves from herbivores
- Is as an FDA-approved fruit preservative and used as food adjunct
- Safe for beneficial microbes, natural enemies, honey bees, earthworms, and humans
- Increases shelf life of several fruit species
- Biodiversity of the orchard ecosystem is conserved without any ill effects

Several independent tests have been done to ensure the safety of hexanal to beneficial microorganisms in soil, earthworms, natural enemies (predators and parasites), honeybees, aquatic organisms (zebra fish), **and human (using human cells lines)**. In order to test the biosafety of hexanal formulations, internationally acceptable protocols developed by Organization of Economic Cooperation and Development (OECD) were used to test hexanal.

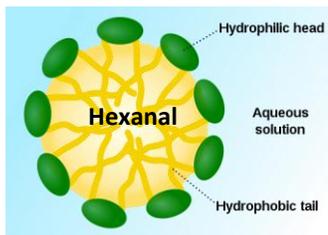
Nano-emulsion hexanal

Hexanal is a six-carbon atom compound ($C_6H_{12}O$) that is highly volatile and is a component of flavor volatiles produced during ripening. This naturally-produced volatile compound has a beneficial effect on fruit ripening process when it has been applied as an external spray. Hexanal inhibits an enzyme in the skin of the fruits thus slowing down the production of ethylene during the ripening process. This physiological mechanism facilitates the preservation of perishables during storage and also greatly reduces the post-harvest diseases (Parthasarathy et al., 2016). In order to entrap the volatile compound so it can have its desired effect, a micelle was developed using surfactant and a co-surfactant at suitable proportions to achieve a nano emulsion. We have found that hexanal-based freshness formulation used as pre-harvest sprays or dip treatments can extend fruit freshness for 2-3 weeks of storage without any loss to fruit quality. Hexanal in vapour form also has the potential to alter the ripening time of fruits that can result in the extension of their shelf life. Such shelf-life extension will help developing countries where fruits and vegetables are produced aplenty but their per capita availability is just 50% of the daily requirement due to post-harvest losses.

Wounding



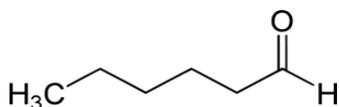
Micelle



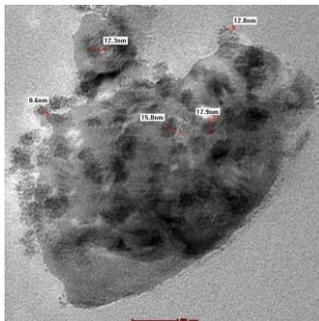
EFF Product (Enhanced Freshness Formulation)



Hexanal



TEM image



Summary

1. Safe for the ecosystem

The safety tests were done as stipulated by the OECD. To test the safety of the product, a set of protocols were adopted to examine the ill effects against beneficial microorganisms, honey bees, natural enemies, earthworms, fish, and human cell lines (Rajkishore et al., 2013; Gunasekaran et al., 2015). The data have unequivocally demonstrated that hexanal and its formulations had no ill effects against all trophic levels even at 300% of the recommended dose. The orchards in which hexanal spray was used did not notice any adverse impact on honey bees or natural enemies. The exposure of hexanal to various human cell lines such as cancer cells, liver cells, and epithelial cells to ensure that the hexanal formulations are safe. There was no adverse effect on any of the cell lines tested. These tests help us to understand the safety of humans in the event of direct exposure to enhanced freshness formulation (EFF).

2. Secures soil microbes

In soil, two groups of living creatures, microbes and earthworms, are generally seen as the biological indicators to reflect any disturbance in the ecosystem. Seventeen microorganisms have been identified by the OECD to be used in these safety tests. Our studies have clearly shown that the formulation had no detrimental effect on these microbes as indicated by total microbial activities using an enzyme assay. In some instances, microorganisms were possibly using the formulation as a food source as they grew much faster in sprayed orchards.

3. Earthworms benefit from EFF

Earthworms are “farmers’ friends” and a good indicator of soil health. Use of agro-chemicals or other synthetic foreign materials added in excess to the soil can cause a decrease in the earthworm population. Earthworms introduced into soils treated with the formulation has shown that there was no negative impact on the general life cycle of earthworms. These biological measurements indicate that the formulation is safe for earthworms and thus the soil.

Robust Earthworms in EFF Treated Soil



4. Nurtures natural enemies

Natural enemies are important constituents of insect ecology and hence we looked at the insect ecology in sprayed orchards. Predators and parasites feed on detrimental insect pests and keep the crop free from their harm. Predators are usually larger insects than the smaller ones that are their prey. For instance, the lace wing bug (*Chrysoperla* spp.), eats the smaller pests such as aphids. Parasites although smaller than their prey, can feed on the eggs and larvae of the insect pests.

Predator and Parasite in Sprayed Orchard



5. Attracts honey bees, distracts fruit flies

Honeybees are unique creatures capable of naturally avoiding unhealthy environmental conditions. It is widely believed that honeybees exploit nanotechnology for their own survival. Honey bees have to visit and siphon off nectar from at least 1,000 flowers to synthesize one drop of honey. Consequently, honey has a long natural shelf life without any loss in quality. Honeybees are often used as a model system to study the toxicity of metal oxide nanoparticles. We tested 4 species of honeybees with our formulation and they seem to be attracted to the formulation. However, one of the major pests, the fruit fly, has been deterred by the formulation (Karthika et al. 2015).

Honey Bees Colonize on EFF Sprayed Trees



6. No ill effects on zebra fish

Many agricultural inputs either sprayed on trees or applied to the soil get into local bodies of water through run off that eventually affects aquatic flora and fauna including fish. In order to test if there is any such aquatic toxicity, the OECD suggests testing it on zebra fish (*Danio rerio* Hamilton) because they are very sensitive organisms to any foreign material. We have shown that the formulation had no ill effects on zebra fish even at 1000% above the recommended concentration.

7. Safe for humans

The safety of any agricultural inputs to humans is tested using cultured cells of target sites. The OECD has evolved protocols to test the intensity of toxicity using cultured cells. Target sites such as skin, lungs, kidneys, and liver are likely to be exposed to the formulations directly or indirectly. Consequently, cultured cells of these organs are used to assess the likely toxicity at the standard concentrations using cytotoxicity and genotoxicity tests. We examined the likely impacts and interactions of the formulation on human cell lines and found no adverse effects.

8. Enhances fruit quality

Extensive studies have been done on residue of hexanal formulation in fruits and vegetables. The data revealed that there was no detectable elevation in the levels of hexanal on treated fruits 48 hours after application. Only the basal amount of hexanal that is naturally present in the fruits can be detected. Hexanal-treated fruit retained its quality for a longer period, thus helping to reduce the post-harvest wastage.

Conclusion

The hexanal formulation had no ill effects in the orchard ecosystem or under other test conditions. Tests with beneficial microbes and earthworms in the soil, natural enemies (predators and parasites) and honeybees on the sprayed trees, and human cell lines unequivocally demonstrated that the formulation is safe for the full spectrum of constituents in the ecosystem. Further, the quality of fruits in sprayed orchards improved and no residues were detected thus fulfilling the safety requirements of domestic and international markets.

References

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