FINAL TECHNICAL REPORT / RAPPORT TECHNIQUE FINAL

ENHANCED PRESERVATION OF FRUITS USING NANOTECHNOLOGY (CIFSRF-PHASE II)

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ENHANCED PRESERVATION OF FRUITS USING NANOTECHNOLOGY (CIFSRF- PHASE II)

IDRC PROJECT NUMBER: 107847

RESEARCH ORGANIZATIONS INVOLVED IN THE STUDY:

1. University of Guelph, Guelph, Canada
2. Tamilnadu Agricultural University, Coimbatore, India
3. Industrial Technology Institute, Colombo, Sri Lanka
4. University of Nairobi, Nairobi, Kenya
5. Sokoine University of Agriculture, Morogoro, Tanzania.
6. University of West Indies, St. Augustine, Trinidad and Tobago.

LOCATION OF STUDY:

1. Milton, Elora, Campbellsville, Simcoe, Vineland and Different locations in the Niagara Peninsula, Ontario, Canada
2. Theni and Dharmapuri Districts, State of Tamilnadu, India
3. Colombo , Ellawala, Dambulla, Udawallawe, Vavunia and Jaffna Provinces , Sri Lanka
4. Muranga, Meru and Machokos counties, Kenya
5. Coast, Tanga, Morogoro regions, Tanzania
6. Carnbee, Orange Hill, Richmond in Tobago/Morugo, Arima and Mt. Hope in Trinidad

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LIST OF ACRONYMS

3MT: 3 Minute Thesis
AFS: Agriculture and Food security
AMAGRO: Association of Mango Growers
BFW: Banana fibre Fruit wrap
CEC: County Executive Chief
CEPA**:** Center for Excellence in Poverty Analysis
CFIA: Canadian Food Inspection Agency
CBO: Community Based Organisations
CHC: Canadian High Commissioner
DOA: Department of Agriculture
DBH: Days before Harvest
GRAS: Generally Regarded As Safe
EFF: Enhanced Freshness Formulations
FGD: Focussed Group Discussions
FPO: Farmer Producer Organization
FR: Financial Report
FRSC: Farmers Resource & Service Centre
FSSAI: Food Safety and Standards Authority of India
FTIR: Fourier Transform Infrared Spectroscopy
HICM: Hexanal Incorporated Composite Material
HSDS: Hexanal Smart Delivery System
IICA: Inter-American Institute for Cooperation on Agriculture
INR: Indian Rupee
IPHT: Institute of Post-Harvest Technology
ITI: Industrial Technology Institute
JKUAT: Jumo Kenyatta University for Agriculture and Technology
KALRO: Kenyan Agricultural and Livestock Research Organization
KEPHIS: Kenya Plant health inspectorate service
KTW: Knowledge Transfer Workshop
M&E: monitoring and Evaluation
MOU: Memorandum of Understanding
MOF: Metal Organic Framework
MPG: Mango Producer Group
MYRADA**:** Mysore Resettlement and Development Agency
NABARD: National Bank for Agriculture and Rural Development
NAMDEVCO: National Agricultural Marketing and Development Corporation
NFC: Nano Film Cellulose
NPFVGA**:** Niagara Peninsula Fruit and Vegetable Growers
OTFMB**:** Ontario Tender Fruit Marketing Board
PCPB: Pest Control Products Board
PLD: Phospholipase D
PRM: Project Review Meeting
REB: Research Ethics Board
RTE: Ready to Eat
RTS: Ready to Serve
SE: Socio Economic Report
SEM: Scanning Electron Microscope
SFAC: Small Farmers Agribusiness Consortium
SUA: Sokoine University of Agriculture
TFF: Tree Fresh Formulation
T&T: Trinidad and Tobago

TAHA: Tanzanian Horticultural Association
TAS: Tobago Agricultural Society
TNAU: Tamilnadu Agricultural University
UG: University of Guelph
UNGA: United Nations General Assembly
UNDP: United Nations Development Programme
UoN: University of Nairobi
US: United States
UWI: University of West Indies
VCS: Venture Capital Scheme
VKRC: Village Knowledge Resource Center
VRIC: Vineland Research and Innovation Centre

EXECUTIVE SUMMARY:

Post-harvest losses contribute several billions of dollars worldwide, in both developed and developing nations. Fruits and vegetables are one of the most highly perishable produce due to their tenderness that results in very short shelf life. Most fruits develop some sort of post-harvest 'disease' that accelerates the deterioration. In incidents where such disease incidences are prevented, fruits tend to shrivel and their taste attributes start to decline. Thus, minimising membrane damage can lead to extended post-harvest shelf life. Research at the University of Guelph lead to the discovery that a key enzyme called phospholipase D (PLD) triggers the onset of membrane deterioration. Further research in this field lead to the discovery of a key natural product, called hexanal that can inhibit the action of PLD dramatically. Based on these discoveries, a spray formulation with hexanal as the key ingredient (named as Enhanced Freshness Formulation or EFF), was developed and tested on many fruit crops as a pre- harvest spray as well as a post-harvest dip treatment. The results revealed that in addition to the anticipated extension of post-harvest shelf life, fruits in the treated trees were also retained for an additional period. These results made a significant impact with the fruit growers who tested these products, as they could generate a 10-15% additional income, depending on the crop. We have developed prototypes that could deliver hexanal at very small doses and in a sustained manner using 'green nanotechnology'. Through these technologies, the post- harvest shelf life could be extended from more than one point of intervention. As a result of this project, the awareness in this EFF technology has increased from zero farmers in 2012 to over 27,000 farmers in 2017. More than 80% of the farmers who were involved in testing approved the technology and 51% of the farmers got premium price in the market for mango and banana in India. This technology also generated 12-17 days of additional employment for women, due to longer availability of fruits during the season and more packaging opportunities. As an effective outcome of this project, 33 graduate students (15 Male: 18 Female) and 13 research associates/research fellows (3:10, Male: Female) were trained resulting in a sustained pool of highly qualified personnel. The salient results/outcomes during the 40 months of the project are:

1. The 9 Nano technological solutions to enhance the shelf life of fruits were tried in 6 countries and in 15 types of fruits. Irrespective of the crop, varieties and location the products continue to enhance the shelf life of fruits.
2. Seventeen Master's thesis and one PhD thesis have defended or submitted. Four or more MS thesis will be submitted by summer of 2018 (***Annex 1***)
3. Graduate students working in this global project won many national awards including a full scholarship to Cornell University for pursuing PhD.(***Annex 2***)
4. With the research and results from this project so far the team had produced 30+ refereed publications and nearly 60 conference presentations. Some of the publications are provided in the links below and detailed list I n A***nnex 3***.
   https://www.dropbox.com/sh/vf32fpu8x3dbmfy/AACuvB0oOKDWDeYxpCyS4aXta?dl=0
   DOI: 10.1080/15440478.2015.1029195 ,
   https://doi.org/10.17660/ActaHortic.2018.1201.8
   https://www.nature.com/articles/hortres201742
   http://dx.doi.org/10.1139/CJPS-2016-0351.
   http://dx.doi.org/10.1016/j.scienta.2017.10.031
   http://www.bioone.org/doi/abs/10.1139/cjps-2017-0365

5. A full set of publications (12) are in the typeset form to be published as a special issue in the journal Tropical agriculture, a first for a CIFSRF project (***Annex 4***)
6. Apart from these research outputs in the scientific community, the team had a solid communication output, through which the product (EFF) has reached more than **25,000 farmers directly**) and **30+** government and policy makers, globally.
7. Pre-harvest spray of EFF had extended shelf-life by 14-21 days in mango, 5-6 days in guava, and 4-5 days in grapes under ambient storage conditions. In Tanzania pre harvest spray has reduced the fruit drop in mango by 40%. In India, for Mangoes, the cost benefit ratio is 1:5/tree.
8. Post-harvest dipping of fruits (mango, banana and papaya) in 2% EFF extends shelf-life by 12-18 days which was confirmed in different geographical locations and reduced the disease incidence by 80%
9. Nano products such as nano sticker, sachet or film extend the shelf life of tropical fruits (mango or banana) by 12-18 days.
10. More awareness and acceptance of the product has been created in East Africa- A huge step from inception. In spite of delay in getting the hexanal in East Africa, EFF pre- harvest spray in sweet oranges reduced the fruit drop by up to 50% and reduced fruit drop (45.4 %) and increased fruit retention in mango. This can give significant economic and nutritional boost to Tanzania as their mango production is off season to other mango producing giants like India.
11. In Trinidad and Tobago, using EFF spray the farmers can stagger the time of harvesting papaya, so they could better manage their harvesting time and labour availability.
12. For school nutrition program in T&T, substituting green papayas (with the help of EFF) in place of potatoes would have implications for **national food security and sovereignty, while also supporting farmers** with assured market as the institutional (school board) demand for green papayas is 2746 lbs/month (But the supply is less than 2000 lbs).
13. Electrospun nano-fibre matrix fortified with hexanal (Sticker) and β cyclodextrin inclusion complex (Sachet) have been developed to minimize the post-harvest fruits damage during transport. The cost of technology is around INR.5 (~10 Cents) per piece to protect the fruit box carrying 2-3 kg.
14. Hexanal Incorporated Composite Material (HICM) maintained the marketability of the mango fruits for 21 days and Banana fibre Fruit Wrap (BFW) can be used as an ecofriendly cushioning in packaging as a substitute of Styrofoam.
15. EFF pre harvest spray in Nectarine increased the fruit retention by 15-17% in addition to higher firmness and reduced physiological disorders. This will fetch better price in Canada as against imported fruits from US. Volatile analyses provided conclusive evidence for the presence of hexanal in the fruit and its sustained presence after treating with EFF, thereby resulting in greater membrane integrity.
16. EFF as pre- harvest spray increased the shelf life in berry fruits: Strawberry (up to 9 days), Raspberry (6 days) which make huge impact in the marketability of these soft berries.
17. In hexanal response screening trials, a delay in mold growth and softening in strawberries is noted. Green aroma was also noted in nectarine and peaches which is due to naturally occurring hexanal trapped in the headspace of the packaging/wrap, which is important for preservation and shelf-life extension
18. As reported by Mango farmers in India, they could earn an additional income of INR. 14 000 (CAD 280) per acre with two weeks delay in their crop. This is in addition to the increased yield due to high retention (INR 10000= CAD 200).
19. Pack house meetings and trainings benefited more than 6000 women mango growers and value addition training in Mango to empower women entrepreneurship generated 17-man days of additional employment to women during crop season.
20. There are 90 Mango Producers' Group (MPGs) in the IDRC project sites, of which more than 60 per cent are represented by women compared to 2-5 per cent in other existing Community Based Organizations(CBOs).
21. About 15 per cent of women in MPGs revealed that they were able to partly convince men partners in the farm level decision making.
22. Two major events addressing packers (banana and mango) and growers (mango) were conducted with 120 packers each covering 80-100 farmers (representing over 10000 banana farmers) and 500 mango growers in Tamil Nadu, India.
23. In 2017 season alone, 2000 litres of EFF concentrate (equivalent to 100,000 litres of spray formulations) was produced and supplied to mango growers across the major mango growing domains of Tamil Nadu, Andhra Pradesh and Karnataka.
24. More than 2000 model farms have been set to disseminate the technologies. They represent all the five mango growing districts in Tamil Nadu besides Andhra Pradesh and Karnataka.
25. TNAU received request from 3122 farmers for the supply of EFF to undertake pre-harvest spray covering six major mango growing domains, of which small and marginal farmers constitute 81% (2526 farmers).
26. In India the technologies results in 12-17 days of additional employment for women during the cropping seasons, especially in the packing side. Also with three FRSCs, a total of 4360 farm advisory services were offered in which **32% of beneficiaries were farm women**. 44 Value added trainings given with 83% women participants (926 Women and 183 Men), 35% of the women became small entrepreneurs. Value addition products in Mango alone got an additional income of INR 19200 (CAD 384) per acre

27. The project was showcased in the Market Place, a side event of UN General Assembly in September 2015
28. The project was presented in the Invited talk "Healthy Goats and Tougher Fruits" at GAC, December 2015 and attended by policy makers, representatives from different consulates and high level government officials.
29. With GAC's help, the lead PI was able to give a webinar organized by IICA (Inter-American Institute for Cooperation on Agriculture, Canadian Chapter) which had 160 registrants from 17 countries.
30. During this project's tenure, the team had the opportunity to interact and update the project's progress with Canadian High Commissioner, Trinidad and Tobago, Sri Lanka, Tanzania and Canadian Consulate General, Bengaluru
31. The team has done extensive research and progress in the entrapment of hexanal and smart delivery of hexanal through various nano packaging materials such as sheets, sachets and stickers. US patent (PCT/CA2015/000027) was filed for a nano product developed in UG. A patent application by TNAU for the nano-sticker is currently being evaluated.
32. 'Smart Harvest'- a new licensee from Canada- hired consultants to clear the regulatory hurdles in US, Guatemala, Costa Rica with their existing partners. Focus is more on dip as it presents relatively lesser regulatory constraints.
33. Smart Harvest signed a MOU with TNAU on March 15, 2018 for commercial production and marketing of EFF.Hayley's in Sri Lanka received DOA (Department of Agriculture) clearance and launched their products (Tree Fresh Formulation and Bio wax) on March 22, 2018. These two events were attended by their respective CHC/Canadian Consul General (***Annex 5)***
34. In July 2016 this grant (project) sponsored a session on Food Security and Sustainability in *Joint* Annual conference for the Canadian Society of Agronomy /Canadian Society for Horticultural Science, Montreal, Canada. We also cosponsored the IIHS conference at Kandy in April 2017 and National Banana festival in India (July 21-23, 2017). This event was well attended by 10000 farmers, state ministers, and state and federal policy makers.
35. Skill demonstration on dip treatment of banana at the major banana growing area of Tamilnadu. This event also well attended by five project coordinator for supply chain management, 300 farmers and five policy makers.
36. A two day National conference on Nanotechnology for evergreen revolution was organized by TNAU in October 2017, which was again co-sponsored by this project funds. This conference was inaugurated by Dr. Anindya Chatterjee, Regional director, IDRC, Asia. The PO and lead PI of the project also attended the event.
37. In Trinidad and Tobago, World Food Day (October 16,2017) had a themed celebration "Change the future of migration - invest in food security" organised by Division of Food Production Forestry and Fisheries (DFPFF) attended by policy makers from Tobago
38. Dissemination meetings and events conducted in all project sites and was well received and attended by 2500 people including farmers, extension agents and policy makers.(***Annex 6***)
39. Book on "Postharvest Biology and Nanotechnology of Fruits, Vegetables and Flowers" edited by Gopinadhan Paliyath, Jayasankar Subramanian, Loong-Tak Lim, Avtar Handa, Autar Mattoo and K.S. Subramanian was submitted to Wiley-Blackwell Publishers in January 2018 and expected to release in Summer 2018 (***Annex 7)***
40. The project produced various informal non-scientific outcomes like pamphlets and 3-5 min videos from various project locations (Project output section of this report)

RESEARCH PROBLEM:

Post-harvest loss in fruits adds to several billion dollars worldwide. Decreasing the post-harvest losses can significantly improve the economic return for the farmers. A large proportion of women are directly or indirectly involved in the production and post production related activities (e.g. harvesting, sorting, packing, vending and accounting) in Asia, Africa and The Caribbean. Preventing these losses will ensure increased availability of nutrient-rich fruits and enhance the food security in these countries., Scientists at University of Guelph have identified that hexanal, a GRAS (Generally Regarded As Safe) compound that is produced by plants can help to extend post-harvest shelf life of fruits. This IDRC-CIFSRF project aims to reduce the post-harvest fruit losses using a nanotechnology based hexanal smart delivery system (HSDS). Since the project started, it was clear that hexanal can also be used to retain the fruits in the trees longer. This aspect was deemed very helpful to manage the limited farm labor availability in certain areas. Further, observations by participating farmers revealed the hitherto unknown benefits of this intervention in keeping pests and diseases at bay, indirectly by improving the health of the tree and fruits. As a result of these observations the project evolved into a pre- and post-harvest shelf life enhancing mechanism. The impact of such delay in harvest on the socio-economic impact was also studied.

Although at the start we envisaged only post-harvest shelf life, research during this CIFSRF funded work evolved into a two- pronged approach. Hexanal also helped to retain fruits for longer period, especially in mango and evolved as a powerful mechanism to keep banana fresh for much longer than usual. These observations lead to the adoption of the EFF technology by the mango and banana farmers (including packers). Downstream players in the market stream opine that a steady supply of certain varieties will help to reduce price fluctuation and make fruits available for a longer period. This also opens export market opportunities for countries like India, Kenya and Tanzania. Based on the output and outcome stories that emerged, the use of EFF has been added as a highly

recommended practice to mango growers by the State Department of Agriculture in Tamilnadu, India. In 2012 when the project started, hardly anyone- including researchers- knew hexanal in India. At the end of the project (Mar 2018) the technology has reached ~12,000 farmers. During this project over 60 HQP (graduate students, research associates and research technicians) were trained across the 6 countries. In addition, 2 patents on smart delivery of hexanal using Nano technological approaches have been filed in India.

MILESTONE PROGRESS

Refer **annex 7A** for detailed report on the project's progress over 40 months

SYNTHESIS OF RESEARCH RESULTS AND DEVELOPMENT OUTCOMES:

Nine Nano technological solutions to enhance the shelf life of fruits were tried on 15 types of fruits in 6 countries. Refer to the outcome story, "Nano technological solution to tackle post-harvest loses in fruits" (**Annex 10**). Important facts about these nine nano technological solutions are:

- Enhanced Freshness Formulation (EFF) carrying hexanal as an active ingredient can be delivered in horticulture systems as pre-harvest spray, post-harvest dip and vapor form preserves fruits for 2-3 weeks under ambient storage conditions. The technology is economical and eco-friendly and enabling farmers to earn 15-20% higher income
- Farmers in North America, Asia, Africa and Caribbean can use **pre-harvest spray** of EFF to retain the fruits for 2-3 weeks on trees and extend the shelf-life for another 2-3 weeks in storage conditions. The technology is working well for both tropical and temperate fruits. It was unequivocally demonstrated that treated fruits had lower incidence of post-harvest diseases
- In pack houses and exporters can use EFF **dipping technology** alone or in combination with wax formulation as an intervention within the sequence of dipping treatments to reduce the post-harvest losses. Huge quantity of fruits can be treated in 5 minutes. A solution that is easy for the farmers to adopt.
- **Vapour** form of hexanal is equally effective and various dispensing systems such as nano-stickers, nano-wrappers and nano-sachet were developed, validated and patents filed. This is simple and large quantities can be treated without any associated ill-effects.
- More than 12,000 farmers in India adopted the technology and our survey suggested that 80% of them either benefited from the delayed harvest and higher incomes
- The hexanal technology is not unique to a particular fruit or vegetable and thus it can be fine-tuned to suit a wide array of perishables. The technology is quite robust and powerful to help address the global challenge of post-harvest losses.

Key results for each project specific objectives were analysed and presented below (and in the table)

1*. Test pre- and post-harvest applications of "Enhanced Freshness Formulation", a combination of hexanal and anti-oxidants, in sprays and dips for extension of fruit retention and improved shelf-life of treated fruit.*

EFF has been tested on a range of temperate and tropical fruits in several countries. EFF has worked well as a preharvest spray (usually 2 sprays before ripening) on fruits such as mango, nectarines where the fruits have a thinner outer skin that permits the EFF to penetrate the fruit. On thicker skinned fruit such as banana and papaya which ripen continuously, the dip treatment has been very effective. Results for mango and banana have been consistent over different varieties in the partner countries. Shelf-life has been extended between 12 and 18 days for mango and 10 to 21 days for banana. When other fruits such as guava, grapes, papaya, and lime were sprayed with EFF, the shelf-life was extended and several other positive outcomes were reported including less fruit drop and longer retention of fruit on the tree, improved colour.

The post-harvest dip was able to extend the shelf-life of mango, banana, plantain and papaya while delaying the development of full colour in the fruits. This has significantly extended the window for marketing a fruit such as banana.

Exposure to EFF as a spray or dip had the added benefit of creating firmer fruit with no negative side effects on other quality measurements such as flavour or total soluble solids.

2. *Test exposure of fruit to hexanal as a vapour for longer post-harvest shelf-life.*

EFF can also be applied as a vapour by placing the hexanal in an air tight space and allowing it to volatilize. On strawberry the vapour treatment helped to maintain the integrity of membranes which contributed to a longer shelf life. When used as a vapour treatment on banana, EFF destroyed the spores of important diseases in banana that would affect both pre- and post-harvest quality. This reduction in disease presence and severity has also been noted with other types of applications of EFF in the field.

3. *Develop hexanal impregnated biowax, bio-nanoparticles-based sachets and packaging materials for maintaining freshness of fruit during packaging and shipping.*

Hexanal has successfully been incorporated into sheets of fibres that will act as a vapour treatment for the fruit once the hexanal is released. This release pattern and the triggers for release are being studied now. The shelf-life of banana and mango was extended by 6 and 12 days respectively, when the hexanal was incorporated in a 'sticker' that was affixed to the inside of a carton. Marketability of hexanal incorporated bio was 84% when compared to its control. Similarly HICM helps to increase the marketability of mango up to 21 days at 13.5 ^{o}C.

4. *Assess risks from hexanal-based nano-applications.*

Hexanal and the vehicles used to carry it are all biodegradable and pose no threat to the environment. The hexanal applied as a vapour dissipates very quickly and is broken down to hexanoic acid. Extensive study on the biosafety of hexanal has been studied. It is safer to beneficial microbes, natural enemies, honey bees, earthworms and humans. Biodiversity of the orchard ecosystem is conserved without any ill effects. All these nano products have been evaluated for their suitability to qualify under green nanotechnology. All the products matched the major part of the criteria apart from flammability and energy requirement. Refer to the outcome stories "Biosafety" (***Annex 11***) and "Green Nanotechnologies" (***Annex 12***)

5. *Develop and implement a marketing strategy to ensure that applications reach a large number of small-holder producers.*

The marketing strategy began with exposing as many large and small farmers to the EFF technology as possible. With mangoes it has been effective to target grower organizations who represent many large and small growers. However, with banana, which are brought to central locations by small farmers in each small region, it has been best to target these collection points because each one deals with 100 or more small farmers. In some countries that state agency responsible for extension has been included as a KTW partner. These organizations are able to run local workshops in populated and remote areas. EFF usage in India 2014 was only 720 farmers, but with constant dissemination and trails, the usage rose to 12074 farmers in 2017. Feedback from the users revealed that post-harvest losses had been reduced by 10-12% in the EFF sprayed fields in comparison to control. Farmers got additional 24% income/acre directly due to increased retention (10%) and two weeks delay in harvest (14%). Based on the usage and cost benefit analysis, The Canadian Company, Smart Harvest, signed a MOU with TNAU in March 2018 to commercially produce and distribute EFF in India. In Sri Lanka, Hayley's sublicensed two products (EFF and Bio wax) and the products were launched in March 2018. Refer to outcome stories "EFF delivery at farm gate" (***Annex 13***), "Hexanal technology Cost Benefit Analysis" (***Annex 14***) and "Technology Transfer Experience in Sri Lanka" (***Annex 15***).

6*. Monitor socio-economic impacts on small-scale and marginal fruit growers, particularly women engaged in post-harvest operations*

In India the technologies results in 12-17 days of additional employment for women during the cropping seasons, especially in the packing side which reduces the drudgery, since they work in shade. Also with three FRSCs, a total of 7245 farm advisory services were offered in which **32% of beneficiaries were farm women**. 44 Value added trainings given with 83% women participants (926 Women and 183 Men), 35% of the women become small entrepreneurs. Value addition products in Mango alone got an additional income of Rs 19200 ($384) per hectare (Value Addition Training in ***annex 17***). In 2017 Mango season alone 3122 farmers were supplied with pre-harvest spray covering six major mango growing domains of India, of which small and marginal farmers constitute 81% (2526 farmers). In Trinidad and Tobago trials of EFF spray for papaya demonstrate potential benefits for actors across the value chain, including small-scale producers, processors, caterers, and consumers, due to the potential to use green papaya as a potato substitute. EFF spray slows the ripening of the fruit, retaining the green colour that processors want to see when buying papaya to make value-added products. For the country, the increased use of green papaya has the benefit of reducing dependence on imported goods and lowering national food bills, while supporting local farmers and their livelihoods. In Sri Lanka, Impregnating banana paper with EFF technologies allows it to do double duty – protecting fruit from injury during transportation over long distances, and increasing shelf life. Results from simulated transportation and storage trials carried out at the Industrial Technology Institute (ITI), Sri Lanka, showed no significant difference in fruit quality between polyethylene sleeves and banana fibre-based wraps. Even better, this technology adds further value through employment and income-generating opportunities, especially for women who otherwise have limited *employment options.* (Value Chain Actors in ***annex 16***).

SYNTHESIS OF RESULTS FOR EACH TECHNOLOGY, COUNTRY AND THEIR CROPS

#	Technology/ Country	India	Sri Lanka	Kenya	Tanzania	Trinidad and Tobago	Canada
	Crop	Mango, Guava, Grapes and Banana	Mango and Papaya	Banana and Papaya	Mango and Citrus	Banana and Plantain Papaya and Lime	Strawberry, Raspberry, Blueberry, Haskap and Nectarines, Apple
1	EFF pre Harvest Spray	**Mango**: Additional two weeks retention and Delayed harvest nets better price (up to 40% more) in the market 50% Reduction in fungal disease incidence on fruits Additional net fruit yield of 5 kg per tree (200 kg per acre) Extended shelf- life of fruits in ambient (2-3 weeks) and cold (4-6 weeks) storage Treated fruits are shiny and firm that enable long distance transport and export Amount invested 1 CAD per tree and amount gained 5 CAD per tree **Guava**: One Spray (2%), 30 days before harvest extended the shelf life by 6 days (Ambient) and 21 days (Cold Storage). Despite the shelf-life extension is shorter, it assists the farmers to transport the fruits to farthest market **Grapes**: One spray (1.0%) 15 days before	**Mango**:EFF 2% spray with three treatments: 20,40,60 DBH on varieties TJC & KK Retained consistent firmness for 4 weeks. Harvesting season of KK variety of mango and TJC variety of mango could be extended up to 4 weeks and 6 weeks respectively using 3 time EFF spray treatment.	**Banana**: 2% pre harvest spray (15&30 DBH) had delayed fruit ripening, 14 days delay in fruit retention and 6 days delay in shelf life after harvest. Also delay in peel softening **Papaya**: 2% pre harvest spray (15&30 DBH) had delayed fruit ripening, 13 days delay in fruit retention and 6 days delay in shelf life after harvest. Also showed better color development before & after harvest, increased firmness, titratable acidity, enhanced Beta Carotene, and reduced ethylene peaks. Also noted less incidence of diseases. Hence more farmers in Machakos County have deep interest in this technology	**Mango**:EFF 2% & 4% Spray reduced the fruit drop 10.4% (Apple Var), 40% (Palmer), pest incidence 10.8% (Apple), 19.49 % (Keitt). Increased the Marketable yield 13.45 % (Apple), 23% (Palmer), Shelf life Increased by 7 days. Also noticed increased fruit firmness, reduced physiological weight loss (PWL) and nutritional value **Sweet Orange**: EFF 2% improved the quality and shelf life by 7 days in Ambient conditions. Increased fruit firmness and reduced physiological loos in weight when compared to control and smoke treatment (most commonly used in Tanzania)	**Papaya**: 2% EFF spray resulted in fruit retention in trees for 2 months and significant reduction of post-harvest diseases **Lime**: 4% EFF pre-harvest sprays biweekly or weekly with 30 days of harvest extended the fruit retention to 99 days. Senescence (color change from green to yellow) took in excess of 120 days.	**Strawberry**: EFF spray did not significantly slow down ripening, but fruits from the sprayed plants were firmer and kept at least 2-3 days more than untreated. Genetic analysis revealed that several genes involved in ripening regulation were slowed down as a result of EFF spray. EFF reduced the transcript levels of Phospholipase D (PLD) **Raspberry**: No significant slowdown of ripening but fruits from the sprayed plants were firmer and kept at least 2-3 days more than untreated. Genetic analysis revealed that several genes involved in ripening regulation were slowed down as a result of EFF spray. A key finding of this study was the involvement of calcium in delaying the ripening process. Induced calcium depositions which helps in maintaining membrane integrity. **Blueberry**: Increase in firmness and soluble solids. Metabolomics revealed that genes involved in carbohydrate and stress response pathways were upregulated (increased) and genes involved in ethylene synthesis and cell wall integrity were decreased (downregulated). **Nectarines**: EFF 2% spray extended the shelf life by 9 - 12 days, reduced internal browning and mealiness by

		harvest extended the shelf life by 4 (ambient) and 31 days (cold storage). One of the significant observation made was greenness of the fruit stalk and retention of fruits that facilitate intact of fruit bunch and enables long-term transport.					8-10 days, Further EFF shifts all the volatiles by few days longer thus keeping them 'fresh'. **Haskap:** Very inconclusive with Haskap a new berry crop of Canada, because identifying the right stage of harvesting is tough as haskap turns dark even when it is unripe. EFF did delay the fruit drop from the bushes which can help the haskap growers. **Apple**: Fruit retention was very compelling and the grower also attests to the fact that the fruits in the sprayed trees had very less bitter pits. EFF treated apples also stored very well – into March 2018 (after harvesting in Sep 2017), which was a welcome result for apple growers
2	EFF Post Harvest dip	Dipping EFF (2%) for 5 mins & shade dried for 30 mins extended the shelf-life of mango, banana, guava and grapes, 13-15, 11-12, 4-6, 2-4 days respectively under ambient storage conditions. The EFF dipping works well for green banana where chips makers wish to take advantage of the fruit preservation.	N/A	Banana:2% EFF dipping for 5 minutes increased the shelf life to 9 days. Also increase in physical and chemical parameters tested (firmness, ethylene production, respiration, total titratable acidity and total soluble solids.) Papaya: EFF (1&2%) dip for 5 mins increased the shelf life by 6 days, retained fruit firmness (9 days), decrease in color break down, Vitamin C degradation		Banana: EFF dip 2% and 4% in commercial banana (William) firmness extended by 3 days. Papaya: 2% EFF dip delayed shelf life by 14 days, reduced post-harvest diseases and increased sugar (ideal for packers)	N/A
3	EFF vapour	Hexanal vapour at 800 ppm for 3 hrs totally reduced anthracnose and stem end rot in	N/A		N/A		Helps to keep the membrane integrity in greenhouse grown strawberries, thus enhancing shelf life.

		Banana. In Mango and banana extended the shelf life for 15-20 days.					
4	Electro Spun Wrapper	N/A	N/A	N/A	N/A	N/A	Simultaneous release hexanal, benzaldehyde, and/or salicylaldehyde from their precursors are effective in delaying the delaying the ripening of pear and nectarine fruits.
5	Hexanal entrapped Sachet (cyclodextrin inclusion complex)	58% hexanal entrapment using (MOFs)". Microwave synthesis took less time to get the crystals of MOFs. MOFs were made as pellets and each pellet of 1g can preserve a pack of fruit (2-3 kg)	N/A	N/A	N/A	N/A	N/A
6	Sticker (Electrospun nano-fibre matrix)	Nano-fiber matrix (developed through electrospinning which traps hexanal) 5 x 5 cm was affixed in a box of fruits (2-3 Kgs). The shelf-life extended by 6 (Mango) and 12 (Banana) days, under ambient storage conditions.	N/A	N/A	N/A	N/A	N/A
7	Banana fibre nano-film (Nano Fibre Cellulose) NFC	A combination of 5% polyvinyl alcohol, 5% poly acrylic acid and 1% NFC resulted in perfect biodegradable nano-film. This extends shelf life of tomatoes up to 18 days under ambient storage.	N/A	N/A	N/A	N/A	N/A
8	Bio wax	N/A	**Mango & Papaya:** storage life of bio-wax treated papaya	N/A	N/A	N/A	N/A

			could be extended for more than 21 days. Marketability of bio wax treated TJC Mango fruits was 84 % compare to 58 % of un-waxed fruits				
9	HICM (Hexanal Incorporated Composite Material)	N/A	**Mango:** HICM helps to increase the marketability of mango up to 21 days at 13.5 ^{o}C.	N/A	N/A	N/A	N/A

Summary statement: EFF has really taken off well and is expected to be available commercially in India and Sri Lanka in 2018-19 season itself. In Canada and US it is expected to be made available in late 2019 or 2020 as the regulatory process will take that long. In Africa it requires some more data for regulatory clearance and a suitable partner to produce it commercially. This is for both spray as well as dip treatments. Hexanal impregnated nano-sachet and nano-sticker technologies have been filed for patent in India and once the patent is issued it is expected to be commercially produced and made available to packers. This technology is expected to reach other countries also as there is no direct contact of hexanal with the fruits and hence the regulations will be much less than EFF spray or dip. Electrospun wrapper is waiting for patent clearance and already the technology is being discussed with prospective suitors in Canada. Once the patent is issued this may be taken up for commercial production in Canada and other places. Biowax is commercially available in Sri Lanka from next year onwards hexanal impregnated biowax is also expected to be made available through Hayleys, it is licensee.

We anticipate that the overall 'hexanal nanotechnology' adoption will take up in the coming years. As of now the EFF is popular in India (only one state) and Sri Lanka, other than Canada and about 12000 farmers are adopting. This is mainly because of the delay in regulatory process that is beyond our reach. The demand for the farmers is quite high and once the regulatory hurdles are removed and the product is produced for distribution in the entire country, we can anticipate at least about 100-150000 farmers/packers using it in various parts of the country. This is a very modest estimate that was developed based on the statistics provided by State Horticultural Department, whose personnel look after the spread of such technologies. It must also be noted that this is a technology for fruits which is only a small percentage of total crops grown and in no way, can be compared with grain crops like wheat, soy or millets for that matter. In short, the demand from farmers, the scientific data from researchers, the licensee to produce it commercially are all there for a substantial growth of this technology. Only thing to be cleared is the regulatory hurdles and an y help to accelerate this process will be of immense use.

RESEARCH PARTNERSHIPS

The team has partnered with many institutions to conduct their field trials, socio economic work and for disseminations.

No	Country	Team	Partnered With
1	India	TNAU	MYRADA-Mysore Resettlement and Development Agency
2	Sri Lanka	ITI	CEPA-Center for Excellence in Poverty Analysis IPHT- Institute of Post-Harvest Technology*
3	Kenya	UoN	KALRO-Kenyan Agricultural and Livestock Research Organization JKUAT- Jomo Kenyatta University for Agriculture and Technology Meru farmers Association* and Kevian (Largest Indigenous Fruit Juice Processor in Kenya)*
4	Tanzania	SUA	AMAGRO- Association of Mango Growers TAHA-Tanzanian Horticultural Association
5	Trinidad and Tobago	UWI	NAMDEVCO- National Agricultural Marketing and Development Corporation Division of Food Production, Forestry and Fisheries, Tobago House of Assembly
6	Canada	UG	OTFMB- Ontario Tender Fruit Marketing Board* NPFVGA- Niagara Peninsula Fruit and Vegetable Growers* VRIC- Vineland Research and Innovation Center*

(*) Public and Private Sector Partnerships\

Results of research conducted by University Guelph in temperate fruits enabled the adaptation of EFF spray and dip technology in India, Sri Lanka, Kenya, Tanzania and the Caribbean. Based on the results in India, similar trials were conducted in Sri Lanka (TNAU and ITI). Hexanal entrapped Sachet, a nano technological intervention developed by TNAU will be sent out to Kenya to study the effect in Papaya (TNAU and UoN). Smart Harvest, a Canadian company had signed a MOU with TNAU on March 15, 2018 to commercially produce EFF in larger scale. Similarly, Hayley's in Sri Lanka, sublicensed for the commercial production of EFF and Bio wax for their market. All the teams have been benefited from capacity building in terms of equipment and human resources (through RA's, Post docs and graduate students). As an effective outcome of this project, 33 graduate students (15 Male: 18 Female) and 13 research associates/research fellows (3:10, Male: Female) were trained resulting in a sustained pool of highly qualified personnel

Younger team members from TNAU and ITI had their training and lab exposure at Canadian University (UG). These researchers are being trained in the fields of horticulture, post-harvest science and nanoscience that would help them in career growth. With a consistent and solid financial support for the past four years, the TNAU team is well positioned to take a lead in nanotechnology in India.

The ITI is now collaborating with the Institute of Post-Harvest Technology (IPHT) Sri Lanka, to expand the scope of the EFF spray treatment and the Bio-Wax application for use on limes (to extend the crop season and post-harvest loss). The IPHT will also promote the use of the fruit wrap paper in the collection and distribution centres in place of the Styrofoam sleeves currently being used to protect fruits from abrasion and vibration damage. UoN is in the process of making a partnership with Kevian, Kenya, a largest indigenous fruit juice processor and with Meru farmers association to procure and process the fruits.

Graduate from UoN, Ms. Peninah M. Yumbya working in this project had been awarded Kshs. 2 Million from the Kenya National Council for Science and Technology to support her future work. Dr .K. S. Subramanian, PI of TNAU team has been awarded the position as the NABARD Chair Professor since April 17, 2017 to take forward the

emerging technologies beyond the project period. Graduate students from this project won several national and international awards (***Annex 2***)

Partnering Institutes benefitted greatly through this project. Apart from financial benefits, researchers-especially students- from these countries were exposed to the functioning of large, multinational project which helped them immensely in their career development. Several students working on this project, won awards and scholarships at International levels as detailed in the output section. This has also helped for newer partnerships which are taking place between different countries as there are joint project proposals submitted for taking these result to next level. In Asia EFF demonstrated through this project is expected to be available commercially soon. In Africa, further research proposals have been submitted to expand this work so as the regulatory hurdles will be cleared and the product will be available for commercial use. TNAU is continuing to work on spin-off technology such as sachet and sticker for further use. This project has helped UoN and TNAU to forge better collaborations with UG and possible student exchange can happen soon.

Governance: This project provides plenty of opportunities for the women to get involved in cutting-edge research that help them in career building. The selection is purely on merit basis and the outcome of their theses are being published or presented in international conferences.

Research ethics: The project follows their respective Institutional Ethics Board and is already in place. All the personal information gathered with consent as per the stipulated guidelines.

Use of research results: In mango and banana, had made a deep impact on the entire value chain in India and Sri Lanka. Because of the overwhelming impact mango farmers, banana packers, banana chip industry and exporters are eagerly awaiting the availability of the product in the market. At the end of the project, EFF has been launched as a commercial product through a private company in Sri Lanka and a MoU has been signed to produce EFF for limited use in India.

In Kenya, Tanzania and West Indies, hexanal was not something that is known to even researchers till the inception of this project. Currently fruit growers have been sensitized through on farm trials and are convinced of the benefits of hexanal for improving fruit growing and shipping in those countries. In Trinidad, green papaya is being substituted in the school meals instead of potato (which tends to be expensive there) and EFF plays a key role in keeping papaya from ripening for an extended duration.

It is anticipated that EFF will be available and used by well over 100000 fruit farmers in India alone within the next 3 years. We also anticipate that the sensitization of growers in Africa and Caribbean will lead to a commercial product (EFF) within the next three years.

SYNTHESIS TOWARDS AFS THEMES

This project focused on the research towards outcomes developed for improvements in food, income and nutrition security. Considerable progress has been done in the following directions:

NEW AND IMPROVED AGRICULTURAL SOLUTIONS THAT INCREASE FOOD PRODUCTIVITY, IMPROVED INCOME OPPORTUNITIES AND IMPROVING GENDER SPECIFIC CONSTRAINTS

EFF interventions have improved food productivity both directly and indirectly. Farmers can get better price for their produce, since they are able to exercise better control of fruit harvest and ripening. Another advantage for growers stems from quality improvement—uniform ripening, better appearance and fruit maturity parameters (e.g. color and size) will allow farmers to fetch premium price.

For example in India, in mango cultivation with Good Agricultural Practices (GAP) along with EFF intervention demonstrated at 2000 model farms in Tamilnadu. In addition to EFF spray on model farms, farmers have been trained to use nets and hooks to harvest fruits (GAP), in order to minimize losses related to falling and bruising.

- With EFF technology there is increased harvest of at least 5 kg fruits per tree which gives a net gain of INR 10000/acre ($200). Additionally delayed harvest (EFF keep fruit fresh for 2 weeks) assists in additional income due to late arrivals in the market. According to farmers 1-day delay = INR. 1000 gain (CAD20/day) and 2 weeks delay = INR. 14,000 gain (CAD280)

- The post-harvest dipping of banana in EFF extended shelf-life up to 2 weeks under ambient and 4 weeks under cold storage conditions. At least 15% of fruits rejected at the pack houses can be avoided with EFF dipping. Such delayed ripening circumvents price volatility while ensuring higher farm income.
- EFF intervention in banana is almost exclusively done by women, round the year, whose families are farm families. In mango farms spraying is done by men while sorting, grading and packing are done by women. Thus, the technologies provide improved livelihood for these farmer families, women included.
- Continued availability of fruits for an extended time due to EFF intervention (spray or dip) directly results in additional employment especially for women between12-17 days during each cropping season. Since, pack houses would primarily be women's jobs, there is increased employment opportunities for women.
- The potential for farmers, particularly smaller-scale farmers, to generate additional income streams from fruit species they have previously avoided. Since the fruits such as banana, mango and papaya are highly perishable the short shelf-life deters smaller-scale farmers to grow it. EFF interventions are beginning to convince such small scale farmers, especially in Africa to re-think and expand fruit growing in these regions. Keeping color as in limes or green papaya can help to expand growing these crops where there is such need (e.g. the Caribbean).
- Value-addition training has potential for women to make use of the additional fruit availability for which the team has given 44 value addition training with 83% women participants (926 Women and 183 Men), 35% of the women become small entrepreneurs. Value addition products in Mango alone got an additional income of INR 7500 (CAD150) per acre.
- In Sri Lanka training programs on banana cultivation and fibre production were conducted, with the goal of enhancing income for products as well as generating new income through selling banana stems for fiber production (SLR 15,000/acre- CAD150.00/acre). Further expansion of banana fiber paper production will lead to additional employment for women and utilize the bio-waste produced.

CONTRIBUTING TO ENVIRONMENTAL SUSTAINABILITY, AND CONSIDERING THE POTENTIAL ENVIRONMENTAL IMPACTS, BOTH POSITIVE AND NEGATIVE, OF THE APPLICATIONS BEING DEVELOPED.

Hexanal is a natural product produced by all plants. It is a GRAS (Generally Regarded as Safe) compound and has no known or observed ill effects on the environment. Further due to its volatile nature, the product evaporates within 24 hrs leaving no trace in the fruits as well as gets broken down quickly into basic molecules in the atmosphere. Risk assessment and environmental safety studies were done in detail using subjects from various trophic levels -microbes, predators, parasitoids, honey bees, earthworms, zebra fish and human cells and none of them was affected by hexanal even at 300% more concentrations than we recommend (Paliyath and Subramanian, 2008). The tests were done for some of the organisms till F1 generation. The results have clearly demonstrated that the EFF formulation is quite safe to all trophic levels even at three times of concentrations recommended for pre-harvest spray or post-harvest dip. A book encompassing the results has been released already which will help the scientific community, industry and policy makers. Further honeybee and other beneficial insect activity improved in the treated farms.

Banana fiber paper and tray produced is a value-added product from the waste which minimizes post-harvest loss during transportation and distribution by protecting fruit from physical injury. The products are ecofriendly and the fruit wrap is expected to replace the Styrofoam sieving currently used to protect fruits during storage and transport. Thus this eco-friendly product will have a huge positive bearing on the environment.

The project also contributes to environmental sustainability as traditional methods for pre- and post-harvest treatments of fruits involve exposing to smoke in several countries, which requires the use of wood, and thus has negative implications in terms of deforestation. In addition, the traditional smoking method creates partially underground tunnels, which contributes to soil erosion. **Thus, EFF spray appears to have positive resource sustainability implications**. Moreover**, it reduces drudgery for women** because it would mean a reduced need for firewood (which women collect by walking long distances). Finally, crop smoking decreases air quality, which has implications for the health of farmers and their families.

IMPROVING ACCESS TO RESOURCES, AND/OR MARKETS AND INCOME (ACCESSIBILITY)

In India three Village Knowledge Resource Centers- VKRC (one each in Krishnagiri, Dharmapuri, and Theni Districts) were established. These VKRC provide essential tools to small and marginal fruit farmers thus enabling them to implement Good Agricultural Practices- GAP. Trainings were conducted on the importance of collective marketing, GAP, EFF interventions, and formation of model farms. The VKRC are serving as a "hub" to promote GAP for mango farmers, answer any concerns about EFF intervention and improve the adoptability of the technology. MYRADA (NGO) is using mobile phones to send SMS on the occurrence of pests and diseases besides price forecast.

IMPROVING NUTRITION

Given the diversity of types of farms (marginal, small-scale, medium-scale, large-scale) in the project, and their diverse sources of farm income, it is expected that household food security is variable. Those vulnerable to food insecurity are most likely to be the marginal and small-scale farmers who rely in farming as the sole or primary source of income. Our data in Sri Lanka suggests that **farmers and the workers of the banana fibre factory are the most vulnerable, and the project activities that are targeted to increase the sustainability of earnings among these groups will likely have significant outcomes for household food security.**

- In the Caribbean, bread fruit is one of the key crops to improve the food and nutrition security. With a shelf life of as little as twenty-four hours at full maturity under ambient conditions, this fruit suffers extremely high levels of wastage despite its potential. Further, the Trinidad and Tobago Government policy has dictated that relevant agencies find ways to utilize breadfruit in the School Feeding Program in which breakfast and lunch meals are provided free of charge to children attending local schools.
- Apart from its direct use as a starchy staple, the breadfruit can be processed into flour for use on its own or in a composite with wheat flour to make many items traditionally used in these meals. Delay in the ripening process will greatly facilitate processing into flour and will set a domino effect on the food, nutritional and economic security of the region. Preliminary test on breadfruit with EFF dip treatment gave promising results and further trails are ongoing.
- In Tanzania, Most farmers consume the banana they produce (99%): 42.1% on a daily basis, 41.3% few times in a week while 16.7% consume these fruits rarely. Enhancing shelf-life will have profound effects on banana consumption given that it is an annual and staple food. Also subsistence farmers can potentially apply the technologies sequentially to spread maturity (over time) thereby smoothing food consumption.
- In India, the best post-harvest management practices and adoption of EFF technology enhanced the availability of fruits per unit area which resulted in improved purchasing power. The consumption survey data suggest that minimized post-harvest losses enhanced the availability of fruits but hardly coincided with increased consumption. Instead, it provides purchasing power to get nutritionally rich foods.

INFORMING POLICY

- In Tamilnadu, in a state level policy decision, EFF technologies were incorporated to reduce losses at the post production stage (January 2016)
- The project was briefed to Canadian Consul General (June 2015) and to Dr. Harshvardhan, Minister for Science and Technology, India (September 2015)
- This project was show cased (one of the thirteen concepts invited) in Market Place, a side event of UN General Assembly in New York in September 2015. Dr. Jay Subramanian, Lead PI of the project explained the progress and its importance in global food security to many policy makers including Hon. Elissa Golberg, Assistant Deputy Minister, and Partnerships for Development Innovation, GAC.
- A presentation entitled 'Healthy Fruits' was delivered by the PI, Dr. Jay Subramanian at the GAC, Ottawa, which was attended by diplomats from several countries as well as policy makers from Canada – December 2015.

- In October 2016, with GAC's help, a webinar on this project was organized by IICA (Inter-American Institute for Cooperation on Agriculture, Canadian Chapter) which had 160 registrants from 17 countries:
- The project and its benefits were explained to Canadian Consul General, Bengaluru, during her visit to TNAU in January 2017
- In February 2017 the seminar presented by Dr. Jay Subramanian at UWI, St. Augustine Campus was well received by Mr. Marlon Thompson, Trade Commissioner, CHC, Trinidad and Tobago and CHC is very much interested in the project updates and wants to involve herself in future events.
- Also during that time Dr. Jay along with few members of UG team and UWI PI/team met Assembly Man Mr. Spencer and explained the project and its implications in Tobago
- Presentation to the Secretary for Food Production, Forestry and Fisheries in Trinidad and Tobago; Tobago House of Assembly, and discussions with NAMDEVCO
- Collaborations with District Agriculture, Irrigation and Cooperative Offices in Tanzania
- Results shared with Kenyan Cabinet Secretary for Agriculture at a March 2017 conference.
- In March 2018, Consul General of Canada, Bengaluru presided the MOU signing event in TNAU and CHC, Sri Lanka, presided the product launch event in Sri Lanka.

PROJECT OUTPUTS:

1. Book on "Biosafety of Hexanal", written by the team members formerly released during the inception in January 2015, by Mr Sydney Frank, Canadian Consul General, Bengaluru (***Annex 18***)
2. Project was show cased in Market place, a side event of UN assembly in September, 2015 https://www.uoguelph.ca/research/discover-our-research/photo-friday/2015-10-02 https://www.guelphmercury.com/opinion-story/5962693-driving-toward-the-zero-hunger-generation-/ Epoch Times: Canadian Innovations show cased in UN http://printarchive.epochtimes.com/a1/en/edition.php?dir=ca/yow/2015/10-Oct/02
3. In India: http://www.thehindu.com/todays-paper/tp-national/tp-tamilnadu/life-of-temperate-fruits-in-orchards-extended-thanks-to-nanotech/article8038916.ece http://www.thehindu.com/todays-paper/tp-national/tp-tamilnadu/new-technologies-will-enhance-income-of-farmers/article8038958.ece
4. In Sri Lanka: http://www.sundaytimes.lk/160508/news/longer-life-for-lankan-fruits-193250.html http://www.ft.lk/it-telecom-tech/Advancing-Dialog-s--Govi-Mithuru--service-in-partnership-with-Industrial-Technology-Institute/50-642946
5. In Tanzania: http://www.habarileo.co.tz/index.php/habari-za-kitaifa/368-dawa-kuzuia-matunda-kuoza-yaja (Swahili)
6. In Australian Broadcasting Center (Radio): http://www.abc.net.au/news/2015-03-17/nanotechnology-mangoes-india-srilanka-canada/6325346
7. IDRC Newsletters: https://www.idrc.ca/en/stories/mango-saving-molecule
8. In Vice Media: https://motherboard.vice.com/en_us/article/4xan8n/how-nanotechnology-will-keep-your-bananas-and-mangoes-from-rotting
9. In October 2016, with GAC's help a webinar on this project was organized by IICA (Inter-American Institute for Cooperation on Agriculture, Canadian Chapter) which had 160 registrants from 17 countries: http://www.iica.int/en/events/affordable-natural-product-extend-fruit-shelf-life
10. Nanotechnology makes mango farming more profitable in Krishnagiri, By Anupam Srivastava,IDRC Regional Office, New Delhi (***Annex 19***)
11. 10 things about Hexanal: https://www.uoguelph.ca/research/discover-our-research/publications/10-things-to-know
12. Hexanal Project: https://www.uoguelph.ca/research/discover-our-research/publications/strategically-themed-newsletters
13. Special Issue on Tropical Agriculture Journal, featuring 12 research articles from this project which will be formerly released in Trinidad and Tobago, with their CHC, in summer 2018 (***Annex 6***)

14. Book on "Postharvest Biology and Nanotechnology of Fruits, Vegetables and Flowers" edited by the team members and published by Wiley- Blackwell Publishers, expected in Summer 2018 (***Annex 5***)
15. Project coverage in Canadian National geography: http://idrc.canadiangeographic.ca/blog/stopping-rot-india.asp
16. Following dissemination videos in various project sites:

    Overall view of the project: https://youtu.be/KOMMXoiT_ZQ
    3MT National Winner Video: https://www.youtube.com/watch?v=TyibZdxfBDw
    Project impact in Trinidad and Tobago: https://uoguelphca-my.sharepoint.com/:v:/g/personal/jsubrama_uoguelph_ca/EePV3VL5iDJDvemHQ-qNkQEB3MzH323xsHAKV_Bu63yS-w?e=P9AoRr
    Project impact in Sri Lanka: https://drive.google.com/file/d/1lMy245cAuCMTzrJliuTJ9C8THxintJU8/view
    Project impact in Kenya: https://drive.google.com/file/d/0BzLNoLD6ommbMFNJeEVXMGRDLXc/view
    Project impact in India: https://www.youtube.com/watch?v=J2P-KpOJYKA

PROBLEMS AND CHALLENGES:

Staff recruitment and retention in a timely manner was an issue throughout the project. This stems mainly due to the unusual start and end dates, especially when student recruitment is concerned.
Lack of knowledge on clearing regulatory hurdles by all the parties involved. Researchers were not familiar with the regulatory process in various countries but tried their best to sort it out. Usually such regulatory processes will be taken up by licensee with technical help from researchers and we had our licensee only in the later stages of the project. We sought help from GAC in India, but it was even a challenge to them.
Availability of hexanal was an issue in Africa and Caribbean and this cost us almost 8 months to start the project as the company who supplied for Africa was not forthright. Eventually we had to acquire it in Canada and ship it to Africa at a cost.
Farmers were reluctant in Africa to take up anything that they perceive as 'chemicals' if it comes from the West. It took a while to convince farmers to participate in the trials. These are things that we did not anticipate in spite of sufficient planning.

OVERALL ASSESSMENT AND RECOMMENDATIONS:

Based on our project gained experience involving 6 countries with varied currencies, we recommend that in future programming, such multi-country projects may be restricted to 3 or 4 countries at best. Addition of more partners delays reporting which has a domino effect on the project and increases transaction cost to all organizations involved.
We also recommend that in a large project operating in 6 countries, some relaxation on budget and financial reporting would have been beneficial. It is not easy in most countries to get the FR every 6 months. In every place the PIs spent a considerable time with finance department consolidating these frequent reports. Holding back the entire team for one member's delay really set us back. Although we all understand this was a specific requirement for CIFSRF projects, yearly FR would have made everyone's life lot better.
Timing of the project start – December 1-is really an inconvenient time to initiate a project as we had to report in December, which will never be possible due to holidays, breaks etc. This also resulted in delays in reporting.
Given the complexity of the project, certain additional flexibility in budgeting would have been beneficial for the teams. Although IDRC grants provide flexibility to accommodate changes in the work plan when mutually agreed in advance, if at all possible ideally researchers should have the freedom to move funds vertically within the budget.

Student stipends are done differently in different institutions. In various cases students were not able to complete their programs within the project period. Flexibility to pay the students as required by the conditions of their program will help to recruit and retain top students.
We appreciate the efforts from IDRC to give recognition of students and research awardees in IDRC projects, as this will go a long way in their careers.

Thesis Submitted by grad students in IDRC Project # 107847 "Enhanced Preservation of Fruits Using Nanotechnology"

MS Thesis:

1. Amrena Jan, N. 2015. Development of hexanal loaded electrospun polymer matrix to extend shelf-life of mango (Dr. S. Ganapthy) - Defended
2. Vivekumar, R. 2015. Hexanal and beta cyclodextrin complex for slow release of hexanal to enhance shelf-life of mango (Dr. S. Marimuthu) - Defended
3. Shanumugapriya, R. 2016. Development of Electrospun fibre matrix to extend shelf-life of fruits (Dr. K.S. Subramanian) - Defended
4. Jincy, M. 2016. Effects of hexanal application on oxidants and anti-oxidants levels in mango leaves and fruits (Dr. M. Djanaguiraman) - Defended
5. Ajith, T. 2016. Enhanced shelf-life of mango through hexanal dipping (Dr. T.N. Balamohan) - Defended
6. Kanmani, V. 2016. Effect of hexanal on extending shelf-life of banana (Dr. I. Muthuvel) – Defended
7. Chitra, R. (2017) Post-Harvest Fruit Quality and Volatile Organic Compounds in banana (Chairman: Dr. S. Haripriya) - Defended.
8. Nandhini, P. (2017) EFF to extend Shelf-life of Guava (Psidium guajava L.). (Chairman: Dr. T. N. Balamohan) - Defended.
9. Tamizheezham, N. (2017) Hexanal Treatment for Enhancing the Shelf-life of Grapes (Vitis vinifera L.). (Chairman: Dr. I. Muthuvel) Defended..
10. Jayanthi, R. (2017) MOF as a Delivery System for Hexanal to Enhance the Shelf- life of fruits (Chairman: Dr. S. Marimuthu) - Submitted
11. Preetha, S. (2017) Nano-matrix to Extend the Shelf-life of Fruits. (Chairman: Dr. M. Kannan) Submitted.
12. Katrina Ammon Aguillera (2017) A Value Chain Analysis of Selected Fruits in Trinidad and Tobago: Mango, Papaya and Banana (Supervisor - Dr. Sharon Hutchinson)- Submitted and graduated
13. Jash. A. 2017. Triggered Release of hexanal and benzaldehyde from their precursors encapsulated in poly(lactic acid) and ethylcellulose carriers (Dr. Loong Tak Lim)
14. Robert John Ouko (2017) Efficacy of Hexanal as a Post-harvest Preservative of Papaya Fruit. (Supervisors: M.J.Hutchinson, Jane Ambuko and Willis Owino.Robert.)
15. Moses Peter Subert (2017) Perceptions of Enhanced Freshness Formulation technologies and adoption decisions among smallholder banana farmers in Morogoro, Tanzania (Prof. Freddy Kilima)
16. Shi. 2018. Activated release of salicylaldehyde and hexanal by encapsulating imidazolidine precursor in ethyl cellulose-poly(ethylene oxide) electrospun fibers and its application on delaying the ripening of fruits (expect to defend MSc. thesis in Fall 2018)
17. S. Krishna Kumar (2018) Enhancement of post-harvest shelf life of nectarines (*Prunus persica* (L.) Batsch var. *nectarina*) using hexanal (M.Sc. Spring 2018)

PhD Thesis:

1. Mohan, C. 2017. Biosafety of hexanal formulation at different trophic levels (Dr. R. Sridharan) Submitted.

Student Awards won by grad students of the IDRC global project # 107847 “Enhanced Preservation of Fruits Using Nanotechnology”

Following are the major prestigious and national awards won by graduate students of this global project. Apart from this there were many other local awards/prize won by our students.

1. Three-Minute Thesis (3MT) Competition: Mr. Shanthanu Krishnakumar, Master’s student in UG won first place in Canada. He made history by winning first place in both people choice and Judges Choice.
2. Ms. Shanmugapriya, grad student from TNAU, got first place for her poster presentation in the International Society for Horticultural Sciences (ISHS), conference, Kandy, Sri Lanka.
3. Ms. Peninah M. Yumbya from UoN submitted a winning proposal to the Kenya National Council for Science and Technology and was awarded about Kshs. 2 million to support her future work. Also won The African Bioscience Challenge Fund (ABCF) fellowship.
4. Mr. John Robert Ouko's from UoN was awarded the 3rd best poster in Africa 1st ALL Africa Post harvest congress and exhibition, Safari park hotel, Nairobi, 28th -31st March, 2017
5. Three grad students and three research associates from TNAU won different placements for their posters and presentations at the national conference on Nanotechnology for evergreen revolution.
6. Other awards/scholarships won by grad students:

No	Student	Award/Scholarship
1	Mr. Shanthanu Krishnakumar	Mrs. Fred Ball Graduate Scholarship Vineland Centennial Horticultural Scholarship OAC '60 Leadership Development Scholarship Invited to represent International 3MT in USA
2	Ms. Erika DeBrouwer	Margaret and Angus Hamilton Apple Research Scholarship
3	Ms. Karthika Srikandharajah	Arrell Food institute Scholarship
4	Mr. Robert Brandt	Keith R Collver award Mrs. Fred Ball Scholarship

Research Output from IDRC Project # 107847 "Enhanced Preservation of Fruits Using Nanotechnology"

- **Published:**
  Please find the following articles in the link and many have their own DOI links
  https://www.dropbox.com/sh/vf32fpu8x3dbmfy/AACuvB0oOKDWDeYxpCyS4aXta?dl=0

1. Aashwini T., Ganapathy, S. and Subramanian, K.S. (2018) Impact of hexanal vapour treatment on the shelf-life and post-harvest quality of banana var. Poovan. Green Farming 9 (2) : 340-344.
2. Mohan, C., Sridharan, S., Subramanian, K.S., Natarajan, N. and S Nakkeeran (2017) Effect of Nanoemulsion of Hexanal on Honey bees (Hymenoptera; Apidae) Journal of Entomology and Zoology Studies 2017; 5(3): 1415-1418.
3. C Mohan, S Sridharan, K Gunasekaran, KS Subramanian and N Natarajan (2017) Biosafety of hexanal as nanoemulsion on egg parasitoid *Trichogramma* Spp. Journal of Entomology and Zoology Studies 2017; 5(2): 1541-1544.
4. Jincy, M., Djanaguiraman, M., Jeyakumar, P., Subramanian, K.S., Jayasankar, S. and Paliyath. G. 2017. Inhibition of phospholipase D enzyme activity through hexanal leads to delayed mango (*Mangifera indica* L.) fruit ripening through changes in oxidants and antioxidant enzymes activity. Scientia Horticulturae 218: 316–325.
5. Anusuya, P., Nagaraj, R., Janavi, G.J., Subramaniana, K.S*. Paliyath, G. and Subramanian, J. 2016. Pre-harvest sprays of hexanal formulation for extending retention and shelf life of mango fruits fruits. Scientia Horticulturae, 211: 231–240.
6. Parthasarathy S., Thiribhuvanamala G., Subramanian, K.S. and Kuppusamy, K. (2016) Bacterial antagonists and hexanal-induced systemic resistance of mango fruits against *Lasiodiplodia theobromae* causing stem-end rot. Journal of Plant Interactions 11:1, 158-166
7. Gopinathan, P, Subramanian, K.S., Paliyath and Subramanian, J. (2017) Genotypic variations in characteristics of nano-fibrillated cellulose (NFC)
8. derived from banana pseudostem. Bioresource
9. Subramanian, K.S. 2014. Nano Food Packaging to Enhance Shelf-life of Perishables. Food Marketing & Technology, January 18-20
10. Subramanian, K.S., Sekar, C, Suranee Meneka, S and Vijaya Prakash, L2015. Reducing Post Harvest Losses in Mango in South Asia. IDRC Bulletin Pp. 4
11. Karthika, S., Nanda Kumar, NB, Gunasekaran, K. and Subramanian , K.S. 2015. Biosafety of Nanoemulsion of Hexanal to Honey Bees and Natural Enemies . Indian J. Sci. Tech. Vol 8(30), DOI: 10.17485/ijst/2015/v8i31/52668, November 2015
12. Sekar, C., Subramanian, K.S., Subramanian, J and Vijaya Prakash. 2014. Gender Dynamics In Mango Production System In India. Innovare Journal of Social Sciences, 2(4):74-80.
13. Geetha, V and Thirupathi, V. 2015. Effect of Post Harvest Application of Hexanal Vapour on the Quality Attributes of Mango Var. Banganapalli. Trends in Biosciences, 8(2): 464-467.
14. Geetha, V and Thirupathi, V. 2015. Effect of Post Harvest Application of Hexanal Vapour on the Quality Attributes of Mango var. Bangalora. Trends in Biosciences, 8(2): 468-471.
15. Preethi, P., Soorianathasundaram, K and Subramanian, K. 2014. Ultrastructural studies of mango fruit ripening cv.bangalora. Biochemical and Cellular Archieves, 14(2): 1-3.
16. Preethi, P., Soorianathasundaram, K and Subramanian, K. 2014. aroma volatile compounds of mango cultivars neelum and banganapalli. Biochemical and Cellular Archieves, 14(2):5-8.
17. Anusha, B., Prabakar, K. and Thiribhuvanamala, G. 2014. Chemodetection of Latent Infection of Colletotrichum gloeosporioides (Penz.and Sacc.) in Unripe Mango Fruits. Trends in Biosciences, 7(17): 2482-2485.

18. Anusha, B and Prabakar, K. 2014. Purification of mycelial proteins of Colletotrichum gloeosporioides and Lasiodiplodia theobromae of Mango for production of Polycolonal Antibodies.Trends Biosciences, 7(19): 2922-2925
19. Milani, M.D.Y., Samarawickrama, D.S., Dharmasiri**,** G.P.C.A. and Kottegoda, I.R.M. (2016). Study the structure, morphology and thermal behavior of banana fiber and its charcoal derivative from selected banana varieties. *Journal of Natural Fibers.*13:3, 332-342 DOI: 10.1080/15440478.2015.1029195
20. Hewajulige, I.G.N., Wilson Wijeratnam, R.S., Perera, M.G.D.S. and Fernando, S.A. (2015). Extending storage life of commercially important tropical fruits using bio-waxes. *Acta Hortic.* 1091, 283-289 ***DOI: 10.17660/ActaHortic.2015.1091.35***
21. Hewajulige, I.G.N., Wilson Wijeratnam, R.S., Gunethilaka, R.M.S.I., Gunesekara, M.M.N.P., Perera, M.G.D.S., Paliyath, G. and Jayasankar, S. (2018). Pre-harvest 'Tree Fresh' spray treatment reduces disease incidence and extends the harvesting season of 'TJC' mango grown in Sri Lanka. Acta Hortic. 1201, 49-54 https://doi.org/10.17660/ActaHortic.2018.1201.8
22. El Kayal, W., Paliyath, G., Sullivan, J.A., Subramanian, J.(2017) Phospholipase D inhibition by hexanal is associated with calcium signal transduction events in raspberry. Horticultural Research, https://www.nature.com/articles/hortres201742
23. El Kayal, W., El-Sharkawy, I., Dowling-Osborne, C., Paliyath, G., Sullivan, J.A., Subramanian, J., 2017. Effect of hexanal in enhancing shelf life and regulation of membrane associated genes in strawberry. Canadian Journal of Plant Science. http://dx.doi.org/10.1139/CJPS-2016-0351.
24. Jash, A., and L-T. Lim. 2018. Triggered release of hexanal from an imidazolidine precursor encapsulated in poly(lactic acid) and ethylcellulose carriers. J Mater. Sci. 53:2221-2235
25. Jash, A., Paliyath, G., and L-T. Lim. 2017. Simultaneous Release of Antimicrobial Aldehydes from Precursors Embedded in Nonwoven Poly(lactic acid) (PLA) Carriers. Applied Materials Today (re-submitted in Mar 2018).
26. Krishna Kumar, S., El Kayal, W., Sullivan, J. A., Paliyath, G., Subramanian, J. (2018) Pre-harvest application of hexanal formulation enhances shelf life and quality of 'Fantasia' nectarines by regulating membrane and cell wall catabolism-associated genes. Scientia Horticulturae 229:117-124. http://dx.doi.org/10.1016/j.scienta.2017.10.031
27. L-T. Lim. 2018. Gases and Vapors Used in Food Preservation. In Encyclopedia of Food Chemistry. Peter Varelis, Laurence Melton and Fereidoon Shahidi (ed.). Accepted on 23 Feb 2018.
28. MacKenzie, J.O., Elford, E.M.A., Subramanian, J., Brandt, R.W., Stone, K.E., Sullivan, J.A. (2018) Performance of five haskap (Lonicera caerulea L.) cultivars and the effect of hexanal on postharvest quality. Canadian Journal of Plant Science (http://www.bioone.org/doi/abs/10.1139/cjps-2017-0365)
29. Shi, C., and L-T. Lim. 2018. Controlled release of hexanal and salicyladehyde imidazolidine precursors from ehylcellulose nonwoven carriers produced from free surface electrospinning. In preparation (to be submitted to J Appl. Polym Sci.)

- **Books & Manuals**

1. Gunasekaran, K., Karthika, S., Nandakumar., N.B., Subaramanian K.S., Gopinathan Paliyath and Jayasankar Subaramanian. 2015. Biosafety of Hexanal. Pp:89.
2. Chinnamuthu, C.R., Natarajan, N., Subaramanian K.S and Lakshmanan, A. 2012. Transmission Electron Microscope Basics and Operation. Pp.74
3. Natarajan, N., Gunasekaran, K. and Subramanian, K.S. Scanning Electron Microscope Basics and Operation. Pp.74
4. Soorianathasundaram, K., Subbiah, A., Janavi, G.J., Thiribhuvanamala, G., Subaramanian, J and Vinoth, R. 2015. A technology on Mango Cultivation. (Tamil) Pp.44
5. Soorinathasundaram, K., Subramanian, K.S., Jannavi, G.J. and Vinoth, S. 2014. Mango production and Post Harvest Management, TNAU offset Press, Coimbatore-3. Pp. 333

- **Conference Presentations & Guest Lectures / Lead Talks**

1. Subramanian, K.S., Janavi, G.J and Subramanian, J. Lead paper on Nanotechnology for Reducing Post-harvest Losses in Perishables. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp. 54 -55.
2. Subramanian, K.S. 2017. Nanotechnologies to minimize post-harvest losses in fruits. International Conference on Nano For Agri 2017, TERI, New Delhi, November 13-15, 2017.
3. Subramanian, K.S. 2017. Nanotechnology for Evergreen Revolution. National Seminar on Nanotechnology for Evergreen Revolution. TNAU, Coimbatore, October 5-6, 2017.
4. Rajkishore, S.K and Subramanian, K.S. 2017. Lead paper on Nanotechnology Regulatory Frameworkfor Agriculture. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp. 61 -62.
5. Yoganathan.G and K.S.Subramanian 2017. Abstract on Stability and Characterization of Hexanal Nano Emulsion. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.93.
6. Badal K. Biswal and K. S. Subramanian 2017. Abstract on Controlled release of Hexanal by using Electrospun Fibre Matrix and Post-harvest Ripening Studies of Mango. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.94.
7. Jayanthi R, Marimuthu S, Viji N and Subramanian K S 2017. Abstract on β-Cyclodextrin based Metal Organic Framework: As a Carrier Vehicle for Hexanal Delivery. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.95.
8. PaladuguKrishnadev, K.Gunasekaran, G.J.Janavi, K.S.Subramanian and K.Soorianathasundaram 2017. Abstract on Nano Emulsion to Extend the Shelf-life of Tomato (Solanumlycopersicum L.) Fruits. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp. 96.
9. Ponni. P, Pragalyaa Shree. M.M, Subramanian. K. S, Janavi, G.J. 2017. Abstract on Nano film Developed from Nano-fibrillatedCellulose of Banana pseudostem (Musa sp.) to Extend the Shelf-life of Fruits. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.97.
10. P.Anusuya, G.J. Janavi , K.S.Subramanaian, GopinathanPaliyath, Jayasankar Subramanian 2017. Abstract on Effect of Pre-harvest Sprays of Hexanal Formulation on Alphonso Mango (*Mangifera indica* L.). 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.98-99.
11. Shanmugapriya, R., Mohanraj, J., Subramanian, K.S. 2017. Abstract on Nano Fibre Matrices for Fruit Preservation. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. Pp 100-101.
12. Sangeetha.V, P.Ponni, S.SriVignesh, K.S.Subramanian, G.J.Janavi 2017. Abstract Enhanced Freshness Formulation (EFF) Dip Technology to Extend Shelf-life of Mango. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.102-103.
13. M. Jincy, M. Djanaguiraman1, P. Jeyakumar and K.S. Subramanian 2017. abstract on Effect of Post- harvest Treatment of Hexanal on Ripening of Mangoes by Inhibition of PLD Enzyme Activity. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp 104.
14. Pushpalatha, M, C. Sekar, K.S.Subramanian 2017. Abstract on Post- harvest Losses of Mango in Value Chain in Indian Context. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution.

Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.105-106.

15. Sivapriya, M, C. Sekar, Vijaya Prakash and K.S. Subramanian 2017. Abstract on Impact of Mango Value Addition Training on Women Empowerment. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.107-108.
16. Priyadharshni, R, C. Sekar, Vijaya Prakash and K.S.Subramanian 2017. Abstract on Acceptance Behaviour of EFF Technology Among Mango Farmers of Tamil Nadu. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.109-110.
17. Kanmani.V, Muthuvel, I., Subramanian, K.S. G.J. Janavi 2017. Abstract on Effect of Hexanal to Extend the Shelf-life of Banana (Musa sp.). 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.111-112.
18. Mohanraj, J., Balasupramani, P., Subramanian, K.S., Janavi, G.J 2017. Effect of Hexanal abstract on Packaged Alphonso Mango Fruits. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.117-118
19. Chithra, R., NilanDharun, V., and HaripriyaShanmugam 2017. Abstract on Nanoencapsulation and Characterization of Bioactive Polyphenols from Fruit Peel of Banana cv. Red Banana (Musa accuminata). 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp. 119-120
20. Preethi,P, K.Soorianathasundaram, A. Sadasakthi, K.S.Subramanian G. Paliyath and J. Subramanian 2017. Abstract on Changes in Enzymatic Activity Pattern of Mango Fruits During Storage Influenced by Hexanal Formulation. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp. 121-122
21. Preetha, S., Kannan, M., Subramanian, K.S. 2017. Abstract on Multilayer Electrospun Matrices for Controlled Release of Hexanal to Extend the Shelf-life of Fruits. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.124
22. Srivignesh, S., Durgadevi, D., ArumukaPravin, I., Janavi, G.J. and Subramanian, K.S. 2017. Abstract on Metagenomic profiling of Enhanced Freshness Formulation (EFF) Treated Fruits of Mango var. Alphonso. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.127-128
23. C. Mohan., S. Sridharan., K. S. Subramanian., N.Natarajan and S.Nakkeran 2017. Abstract on Impact of Nano Emulsion of Hexanal on Fruit fly Bactrocera dorsalis in Mango. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp. 135-136
24. Karthika, S and K. Gunasekaran 2017. Abstract on Biosafety of Hexanal Formulations to Earthworms and Fishes. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp.139
25. Prasangi. Sathi Raju and K.S.Subramanian. 2017. Abstract on Nano-composite for Multiple NutrientDelivery in Maize. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp. 145 -146.
26. Balakrishnan N, S. Marimuthu and K.S. Subramanian. 2017. Abstract on Synthesis of Bionano Particles from Banana Fibres. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp. 147 -148

27. Durgadevi. D, Arumukapravin. I, Srivignesh. S, Subramanian. K.S, Janavi. G.J, GopinathanPaliyath, Jayasankar Subramanian. 2017. Abstract on HexanalVapour to Reduce Major Post-harvestPathogens of Banana (*Musa* sp.). 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp. 194 -195
28. ArumukaPravin. I, D. Durgadevi, S. SrivigneshK. S. Subramanian and A.S. Krishnamoorthy. 2017. Abstract on HexanalVapour Treatment for the Reduction ofPost-harvest Diseases of Mango. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp. 198-199
29. Amsa, T, S.Sithanantham and K.S.Subramanian. 2017. Abstract on Pilot Field Study on Effect of SprayingNano-emulsion of Hexanal on ParapheromoneTrap Catch of Mango Fruit Flies. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp. 205
30. R.Parthasarathy, B. Anusha, K. Sathya, G. ThiribhuvanamalaK.S. Subramanian and K. Prabakar. 2017. Abstract on Efficacy of Hexanal and Bacterial Antagonists for theManagement of Mango Anthracnose in Tamil Nadu. 2017. Proceedings of National seminar on Nanotechnology for evergreen revolution. Published by Department of Nano Science and Technology, Directorate of Natural Resource Management, TNAU, Coimbatore. pp. 208-209
31. Subramanian, K.S. 2015"Nanotechnology in Agriculture" at the National Seminar on "New Frontiers in Agricultural Biotechnology" during March 3-4, 2015, Kerala Agricultural University, Thrissur, March 3-4, 2015
32. Subramanian, K.S. 2016. Nanotechnology for fruit preservation. Lead talk at the 7th Horticulture Congress, IARI, New Delhi, Nov. 15-18, 2016
33. Hewajulige, I.G.N., Wilson Wijeratnam, R.S., Perera, M.G.D.S. and Fernando, S.A. (2015). Extending storage life of commercially important tropical fruits using bio-waxes. Proceedings of the International Conference on 'Managing Quality in Chains' 2-5 September 2013, Cranfield, UK.
34. Hewajulige, I. G. N., Wilson Wijeratnam, R. S., Gunathilaka, R. M. S. I., Gunasekara, M. M. N. P., Perera, M. G. D. S., Paliyath, G. and Jayasankar, S. (2017) Pre-harvest "tree fresh" spray treatment reduces disease incidence and extends the harvesting season of mango "TJC" grown in Sri Lanka. Proceedings of the VII ISHS International Conference on Managing Quality in Chains (MQUIC), Stellenbosch, South Africa. 4th -7th September 2017. pp 26.
35. Wilson Wijeratnam, R. S., Fernando, K. and Hewajulige, I. G. N. (2017) Models adopted for commercializing new postharvest technology for minimizing post harvest loss of fruits in Sri Lanka. Proceedings of the VII ISHS International Conference on Managing Quality in Chains (MQUIC). Stellenbosch, South Africa. 4th -7th September 2017. pp56.
36. Wilson Wijeratnam, R.S., Milani, M.D.Y., Samarawickrama, D.S. and Hewajulige, I.G.N. (2017) Production and commercialization of eco friendly packaging material for transportation of fruit and vegetables. Proceedings of the IV International Conference on Postharvest and Quality Management of Horticultural Products of Interest for Tropical Regions, Kandy, Sri Lanka. pp 2.
37. Gunasekara, M.M.N.P., Gunathilaka, R.M.S., Wijeratnam, R.S.W., Perera, M.G.D.S., Sarangi, K.A.N. and Hewajulige, I.G.N. (2016). Effect of new edible wax formulations on postharvest quality of mango (*Mangifera indica*) var. Karthakolomban. Proceedings of the Wayamba University International Conference, Sri Lanka, pp158.
38. Kemali, R.W., Hewajulige, I.G.N. and Rathnayake, R.H.M.K. (2015). Effect of Enhanced Freshness Formula (EFF) on control of stem end rot disease caused by *Bortrydiplodia theobromae* in Papaya (*Carica papaya*). Proceedings of the 14th Agricultural Research Symposium, Wayamba University of Sri Lanka, pp15-19.
39. Pavithra, K.G.N., Hewajulige, I.G.N. and Ratnayake, R.H.M.K. (2015). Effect of Enhanced Freshness Formula (EFF) on control of stem end rot disease caused by Colletotrichum gloeosporioides in Papaya (*Carcia papaya*). Proceedings of the 14th Agricultural Research Symposium, Wayamba University of Sri Lanka, pp 25-29.
40. Milani, M.D.Y., Samarawickrama, D.S., Dharmasiri**,** G.P.C.A. and Kottegoda, I.R.M. (2015). Characterization of banana fiber extracted from common banana varieties in Sri Lanka. Proceedings of the 2nd Biennial Research Symposium 2015, Industrial Technology Institute, Sri Lanka. pp 45.

41. Wilson Wijeratnam, R.S., Gunasekara, M.M.N.P., Gunathilaka, R.M.S., Perera, M.G.D.S. and Hewajulige, I.G.N. (2015). Improving quality of mango (*Mangifera indica*) var. Karuthakolumban by postharvest application of new edible wax formulations. Proceedings of the 2nd Biennial Research Symposium 2015, Industrial Technology Institute, Sri Lanka. pp 12.

42. Samarawikrama, D.S., Weerathunga, H.D., Gunasekara, M.M.N.P., Milani, M.D.Y., Hewajulige, I.G.N., Wilson Wijaratnam, R.S. and Dissanayaka, D.P. (2017). Headspace characterization of Hexanal incorporated composite material (HICM) for postharvest application of mango fruits. Proceedings of the 3rd Biennial Research Symposium 2017, Industrial Technology Institute, Sri Lanka. pp 58.
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44. Champa, W.A.H., Gunasekera, M.M.N.P., Wilson Wijeratnam, R.S., Hewajulige, I.G.N., Weerasinghe, W.M.S.S.K. and Kumara, B.A.M.S. (2017). Biowax coating improves quality and extends storage life of lime fruits (Citrus aurantifolia L.). 03rd Annual Research Session of the IFSTSL, 05th August 2017, Mihilaka Medura, BMICH, Colombo, Sri Lanka. (in press)
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Papers accepted and their order for *Tropical Agriculture* Special Issue

1. **Post-harvest dip of enhanced freshness formulation to extend the shelf life of banana (*Musa acuminata* cv. Grand Naine) in India**
   Kanmani Venkatachalam[1], Iyamperumal Muthuvel[1], Srivignesh Sundaresan[2], Kizhaeral S. Subramanian[2]*, Janavi Gnanaguru Janaki[2], J. Alan Sullivan[3], Gopinathan Paliyath[3], Jayasankar Subramanian[4]

2. **Efficacy of Hexanal Application on the Post-Harvest Shelf life and Quality of Banana Fruits (*Musa* spp.) in Kenya**
   P. M. Yumbya,[1]* M. J. Hutchinson,[1] J. Ambuko,[1] W. O. Owino,[2] J. Alan Sullivan[3], G. Paliyath[3] and J. Subramanian[4]

3. **Effects of pre- and post-harvest treatments with hexanal formulations on time to ripening and shelf life of papaya (*Carica papaya* L.) fruits**
   Nirmalla Debysingh, Lynda Wickham [a,] Majeed Mohammed [a], George Legall [a], Gopinadhan Paliyath [b], Jayasankar Subramanian [c]

4. **Effects of hexanal dip on the post-harvest shelf life and quality of papaya (*Carica papaya* L.) fruit**
   M. J. Hutchinson[1], J. R. Ouko[1], J. Ambuko[1], W. Owino[2], and J. Subramanian[3]

5. **Effects of smoke, hexanal, and calcium chloride on post-harvest quality of oranges** *(Citrus sinensis*) cvs ***Msasa*** **and *Jaffa* under different storage durations and conditions in Tanzania**
   Anna Baltazari, [1, 3*] Hosea Dunstan Mtui, [1] Maulid Walad Mwatawala, [1] Lucy Mlipano Chove, [2] J Alan Sullivan[4], Gopinadhan Paliyath[4] and Jayasankar Subramanian[4]

6. **The effects of pre-harvest application of hexanal formulations on time to ripening and senescence and fruit retention time in limequat (*Citrofortunella floridana* J.W.Ingram & H.E.Moore)**
   Nirmalla Debysingh, Lynda Wickham [a,] Majeed Mohammed [a], George Legall [a], Gopinadhan Paliyath [b], Jayasankar Subramanian [c].

7. **The effects of pre-harvest treatments with hexanal formulation on selected postharvest quality parameters of limequat (*Citrofortunella floridana* J.W.Ingram & H.E.Moore) fruits**
   Nirmalla Debysingh, Lynda Wickham [a,] Majeed Mohammed [a], Stephan Moonsammy[b] , Gopinadhan Paliyath [c], Jayasankar Subramanian [d]

8. **Extending storage life of mango (*Mangifera indica* L.) using a new edible wax formulation incorporated with hexanal and cinnamon bark oil**
   M. M. N. P. Gunesekara[1]*, R. S. Wilson Wijeratnam[1], M. G. D. S. Perera[1], I. G. N. Hewajulige[1], R. M. S. Gunathilaka[1], G Paliyath[2], J. Subramanian[2]

9. **The effects of hexanal incorporated composite material (HICM) made of banana fibre and polymers on extending the storage life of mango fruit (*Mangifera indica* L. var TEJC) in Sri Lanka**
   *D. S. Samarawickrama[1], M. D. Y. Milani[1], P. S. D. Perera[1], H. D. Weeratunge[1],
   R. S. Wilson Wijeratnam[1], D. P. Dissanayake[2], I. G. N. Hewajulige[1], L-T. Lim[3],
   G. Paliyath[4], and J. Subramanian[4]

10. **Women's prospects to adopt enhanced freshness formulation (EFF) technologies for banana in Morogoro rural district, Tanzania**
    Moses P. Subert[1], Fredy T. M. Kilima[1*], Maulid W. Mwatawala[2], Theodosy Msogoya[2], and Hosea Mtui[2]

11. **Factors influencing gendered intra-household allocation of land and capital assets in banana (*Musa* spp.) production: the case of Meru County, Kenya**
    Violet Nyabaro,[a] John Mburu,[a]* and Margaret Hutchinson[b]

12. **A Study on Gender Participation in Post-production Operations of Selected Fruits in Trinidad and Tobago**
    Katrina Ammon-Aguillera[1], Lynda D. Wickham[2*] and Stephan Moonsammy[1]

Commercialization Progress

MOU signed between TNAU and Smart Harvest Agri, Canada

to Promote Technologies to Reduce Post-Harvest Losses in Perishables

India loses about **Rs. 2, 40,000 Crores** from the post-harvest losses in perishables annually due to lack of improper harvesting, handling and transport of fruits besides inadequate cold storage facilities. In order to address the global challenge of post-harvest losses, the Global Affairs Canada (GAC) and International Development Research Center (IDRC) jointly funded project on "*Enhanced Preservation of Fruits using Nanotechnology*" to TNAU for the past five years (2012-2018) with a financial outlay of **Rs. 11.4 crores**. The Tamil Nadu Agricultural University, Coimbatore, collaboratively worked with University of Guelph, Canada, and adopted their technologies such as pre-harvest spray or post harvest dip of hexanal formulation, hexanal vapour and developed nano-stickers, nano-pellets and nano-film developed from banana pseudostem. Hexanal is a biomolecule derived from plants possessing the capability of inhibiting phospholipase D enzyme and slowing down ethylene thereby shelf-life of fruits and vegetables get extended besides minimizing post-harvest losses. All these technologies singly or in combinations can reduce the post-harvest losses to the tune of 10-15% which is an economic boon to the country.

Ms. Jennifer Daubeny, Consulate General of Canada, delivered the special address narrating the significance of Canadian funding in developing nanotechnologies to reduce post-harvest losses that enables food security in Asian Countries. **Dr. K. Ramasamy**, Vice Chancellor, Tamil Nadu Agricultural University, Coimbatore presided over the function and highlighted the role of TNAU in knitting nanotechnology research framework and serving as a torch bearer in the country. He emphasized that the GAC-IDRC Project helped more than 60 students and researchers, developed two technologies, filed patents for two inventions, extensive infrastructure development besides helping more than 12,000 fruit growers in the State of Tamil Nadu. **Dr. Jayasankar Subramanian**, Professor, University of Guelph, Canada, explained the evolution of the project till reached the stage of technology delivery to benefit farmers. **Dr. K.S. Subramanian**, NABARD Chair Professor, TNAU, Coimbatore, lead Principal Investigator of the Project for India presented nanotechnologies developed to assist in the entire value chain from the farm to fork. **Mr. Arun Nagarajan**, President, Tamil Nadu Fruit Growers' Association, explained that the fruit growers are eager to use the technology to improve their farm income. **Mr. Terence Park**, Managing Director, Smart Harvest Agri, Canada, bestowed interest to take forward the technologies to the farm gate and signed MOU with TNAU for the Commercialization of the Hexanal Formulation. **Dr. G.J. Janavi,** Professor & Head, Department of Nano Science & Technology, TNAU, Coimbatore welcomed the gathering and **Dr. C. Sekar**, Dean, Imayam Agricultural College, Turaiyur, and Co-PI of the Project proposed a formal vote of thanks.

The Canadian Consul General **Ms. Jennifer Daubeny** visited all the exhibits and interacted with students, scholars and researchers besides the NGO partner Myrada. She was very impressed with the technologies developed by TNAU in collaboration with University of Guelph, Canada, and looking forward to support research programs in the near future. More than 200 Scientists and Diplomats from Canada, students, scholars, university officials participated in the event.

Products launch by ITI, Colombo

Two of the project's technology outputs - hexanal incorporated ITI Bio-wax and the Tree Fresh Formulation spray were transferred to Hayleys Agriculture Pvt. Ltd., a reputed Agro Service provider in Sri Lanka. The products were launched on 22nd March 2018 at the Taj Samudra Hotel, Colombo. The chief guest at the event was the Hon. Susil Premajayantha, Minister of Science Technology and Research (Min. ST&R). The guest of honour was H.E. David McKinnon, High Commissioner for Canada in Sri Lanka. Others present included the Secretary to the Min. ST&R, The Chairman and Director General, ITI, Mr Rizvi Zaheed, Hayleys Agriculture and his team, the Chairman, National Science Foundation, Sri Lanka, representative of the Chairman Sri Lanka Export Development Board, representatives from the Dialog mobile service provider, the Registrar of Pesticides, representing the Dir. Gen., of Agriculture, President of the Lanka Fruit and Vegetable Producers, Processors and Exporters Association, leading large scale mango, papaya and pineapple growers, several export and fruit processing company representatives, senior officials from the ITI, the multi-disciplinary ITI research team and our partner from CEPA. The press was also well represented and a total of 100 persons were present on this occasion. The Managing Director Hayles, the two PIs' of the project, the High Commissioner for Canada, The Minister and for ST&R and the Secretary to the Ministry addressed the gathering and the new video clip on the project was viewed. The new products were jointly uncovered for display by the Hon. Minister and H.E., the High Commissioner. Samples of the products were distributed to the President of the Lanka Fruit and Vegetable Producers Processors and Exporters Association and to two leading mango growers. The Project team also took this opportunity to run a presentation on the various stages of the project and related activities, display posters on their research findings and to print and distribute the pamphlets on the same as well as on hexanal, the latter as prepared by our partners from the University of Guelph. The launch ended with a time of fellowship providing a useful opportunity for networking.

https://youtu.be/E7Azo1McMeU -

Book on "Postharvest Biology and Nanotechnology of Fruits, Vegetables and Flowers" edited by Gopinadhan Paliyath, Jayasankar Subramanian, Loong-Tak Lim, Avtar Handa, Autar Mattoo and K.S. Subramanian by Wiley- Blackwell Publishers

Chapter Title
Enhancing Food Security Through Postharvest Technology- Current and Future Perspectives
Ripening and Senescence of Fleshy Fruits
Ethylene signal transduction during fruit ripening and senescence
Preharvest and Postharvest technologies based on Hexanal - An Overview
Nitric Oxide Signaling in Plants
Postharvest uses of ozone application in fresh horticultural produce
Active and Intelligent Packaging for Reducing Postharvest Losses of Fruits and Vegetables
Application of hexanal-containing compositions and its effect on shelf life and quality of banana varieties in Kenya**.**
Hexanal compositions for enhancing shelf life and quality in papaya
Effect of Hexanal composition treatment on wine grape quality
Benefits of application of hexanal compositions on apples
Preharvest spray application of blueberry fruits with hexanal formulations improve fruit shelf life and quality
Improving shelf life and quality of sweet cherry (*Prunus aviuum.* L*)* by preharvest application of hexanal compositions
Hexanal effects on greenhouse vegetables
Reduction of pre- and postharvest losses of sweet orange (*Citrus sinensis* L. (Osberck) using hexanal in Eastern Tanzania
Post-harvest technologies in Tender Fruits – Peach, Nectarine, Plum, and Apricot
Effect of Hexanal Compositions on Guava Fruits
Effect of hexanal vapour treatments on delay of flower senescence
Applications of Nano- and Microstructured Materials in Postharvest Packaging of Fresh Fruits and Vegetables
Economic Impact of Hexanal-based nanotechnology on Mango value chain in Tamil Nadu State, India
Cyclodextrin Inclusion Complex as a Smart Delivery of Volatiles in Nano-Food Systems

IDRC Project # 107847 (Enhanced Preservation of Fruits Using Nanotechnology)
Dissemination at Project sites.

No	Site/team	Date	Event	# Farmers & others attended	Talks Presented/Handouts given	Comments/feedback by farmers and policy makers
1	Canada/UG	24th November 2017	Project Dissemination in Niagara Peninsula, ON	Farmers, students, stake holders, policy makers 50	Three talks with project outcomes Ten things about Hexanal pamphlet	Suggestions given to fast track the commercialization of the product in US and Canada Positive feedback by apple grower Significant progress in regulatory clearance after the meeting and the license Smart Harvest is taking care of that.
2	Kenya/UoN	5th- 9th February 2018	Project Dissemination Workshop, Meru and Machakos county	Farmers, students, stake holders, policy makers 500	Briefing to Agricultural CEC Interactive workshop involving farmers, stakeholders from KALRO, representatives from KEPHIS Team met with Governor of Meru County, Hon. Kiraitu Murungi and the CEC Agriculture in Meru County	Agricultural County Executive Chief (CEC) of Machakos county promised the technology fully registered and made available the farmers. Government support to adopt and scaling up the hexanal technologies were discussed Availability was addressed by Mr. Erick, from KEPHIS which is the government mandated body responsible for authorization of new compounds and technologies. He assured the farmers that they were fully aware of the technology from its inception, and have monitored and verified that the technology is safe to use. He stated that they currently fulfilling all the government requirement to see Hexanal readily available in the local agrovets ASAP. He encouraged the farmers to consider safer technologies like the use of Hexanal to facilitate smooth trade of their produce both locally and internationally. Farmer narrated with much confidence and joy how the use of Hexanal for his papaya farm saved him from the exploitation of middlemen and resulted into a significant increment of his Income. The passionate support and corporation of farmers was recognized with a certificate of appreciation
3	Sri Lanka/ITI	21 December 2017 8th February 2018	Awareness Program@ Rajarata Ellawala Farms, Galkiriyagama, Dambulla Awareness Program@Jaffna, Nirveli Banana Producers and processors Association.	500 small holder farmers 45	EFF and Hexanal incorporated Bio wax were discussed to farmers. Pamphlets on EFF, Biowax and HICM were given Members of the association were briefed on the purpose of producing the banana fibre based fruit wrap.	Participants were informed that the product would be launched in the near future and would be marketed and distributed by Hayleys Agriculture (Pvt) Ltd. The possibilities for generating income and employment Opportunities for women in this banana producing area was discussed and the need for cost effective production was explained.

4	Trinidad and Tobago/UWI	March 19-28, 2018	Final Dissemination Seminars in 8 different locations of Trinidad and Tobago at NAMDEVCO (National Agricultural Marketing and Development Corporation) farmer's market meeting Two seminar series in Tobago @ Tobago House of Assembly (THA)	500 Farmers and Stakeholders 177 (Stake holders including farmers, agro-processors, home gardeners, agri-business cooperative members and Extension Officers	Each seminar took the form of presentations of the actual trials and a summary of the findings with respect to papaya and lime. The use of the hexanal as a post-harvest dip was introduced to the packinghouse staff in the Division of Food Production, Forestry and Fisheries. Separate special presentations were made to policy makers.	The farmers were more comfortable on the GRAS certified project and eagerly await the availability of the product in Trinidad and Tobago. The main advantage of the product as expressed by farmers was the extension in fruit retention time in trees.
5	Tanzania/SUA	March 22 – 23, 2018	Final dissemination meetings in Muheza region in Tanga district (Orange) and in Dar-e-salaam (Mango)	60 farmers, students, policy makers	EFF preharvest spray demo on Mangoes- Grad student Mr. Jasper EFF Post Harvest dip demo on Oranges- Grad Student Ms. Anna.	Muheza Meeting: officiated by District Agriculture Irrigation Officer (DAICO) Mr Anthony Senkoro Dar-e – salam meeting: Officiated by Chairperson of the Association of Mango Growers (AMAGRO) Mrs Fatma Riyami. Fast track the registration of hexanal so that it can be commercially available
6	India/TNAU	March 12, 2018	A mega Pack house Workshop to disseminate EFF Dipping technology to extend shelf-life of fruits	400 farmers, stake holders, Department Officials, traders, scientists and students	Two presentations on EFF technology for fruit preservation and nanotechnologies to minimize post-harvest losses besides a live demo on dipping	Mr. Sitarasu, President of Mango Growers Association of Krishnagiri, expressed that the technology is very good for exporters. He will recommend it to additional 1500 farmers. Mr. Santhakumar explained his involvement in the project for the past five years and he finds unique advantage of the technology in fruit preservation while reducing the incidence of post-harvest diseases. Mr. Pugazhlendi, owner of packhouse elucidated how this technology is powerful in minimizing losses of fruits in pack houses where huge quantities of fruits are being assembled and exported to various countries.
		March 15, 2018	Final Dissemination Workshop for the GAC-IDRC Project	200 Farmers, students, scientists besides Ms. Jennifer Daubeny, Consul General of Canada, Dr. Jay Subramanian, Overall PI, Mr. Terence Park, Smart Harvest Agri, Canada	There were three presentations. Dr. Jay Subramanian gave a overall achievements of the project. Dr. K.S. Subramanian narrated the technologies developed, patents filed, publications made and ways forward beyond project period	Mr. Arun Nagarajan, President of Tamil Nadu Fruit Growers Association offered his feedback on the EFF technology. Ms. Jennifer Daubeny, Consul General of Canada, offered her felicitations and expressed her interest to support to take forward the technologies to the next phase. Dr. K. Ramasamy, Vice Chancellor of TNAU and Mr. Terence Park, Smart Harvest Agri, Canada, signed the MOU that enables production and distribution of EFF

The following handouts and videos were shared during the dissemination events

1. EFF Spray ITI (Annex 20)
2. Bio Wax (Annex 21)
3. Hexanal Incorporated Composite Material (HICM) (Annex 22)
4. 10 things about Hexanal: : https://www.uoguelph.ca/research/discover-our-research/publications/10-things-to-know
5. Overall view of the project: https://youtu.be/KOMMXoiT_ZQ
6. 3MT National Winner Video: https://www.youtube.com/watch?v=TyibZdxfBDw
7. Project impact in Trinidad and Tobago: https://uoguelphca-my.sharepoint.com/:v:/g/personal/jsubrama_uoguelph_ca/EePV3VL5iDJDvemHQ-qNkQEB3MzH323xsHAKV_Bu63yS-w?e=P9AoRr
8. Project impact in Sri Lanka: https://drive.google.com/file/d/1lMy245cAuCMTzrJliuTJ9C8THxintJU8/view
9. Project impact in Kenya: https://drive.google.com/file/d/0BzLNoLD6ommbMFNJeEVXMGRDLXc/view
10. Project impact in India: https://www.youtube.com/watch?v=J2P-KpOJYKA

	MILESTONE PROGRESS IN THE ENTIRE PROJECT TERM (DECEMBER 2014- MARCH 2018)		
No	Milestone Description	Progress and Evidence	Comments
1	Inception workshop with key partners and stakeholders held (New Delhi/Coimbatore) and report shared (Guelph/TNAU).	Completed Refer MN 06 joint technical report	Successfully completed in January 2015 @ Bangalore
2	Recruitment of project staff, including research fellows and associates (12) and enrolment of post-graduate students (three each for Masters, MPhil and PhD) completed and essential equipment purchased (UG, SUA, UWI, UoN and ITI).	Completed Refer MN 06 and MN 18 joint technical report	Though some delays in purchasing equipment's but completed on or before MN18 reporting period
3	Development of key project strategies including: (i) M&E framework, (ii) communications strategy, (iii) gender strategy and (iv) scaling-up approach completed (UG /All partners)	Completed Refer MN 06 and MN 18 joint technical report	Strong internal and external communication strategy was developed and followed throughout the project term. Scaling up approach by large scale trials and resulted in a Canadian company investment. Gender strategy reflected in Socio economic models
4	Bio-safety report on hexanal completed and policy brief developed (UG /TNAU)	Completed Refer MN 06 technical report	See the outcome stories (Green Nanotechnology and Biosafety of nano-products) and publications
5	Research protocols for baseline surveys approved by respective ethics boards (All)	Completed Refer MN 06 technical report	UG, UoN and TNAU got their REB clearance in 2015. ITI, Sri Lanka, SUA, Tanzania and UWI, Trinidad and Tobago don't need a REB clearance, and hence it is proposed that they are covered under UG REB clearance.
6	Identification of farmers, fields or industry outlets (for postharvest studies) for trials completed. Begin on-farm activities where applicable (UWI, UoN, SUA, ITI)	Completed Refer MN 06 technical report	
7	Extend MoU on intellectual property rights for UG, TNAU, and ITI and create related MoU for the new partners (UG).	Completed Refer MN 06 technical report	
8	Initiation of licensing activities and Identification of suitable partner[s]/mechanism for commercialization of Enhanced Freshness Formulation (EFF) sprays in India (UG / TNAU	Completed Refer MN 18,24,30 and 36 technical report	Initial licensing agreement signed with Harvest one Agritech (Canadian Company) and it got switched Novus Merchants who licensed this product under Smart Agri
9	Financial reports submitted (UG /TNAU/ ITI).	Completed, Refer MN(06) Financial report	

10	Baseline survey including gender disaggregated data/indicators, completed by the three new partners (UoN, SUA, UWI with help from UG/TNAU).	Completed Refer MN 12 and MN 18 joint technical report	Delayed in UoN and UWI but achieved by MN 18 Data presented, gaps identified and midterm correction done in PRM Colombo detailed report in MN 18 report
11	Fine tuning of pre-harvest hexanal spray and testing with mango, banana, grapes and tender fruits demonstrations in East Africa and the West Indies (UG with UoN, SUA, UWI)	Completed Refer MN 12,18 and 24 Joint Technical reports	Delayed in Africa due to the non-availability of hexanal but was mitigated by shipping hexanal from Canada
12	Fine tuning of hexanal based post-harvest treatments including, dip treatment for banana and grapes, vapor treatment and feasibility studies in mango, banana, grapes and tender fruits (UG-leads: TNAU, UoN, ITI, SUA, UWI) and hexanal impregnation in biowax (ITI).	Completed Refer MN 12,18 and 24 Joint Technical reports	Though there were some delays due to season, crop failures and non-availability of hexanal in Africa, but everyone caught up and archived their targets over and beyond
13	Completion of nanotechnology approaches for 'smart packaging systems': electrospinning methodology and filing patent for wraps (UG); and invention disclosures filed for producing bio-nanoparticles from banana fibres and hexanal retention studies in bio-nanoparticles (TNAU-UG)	Completed Refer MN 12 and MN 18 Joint Technical reports	Filed US Patent on January 15,2015 PCT/CA2015/000027 TNAU got their institutional approval to file a patent for their Nano sticker
14	Develop marketing strategy and evaluate institutional and financial options (e.g. venture capital, social enterprise funds) for commercialising technology at scale. (UG /TNAU/ITI).	Completed Refer MN 12 and MN 18 Joint Technical Reports	Marketing strategy presented in PRM 1 in Tanzania and scale up appraised by Dr. Alvaro Paz in October 2015 at UG
15	First Monitoring and Review meeting to be held in Tanzania by August 2015 (UG /SUA).	Completed Refer MN 12 Joint Technical Report	Meeting held in July 2015 in Tanzania, members from all team attended
16	Second financial reports for all partners (UG /TNAU/ITI).	Completed	Refer MN 12 Financial report
17	First post-harvest experiments completed and evaluation of fruit quality, shelf life and nutritional composition (All).	Completed, Refer MN 18 Joint Technical report	For UWI, SUA the experiments were ongoing during MN 18 reporting but completed few months later

18	Evaluation of tests sites with farmers for any concerns (risks) and confirming plots for the second season trials for pre-harvest spray (All).	Completed, Refer MN 18 Joint Technical report	For UWI, SUA it was ongoing during MN 18 reporting but completed few months later
19	Baseline socio-economic analyses including gender analyses completed – South Asia, East Africa and the Caribbean - mid-term report and mid-course corrections done (UG with all partners)	Completed, Refer MN 18 Joint Technical report	Gaps identified and Mid-course correction done at PRM Colombo (May 2016), one month before the Joint report due.
20	Evaluation of commercially manufactured EFF products and feedback from growers and grower organizations (TNAU-UG). First grower interaction workshop on the effects of hexanal intervention on the economic benefits and livelihood of farmers (TNAU, UoN, SUA, UWI).	Completed, Refer MN 18 Joint Technical report	
21	Presentation of results at professional meetings such as the annual Ontario Fruit and Vegetable Convention, Niagara Falls (UG), Scientific Workers Conference (TNAU)	Completed, Refer MN 18 Joint Technical report	Conference presentations in the Research outputs.
22	Third financial reports submitted (UG /TNAU/ ITI).	Completed, Refer MN 18 financial reports	
23	Second field and post-harvest trials including hexanal vapour and wax-dipping initiated (All).	Completed, Refer MN 24 Joint Technical Report	Exciting results in Nectarine (UG), Citrus (Tanzania) and Hexanal vapour Kinetics (TNAU)
24	Production of electro-spun wraps containing hexanal for testing (UG)–to be tested in mango (TNAU, UWI, SUA, UoN), grapes (TNAU), papaya (ITI, UWI), peach (UG) and citrus (SUA).	Completed only 45%, Refer MN 24 Joint Technical Report	Due to issues with the electrospinning machine, this could not be achieved as planned. Further, sending out the papers in mail is not as simple as originally thought and hence this will be restricted to Canada only. In TNAU Hexanal fortified Sticker and Sachet are being fine-tuned for commercial use. In all likelihood this milestone may not be achieved as planned, instead will be done only in Canada and in India
25	Initiation of registration protocols for EFF and electro-spun wraps in Canada, contact federal agencies (UG).	Completed 75% and ongoing with the licensee Refer(Commercialization strategy Progress) in MN 24,MN 30 and MN 36 Technical reports	Registration protocols for EFF has been initiated. The licensee is expected to take it further for obtaining clearance (which is the norm in academic inventions) and we will help them with the logistics. They had hired consultants to clear the regulatory hurdles in US also clearing the regulatory issues in South America, especially Guatemala and Costa Rica with their existing partners. Focus is more on dip as it presents relatively lesser regulatory constraints.
26	Evaluate marketing/distribution of EFF to East Africa, Caribbean and Canada (UG, SUA, UoN, and UWI). Target between 200-300	Completed	Growers are very happy with the EFF spray and now it is being tested on pack lines (Canada). EFF has been produced and supplied

	mango growers, 100-200 banana growers (India), 50 banana growers and 50 citrus growers (Africa) 25 banana growers (Caribbean) and 25 tender fruit growers (Canada) to take up the pre-harvest spray of EFF in their farms.	Refer MN 24 Joint Technical Report	to more than 120 farms (25,000 litres) covering major mango growing domains in Tamil Nadu. MOU has been signed between Smart Harvest and TNAU, so the EFF Production Pilot Plant will start functioning within 2 months to reach the commercial scale.
27	Assess scope and quality of women's participation in farm-level decision making (and related empowerment indicators). Estimate that women's participation will increase by 10-20% in all locales, due to inclusion of women in gender-aware knowledge-transfer (UG / All).	Completed Refer MN 24 Joint Technical Report. Extensive reports by socio economist Dr. Finnis (UG), Dr. Sekar (TNAU)	
28	Preparation of bio-nanoparticle derived packaging systems for testing and shipping (TNAU-UG)	Completed There was interruption due to human resource challenge (RA left) but resumed back with a new hire. Refer MN 24 Joint Technical Report.	Nano-fibrillated cellulose extracted from banana pseudo stem has been successfully done and a manuscript has been accepted for publication in Fibre & Polymer.
29	Presentation to grower groups in appropriate forums (ITI, SUA, UoN, UWI).	Completed over and beyond. Refer MN24,MN30 and MN 36 reports	Project known to more than 20000 farmers during first 24 months alone. The communication was done well ahead of the target
30	Second Monitoring and Review meeting held in Sri Lanka by July 2016 (UG-ITI)	Completed Refer MN 24 Joint Technical Report	Conducted in May 2016 @ Colombo Attended by all the team PI. their SE member and the project manager
31	Fourth financial reports submitted (UG/ TNAU/ ITI).	Completed, Refer MN 24 Financial Reports	
32	Analysis of socio-economic results from use of pre and post-harvest treatments with farmers' groups. Analysis of empowerment and capacity-building among farmers in terms of confidence with technologies, decreases in crop losses and increase in incomes (that are related to decreased losses). Projected income increases (10-15%), and related projected increases to household budget allocations to food/nutrition purchases (10%) tracked and analysed among farmer participants in South Asia and East Africa.	Completed, Refer MN 30 Joint Technical Reports	**TNAU**: Improved post-harvest management practices created about 12-17 additional days of employment during the crop season for women. Value added products made by MPG's resulted in savings in the household expenditure of between Rs. 240-Rs.270/month. ITI: Banana fibre processing unit to make fruit trays and fibre as a value added product. Processing yet to start in large scale as the construction of the facility through UNDP. UoN: Qualitative data has been collected through focus group discussions (FGDs), key informant interviews and participant observations were done UWI: Increase already noted in Lime fruit (2000 fruits/acre/week available for sale and improved marketability through increase shelf life of yellow fruit that would otherwise not be marketed

33	Determine growers' needs regarding demand /supply and costs and apply corrective measures if necessary, to facilitate longer-term adoption of hexanal in all project locales (All). Target at least 20% of mango farmers in Tamil Nadu to take up hexanal use in the near-term.	Completed (75%), During the main mango season 2017, TNAU has supplied more than 2000 Litres of concentrate For additional information refer MN 30 Joint Technical Report	India (EFF Spray): Farmers are willing to pay Rs.750- 1200/acre. In 2017 Mango season 3742 farmers (of which 81% are small and marginal farmers) showed their intent to use EFF. India (EFF Dip): 120 packhouses and each one is supported by 80-100 farmers and quantity of fruits (primarily banana) treated will be 25-30 tonnes per unit per day Growers in Asia (Mango-Seasonal demand, Banana; Year around demand) and Canada want it as soon as possible. In Africa and Caribbean the acceptance is achieved from the initial reluctance. Commercial production of EFF should start at the earliest and steps have been taken by the licensee. However in Africa the major concern is on the availability hexanal. Also they prefer to have the hexanal packaged in small volumes and available at the local agrovet shops. Once the regulatory huddles are cleared by the licensee, they will bring in to Africa as the demand is created by our trials. The demand in the Caribbean farmers is very less (35%) and the market assessment is ongoing. Farmers who had done the trials or exposed to seminars had shown more interest.
34	Evaluation of results from electro-spun wraps and determining their market feasibility (UG-TNAU)	50% Evaluation completed during MN 24 and continuing thereafter. Refer MN 24 Joint Technical Report	UG: After the issue with the machine is fixed the evaluation is done in Canada (MN 24) and is continued thereafter. Marketing is on hold. The product cannot shipped outside of Canada due to technical issues with shipping (Nano Particle excite the security screening machine). As we wait for the patent approval (USP/PCT 15/111,363), interested companies are being apprised on the intervention. Manuscript has been submitted in Polymer. TNAU: Electrospun fibre matrix (Sticker) is ready for testing in India in commercial/field settings, which is anticipated to be done before the end of the project time. TNAU is also sorting out the shipping logistics to send them to UoN
35	Conduct large scale field trials for mangoes with 2-3 Medium/large growers and 5-10 small growers (TNAU, UoN); bananas with10 small growers and 3 packers (TNAU, UWI); citrus with 5 small/medium growers (SUA); tender fruits with 4 growers (UG); following recommended practices of regulatory agencies e.g. Canadian Food Inspection Agency (CFIA) in Canada and demonstration of quality analyses	Completed (75%) and will be ongoing beyond the project's time frame. Refer MN30 and MN 36 Joint Technical Reports	TNAU: Large scale EFF spray on Mango and Dip in pack house (Mango/Banana) was done in July 2017UoN: Hexanal Post harvest dip trials were conducted in Meru County with 20 small scale growers SUA: Final Citrus trail was completed in July 2017UWI: Research focused on shipments of commercially available bananas and trials are ongoing. Also with small-scale lime producers. UG: The reception from growers is great as it extends the shelf life of the tender fruits by 7-10 days which fetches better prices and reduces the US imports. Exciting results in Apple but yet to confirm with one more season (Beyond the project's time frame). Awaiting for the commercial availability of the product

36	Knowledge transfer workshops (KTW) held with mango producer groups in India and mechanisms for peer learning, knowledge diffusion between smallholder farmers identified (TNAU / UG).	Completed. Refer MN 30 Joint Technical Report	In India the three FRSCs, a total of 4360 farm advisory services were offered. Among the users about 32% of beneficiaries were farm women. 21 Value added trainings with 86% women participants (Men- 96 and women- 579). 128 EFF awareness campaigns and all 92MPG members were exposed to EFF technology. FPO for mango in process; Establishment of FPOs and linking farmers with the existing FPOs to get financial assistance from SFAC and NABARD is under way.
37	Prepare at least 5 peer-reviewed, scientific publications based on data from two rounds of field trials, especially on new crops tested – pre-harvest sprays on banana (TNAU), citrus (SUA), papaya (UWI), mango (UoN), berries (UG); post-harvest dip/vapour/wax on mango (TNAU, ITI), banana (TNAU,UWI, ITI).	Completed and achieved more than expected 8 peer reviewed Publications reported in MN 30 Joint Technical Reports	Detail list in Research output (***Annex 3***)
38	Large scale testing of bio-nanoparticle derived packaging systems with mango and banana packers/shippers (TNAU) and tender fruits growers' co-op (UG).	Completed and Refer MN30 Joint Technical Report	After two years of experimentation, nano-fibrillated cellulose (NFC) was successfully extracted from banana psuedostem. This has a unique property of UV-protection and biodegradability. This NFC with poly acrylic acid extended shelf-life of tomato by 18 days even under ambient room temperature conditions
39	Evaluation of biowax impregnated with hexanal for large scale testing by mango exporters and long distance packers (ITI).	Completed and Refer MN30 Joint Technical Report	This trial was conducted in Collaboration with Ellawala Farm and Hayleys Agriculture and repeated under observation for evaluation of results by the Department of Agriculture in Sri Lanka.
40	Fifth financial reports submitted (UG/ TNAU/ ITI)	Completed and Refer MN30 Financial Reports	
41	Thesis submissions from graduate students: 10 Masters students are anticipated to complete by this time	Completed and Refer MN 36 Joint Technical Report for the detailed list	17 MS Thesis and one PhD submitted or defended. At least 6 more PhD and 4 more MS thesis in the pipeline. MS thesis will be defended by mid-2018 (***Annex 1***)
42	International Conference on the use of nanotechnology in agriculture and food safety	Completed Covered in IDRC's Asia Newsletter and Regional TV News. Refer in MN 36 Joint Technical Report.	Due to international security and logistics process instead of International conference a National conference on Nanotechnology for Ever Green Agriculture was Conducted in TNAU (October 5-6, 2017). Dr. Anindya Chatterjee, Regional director, IDRC, Asia inaugurated the conference. Dr. Kevin Tiessen, PO of the project also attended the event.

43	Scale up and commercialization of electro-spun, Hexanal-impregnated paper wrap production for individual fruit through licensing (UG)	Scale-up is completed (60%) with semi-pilot scale free surface electrospinning equipment. Commercialization and licensing is premature at this time as patent is still pending with office action ongoing.	Since the activation and premature release of hexanal is a challenge, but with a hexanal precursor approach (a small add on to Sachet or label) is promising. Graduate students are working on that which will go beyond the project timeline.
44	Third Monitoring and Review meeting at Guelph in June 2017 (UG-UWI)	Completed and reported in MN 36 Joint Technical Report	Successfully completed in June (21-23) in Guelph, Ontario.
45	Sixth financial reports submitted (UG/ TNAU/ ITI).	Completed and refer in MN 36 Financial Reports	
46	Final impact evaluation including socio-economic and gender variables, and farmers' knowledge and capacities regarding the technologies, completed (UG / All).	Completed (90%), in Sri Lanka it is ongoing as waited for the product launch. Refer ***Annex 8***	In India, by using EFF spray farmers earn additional Rs 10000 ($200)/acre and Spraying reduced post-harvest losses to distant markets by 10-12%. Data revealed that that 31% of farmers felt that EFF delayed ripening, and 51% felt it increased the premium on their products. Post-harvest dip at pack houses is performed mostly by women; this provides continuous employment for women during the season, similar view from Tanzania In Tanzania Young women farmers will likely be more willing to adopt technologies than women over 35. In Kenya, Farmer narrated with much confidence and joy how the use of Hexanal for his papaya farm saved him from the exploitation of middlemen and resulted into a significant increment of his Income. In Trinidad and Tobago: The farmers were more comfortable on the GRAS certified project and eagerly await the availability of the product in Trinidad and Tobago. The main advantage of the product as expressed by farmers was the extension in fruit retention time in trees. In Canada: After the dissemination meeting in November 2017, suggestions given by the farmers and OMAFRA staff to fast track the commercialization of the product in US and Canada. Positive feedback by apple grower. Significant progress in regulatory clearance after the meeting and the license Smart Harvest is taking care of that.

47	Preparation of 6-8 peer-reviewed manuscripts from the work done in the project (All)	Completed this milestone in MN 30 itself. Refer MN 30 Joint Technical Report	Detail list in Research output (***Annex 3***)
48	Evaluation of company (Incubator) performance, plan expansion as needed within target countries and internationally where post-harvest losses are an issue. Work through Guelph- East Africa (GEA) in target African countries (UG).	90% Completed, except in Africa. Refer to Commercialization update in the Final report (***Annex 5***)	The licensing is given to Smart harvest, Canada, signed a MoU with TNAU on March 15, 2018 to commercially produce and distribute EFF in the state of Tamil Nadu while they are also actively pursuing clearance for sales in other parts of the world. Canadian consul general , Bengaluru attended this event In Sri Lanka Hayley's launched the product commercially on March 22,2018 and it is hoped that the product will be available for growers from next season. CHC, Sri Lanka attended this event. It will take another 2 years before EFF can be produced commercially in East Africa mainly due to non-availability of Hexanal in these parts, although the governments of both Kenya and Tanzania are exploring to circumvent this.
49	End of project evaluation and impact assessment for a) pre-harvest spray of EFF (on new crops); b) biowax effect on shelf life, c) Electrospun wraps and d) Bio-nanoparticle mediated hexanal packaging. Target at least one company to produce EFF and Biowax in Asia, EFF in Africa and North America and Caribbean and 1 company to produce electrospun wraps in Asia and North America and 1 company for producing bio-nanoparticle based packaging systems.	90% Completed, except in Africa. Refer to Commercialization update in the Final report (***Annex 5***)	EFF production in Asia will be done commercially soon as the MOU has been signed between Smart Harvest Canada and TNAU, Products launched with Hayleys in Sri Lanka. It will take another 2 years before EFF can be produced commercially in East Africa mainly due to non-availability of Hexanal in these parts, although the governments of both Kenya and Tanzania are exploring to circumvent this. Electrospun wraps and bio nanoparticles have been filed for patent in India and it will take at least another year before the patent situation will be known. Thus unfortunately this part of the milestone cannot be met.
50	Large scale production of EFF (to cover 2000 acres of fruits in all countries combined), biowax, electrospun wraps – 1000-2000 sheets per crop and bio-nanoparticle mediated hexanal packaging (2500 cartons).	90% completed except with electrospun wraps and bionano particles as their patent is pending.	In India, pre harvest spray in Mango alone reached 35000 acres, Bio wax produced 200 litres, and product launched and expected to produce 250 litres/day on demand. Refer large scale production of technologies in ***annex 9***
51	Final project dissemination workshop	UG completed in November 2017 itself, all teams completed in multiple locations. Few teams had GAC interaction	Huge impact in Niagara Peninsula growers particularly apple growers Canadian Consul general Bengaluru attended the TNAU dissemination event

		(with their CHC present in their event) Refer to Project Disseminations (***Annex 7***)	Two major events in Sri Lanka followed by Product launch with their CHC presence 4-5 day event in Kenya done in a systematic way 2 day event in Tanzania 9 different meetings in Trinidad and Tobago
52	Final financial reports submitted (UG/ TNAU/ ITI).	Final stages of review and will be submitted soon	

Annex:

Final impact evaluation including socio-economic and gender variables, and farmers' knowledge and capacities regarding the technologies, completed

Country	Activities	Key Findings: General	Key Findings: Gender and/or Age
India	Further workshops to distribute EFF (February Farmer's Day, TNAU, 28 people; Krishnagiri Packhouse meeting March, 281 people)	Farmers (both men and women at a ratio of approximately 4:1) continue to visit and learn from Knowledge and Resource Centres Costs of 2 EFF sprays (mango) = approximately Rs. 10 000/hectare. Assuming this averages to an additional 500kg of fruit/hectare, this means an increase of Rs.20 000 for farmers (net gain: Rs.10 000) Spraying reduced post-harvest losses to distant markets by 10-12% in comparison to control fields Data collection indicates that 31% of farmers felt that EFF delayed ripening, and 51% felt it increased the premium on their products (6% indicated No Difference, 8% did not use EFF)	Post-harvest dip at pack houses is performed mostly by women; this provides continuous employment for women during the season
Kenya	Training and knowledge dissemination about EFF technologies to farmers - Including addressing concerns/questions about health and environmental implications. - Including practical, hands-on demonstrations and sharing samples of treated/untreated fruits for farmers to take and observe in terms of ripening speeds.	Agricultural County Executive Chief (CEC) of Machakos county promised the technology fully registered and made available the farmers. Government support to adopt and scaling up the hexanal technologies were discussed Availability was addressed by Mr. Erick, from KEPHIS which is the government mandated body responsible for authorization of new compounds and technologies. He assured the farmers that they were fully aware of the technology from its inception, and have monitored and verified that the technology is safe to use. He stated that they currently fulfilling all the government requirement to see Hexanal readily available in the local agrovets ASAP. He encouraged the farmers to consider safer technologies like the use of Hexanal to facilitate smooth trade of their produce both locally and internationally. Farmer narrated with much confidence and joy how the use of Hexanal for his papaya farm saved him from the exploitation of middlemen and resulted into a significant increment of his Income. The passionate support and corporation of farmers was recognized with a certificate of appreciation	
Sri Lanka	Final round of SE data collection was not done, related to delays in commercialization (no new data)	No new findings to report	No new findings to report

Tanzania	Working with farmers to educate about EFF and understand attitudes One focus group (23 men; 28 women)	Farmers see EFF as having high consumer appeal because it will affect appearance in terms of colour, freshness, and firmness → might enhance marketability. Work has addressed the safety of EFF to help reassure farmer concerns about health/environmental effects.	Post-harvest dips: more appropriate for women growers/vendors Young women farmers will likely be more willing to adopt technologies than women over 35.
Trinidad & Tobago	8 seminars to share information (March) - attended by farmers and agro-processors - Approximately 500 farmers reached Tobago Dissemination seminars (2; 177 stakeholders attend)	Each seminar took the form of presentations of the actual trials and a summary of the findings with respect to papaya and lime. The use of the hexanal as a post-harvest dip was introduced to the packinghouse staff in the Division of Food Production, Forestry and Fisheries. Separate special presentations were made to policy makers.	The farmers were more comfortable on the GRAS certified project and eagerly await the availability of the product in Trinidad and Tobago. The main advantage of the product as expressed by farmers was the extension in fruit retention time in trees.
Canada	Dissemination meeting in November 2017, attended by growers, OMAFRA staff and license Smart Haverst	Suggestions given to fast track the commercialization of the product in US and Canada Positive feedback by apple grower Significant progress in regulatory clearance after the meeting and the license Smart Harvest is taking care of that.	

Large scale Production of Technologies

TNAU Production

Pre-harvest spray

EFF (diluted) distributed in India - **352,050 litres**

Area coverage = **35,205 acres of mango orchards**

2. Post-harvest dip

Qty. of EFF (diluted) distributed in India - **17,500 Litres for Banana**

Qty of fruits dipped for domestic /export market - **175 tonnes of banana fruits**

Qty. of EFF distributed for other fruit crops (Guava, Grapes, Papaya) in India - **1500 litres**

Qty. of other fruits treated - **15 tonnes**

3. Nano-stickers

Number of stickers used / experimented / distributed - **400**

Number of carton boxes treated - **400 (each box carries 2-3 kgs of mango / banana**

4. Nano-sachet

Number of pellets used / experimented - 100

Number carton boxes treated - 1**00 (each box carries 2-3 kgs of mango / banana**

5. Vapour

Number of carton of boxes exposed

Banana - 150

Mango - 100

Tomato - 10

6. Nano-film developed from bionano-particles (banana fibres)

Number of films developed / used - **25 films**

Sri Lanka (Anticipated) Production

Pre-harvest spray

Tree Fresh Formulation (TFF modified EFF) - 500 litres produced by Hayleys Agriculture Pvt. Ltd., for marketing and distribution in Sri Lanka as of March 2018. 20 litres prepared as samples for distribution at the launch on 22nd March 2018. Projected production 1000 litres per day as per market demand

Bio Wax

Hexanal Incorporated ITI Bio-wax - 200 litres produced by Hayleys Agriculture Pvt. Ltd., for marketing and distribution in Sri Lanka. 20 litres prepared as samples for distribution at the launch on 22nd March 2018.

Projected production 250 litres per day as per market demand.

Hexanal Incorporated Composite Material (HICM)

Number of HICM cards used / experiment - 90

Number carton boxes treated - 90 where each box carried 2-3 Kg of mango.

Banana fibre based fruit wrap

Number of fruit wraps distributed as samples

(Mango collection and distribution centre and large grower) - 900

Nanotechnology Solutions to Tackle Post-Harvest Losses in Fruits

The world is producing annually 675 million tonnes of fruits to meet the nutrition requirement of its population as per the latest statistics available in 2017. The major part of fruits is being harvest in Asia particularly China which contributes 275 million tonnes followed by India with 100 million tonnes. There is a phenomenal growth in fruit production over the years and both India and China had reached the highest level ever achieved. Despite all the rosy statistics, the global population is struggling to meet the daily requirement of fruits due to the huge post harvest losses which is in the range of 30-35% in Asia particularly India and Sri Lanka, 80-85% in Africa, 15-20% in USA and Canada and 25% in Europe. This depicts the extent of post-harvest losses in perishables which is a global challenge to be addressed. The economic loss of such devastation works out in billions of USD annually. Developing countries have a spectrum of a series of issues such as improper harvesting & handling, transport, poor storage and lack of infrastructure. On the other hand, developed countries like USA and Canada face a challenging energy, environmental and health issues.

In order address a global challenge of post-harvest losses across the world, Global Affairs Canada and International Development (IDRC), Canada, had financially supported a project on "*Enhanced Preservation of Fruits using Nanotechnology*" under the Canadian International Food Research Fund (CIFSRF) for more than five years for the research team lead by University of Guelph, Canada. The other partnering institutes include Tamil Nadu Agricultural University, Coimbatore, Industrial Technology Institute, Sri Lanka, University of Nairobi, Kenya, Sokoine University of Agriculture, Tanzania and University of West Indies, Trinidad & Tobago. The team worked together and developed a basket of technologies to reduce the post-harvest losses in temperate and tropical fruits. During the course of project period, 9 technologies had been developed, tested and proved effective in minimizing the post-harvest losses in fruits. All the products had the active ingredient as "hexanal" which is known to extend shelf-life of fruits and vegetables as a consequence of inhibition of phospholipase D enzyme and slowing down ethylene evolution from the produce. These physiological changes collectively contribute for the fruit preservation during storage and transport. The project has brought out hexanal-based nine technologies that include 1. EFF Pre-harvest spray, 2. EFF Post-harvest dip, 3. Hexanal vapour, 4. Wax formulation, 5. Electrospun fibre wrapper, 6. Nano-sticker 7. Nano-pellets (Sachet) 8. Nano-film and HICM (Hexanal Incorporated Composite Material. These technologies either singly or in combination can help to reduce the post-harvest losses vis-à-vis improved the per capita availability of fruits.

Key messages

- Enhanced Freshness Formulation (EFF) carrying hexanal as an active ingredient can be delivered in horticulture systems as pre-harvest spray, post-harvest dip and vapour form preserve fruits for 2-3 weeks under ambient storage conditions. The technology is economical and eco-friendly and enabling farmers to earn 15-20% higher income
- Farmers in North America, Asia, Africa and Caribbean can use **pre-harvest spray** of EFF to retain the fruits for 2-3 weeks on trees and extend the shelf-life for another 2-3 weeks in storage conditions. The technology is working well for both tropical and temperate fruits. It was unequivocally demonstrated that treated fruits had the less incidence of post-harvest diseases
- In packhouses and exporters can use EFF **dipping technology** alone or combination with wax formulation as an intervention within the sequence of dipping treatments to reduce the post-harvest losses. Huge quantity of fruits can be treated in 5 minutes time and it is easier for the farmers to adopt.
- **Vapour** form of hexanal is equally effective and various dispensing systems such as nano-stickers, nano-wrappers and nano-sachet have developed, validated and patents filed. This is simple and large quantities can be treated without any associated ill-effects.
- More than 12,000 farmers in India adopted the technology and our survey suggested that 80% of them either benefited from the delayed harvest or lucrative income
- The hexanal technology is not unique to a particular fruit or vegetable and thus it can be fine tuned to suit a wide array of perishables. The technology is quite robust and powerful to address the global challenge of post-harvest losses.

Emerging outcomes

1. Pre-harvest spray helped the farmer to tide over economic crisis in the country

Mango farmers in one of the major mango growing domains in Tamil Nadu, India, was devastated after the demonetization in 2016. The price of many horticultural commodities including mango had taken a dive reaching the rock bottom. The farmers were in distress and looking for help to retain the fruits on trees for 2-3 weeks that allow enough time to stabilize the price. **Mr. Ramesh**, Mango grower from Pochempalli, Krishnagiri sprayed EFF as per prescription on 5 acres of land carrying more 200 bearing trees. The adjacent 5 acres were not sprayed and was treated as control. After the spray was done, the farmer closely monitored the sprayed and unsprayed trees for a month. He shared his feedback.

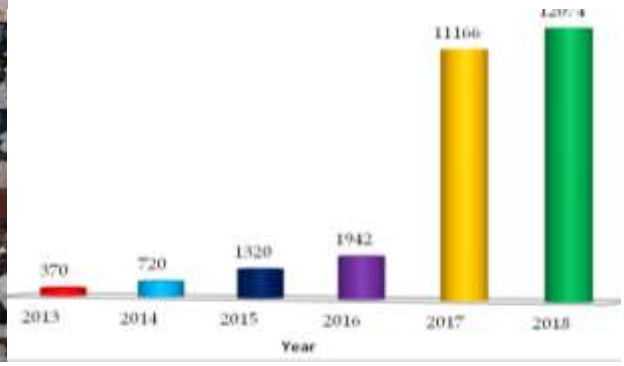
"The EFF technology came out as a savior of my livelihood as I could protect my mango orchards from fruit drop. Fruit retention got extended up to 15 days in comparison to unsprayed trees that eventually resulted in lucrative price for my produce. The profitability of the technology is more than Rs. 10,000 per acre" I should have lost my crop if this technology is not informed during the project period"

Sprayed Mango Trees with Retained 20% more Fruits

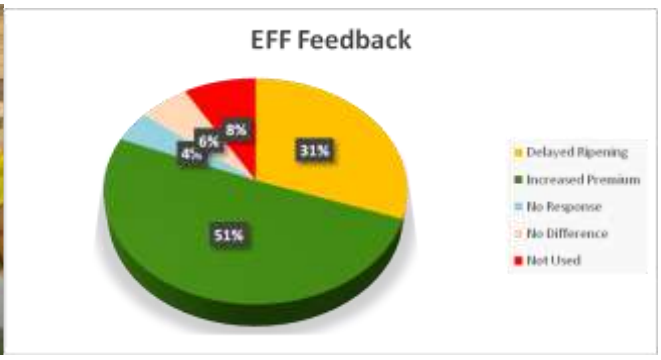
2. Feedback of EFF Technology at the farm gate

More than 12,000 mango growers received EFF from TNAU and used in fruits and vegetables. The feedback survey revealed that post-harvest losses had been reduced by 10-12% in the EFF sprayed fields in comparison to control. Due to the reduction in losses at the field level, the farmers got about 500 kg of additional yield of fruits. Our feedback survey suggested that 55% of the respondents got the premium price for the EFF spray, 31% expressed that the ripening of mango fruits were delayed, and 6% indicated that there was no difference between treated and control. The data clearly suggests that more than 80% of the mango of fruit growers benefitted from the EFF technology.

EFF distribution and its impact



EFF technology is very good for bananas and mangoes in Africa where the post-harvest losses I estimated as > 80%.

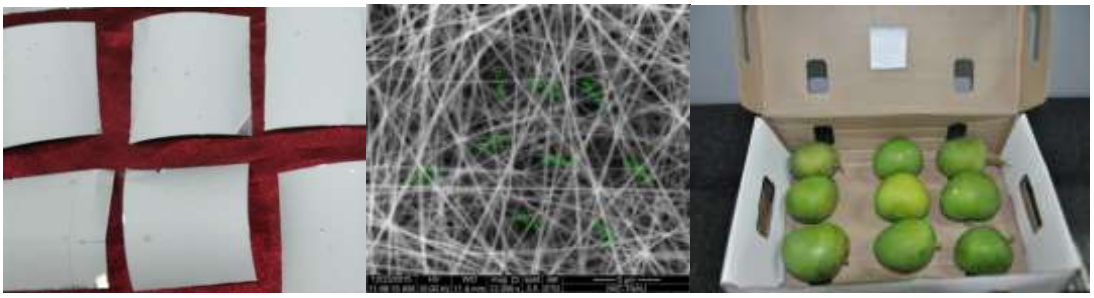


3. Smart delivery systems for hexanal

Hexanal is a highly volatile compound and it vaporizes completely within 2 hrs of exposure to normal room temperature. To regulate the release of hexanal, suitable dispensing system is required. Both University of Guelph and TNAU team worked together to develop four types of delivery systems utilizing nanotechnology approaches. Electrospinning is one of the versatile technologies wherein polymer solution is subjected to high intensity electricity to convert the liquid into a long nano-fibre. It has been estimated that each gram of fibre possesses extensive surface area (> 10 m^2) with a length of > 100 KMs and the dimension in the range of 50-200 nm. Utilizing this principle, the UoG developed a **electrospun fibre wrapper** and the TNAU came up with a **nano-stickers**. In both the cases, hexanal is entrapped in the nano-fibre and the release is regulated. The wrappers have shown to extend shelf-life of high valued temperate fruits. Nano stickers (5 x 5 cm^2) were tested and proved effective in preserving mangoes and bananas. In another case, hexanal is loaded and encapsulated into cyclodextrin inclusion complex (**nano-sachet**) to regulate the vapourization. Further, hexanal can be delivered as a **vapour** directly using a suitable electrified dispensing system. In all the cases, the critical concentrations hexanal can completely eliminate post-harvest pathogens thereby fruits get protected from infestation thereby shelf-life gets extended up to 2-3 weeks. There are simple technologies packaging industries and large scale store houses can adopt. These technologies have been filed for patents.

The packhouses have been provided with nano-stickers for getting the feedback fromfarmers. Even, 50 stickers had been distributed to University of Nairobi, Kenya for getting the farmers views of the technology delivered.

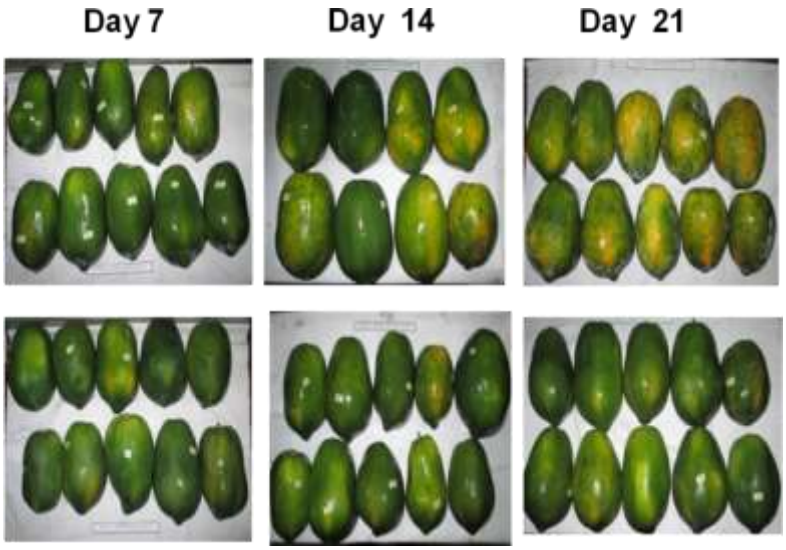
Nano-stickers for fruit preservation



4. Dipping technology enhances shelf-life of fruits

Dipping is one of the simplest technologies wherein fruits are dipped in 1-2% EFF for 5 minutes and the fruits shade dried, packed and transported. This technology was tested in mango, banana, guava, grapes, acid lime, papaya etc. This can be easily fit in the on-going activities of the packhouses. The ITI, Sri Lanka, comes up with wax formulation which is helping the farmers or traders to preserve the fruits up to 3 weeks. The cost of the technology is very cheap (Rs. 2 per kg) and the benefits are many fold. Interestingly, this technology engages primarily women and they get employment in packhouses.

Wax formulation treated fruits



Conclusions

The GAC-IDRC project funded project has helped the team to evolve a series of technologies to reduce post-harvest losses in fruits from the farm till it reaches the consumers. This project has developed bundle of technologies such as pre-harvest spray of EFF for small and marginal farmers to preserve the fruits on trees or off the trees till it reaches the market, post-harvest dipping in EFF or wax for packhouses, nano-packaging (stickers, sachet and wrappers), hexanal vapor for transporters. These technologies singly or in combinations can extend the shelf-life of fruits while minimizing the infestation of pathogens. The outcomes of the project have to be taken forward for extensive adoption and enhance the availability of fruits that ensure nutritional security of the country.

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

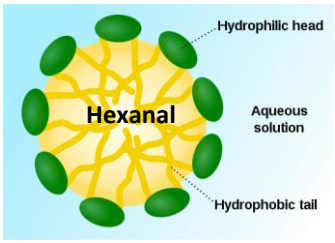
Several independent tests have been done to ensure the safety of hexanal to beneficial microorganisms in soil, earthworms, natural enemies (predators and parasites), honeybees, aquatic organisms (zebra fish)**, and human (using human cells lines)**. In order to test the biosafety of hexanal formulations, 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.

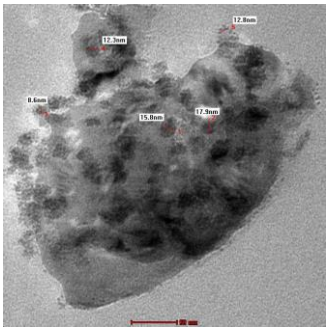
Wounding

Micelle

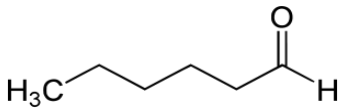


EFF Product (Enhanced Freshness Formulation)

TEM image



Hexanal



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.

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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.

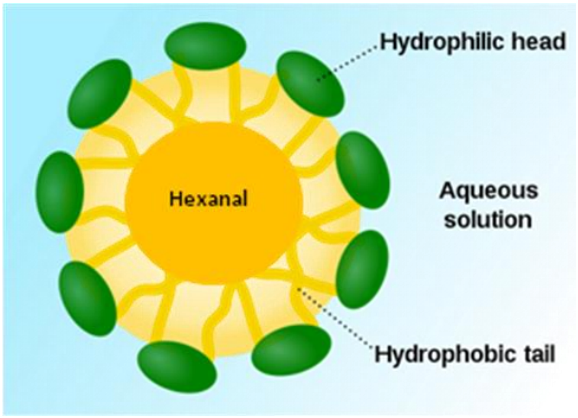


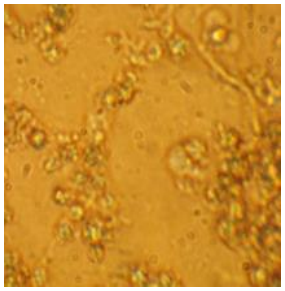
Fig. 1. Micelle of nano-emulsion of hexanal

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.

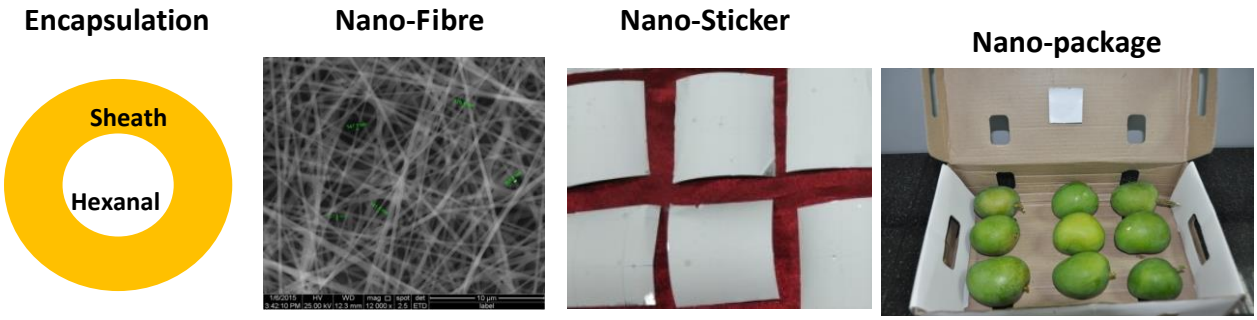


Fig. 2. Nano-matrix (Sticker) for fruit preservation

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.

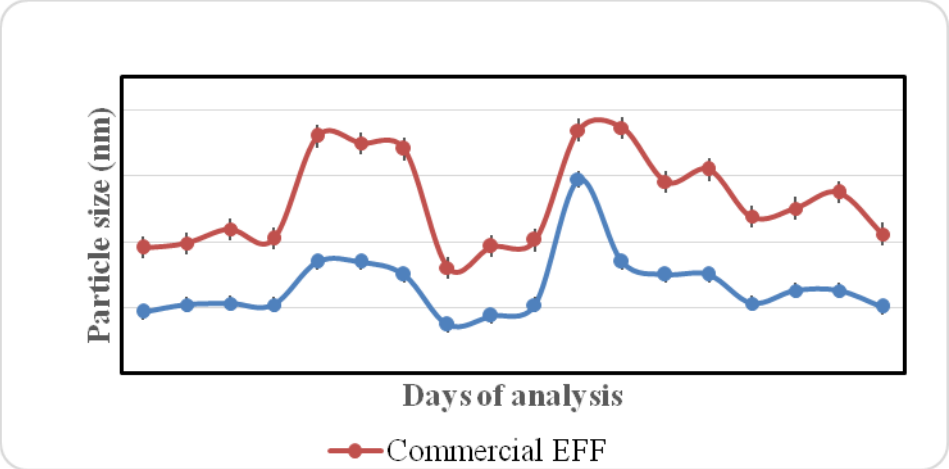


Fig.4. Stability of EFF during storage

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.

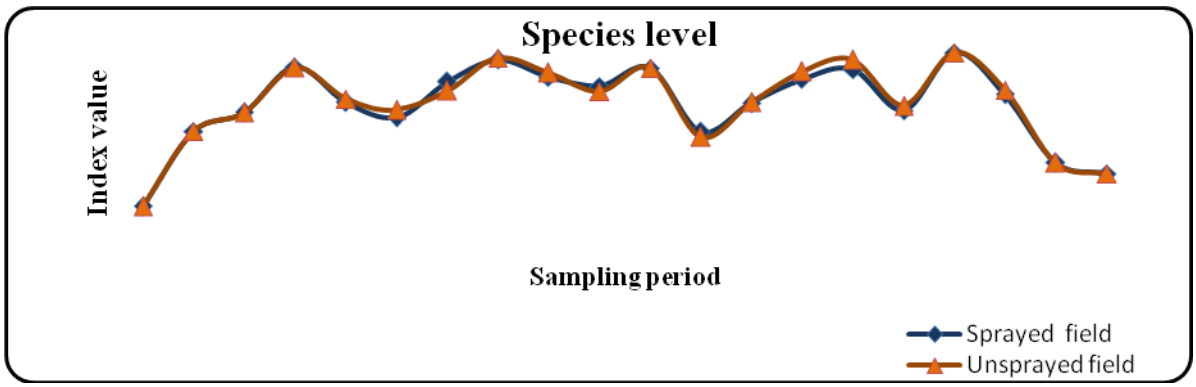


Fig. 5. Species richness in EFF-sprayed and unsprayed ecosystems

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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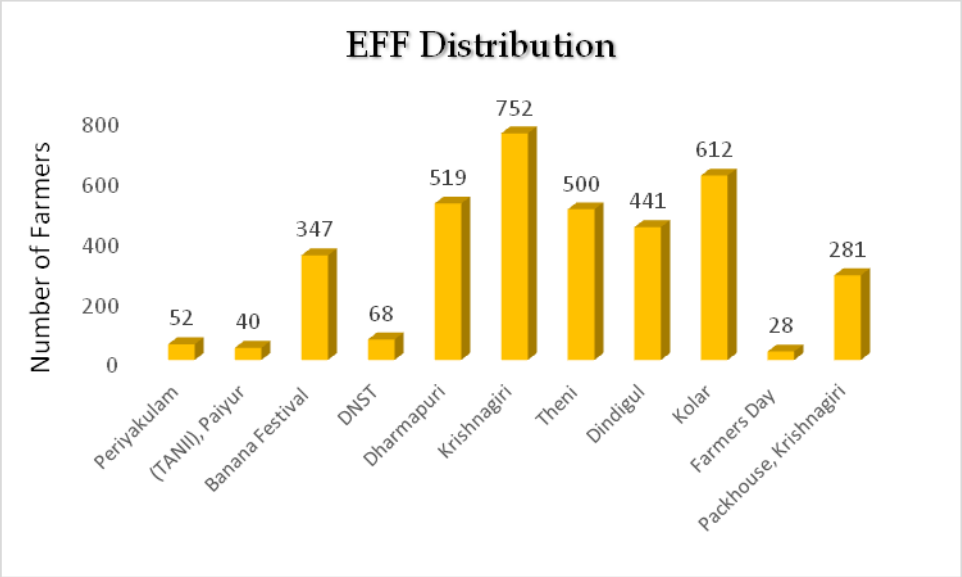
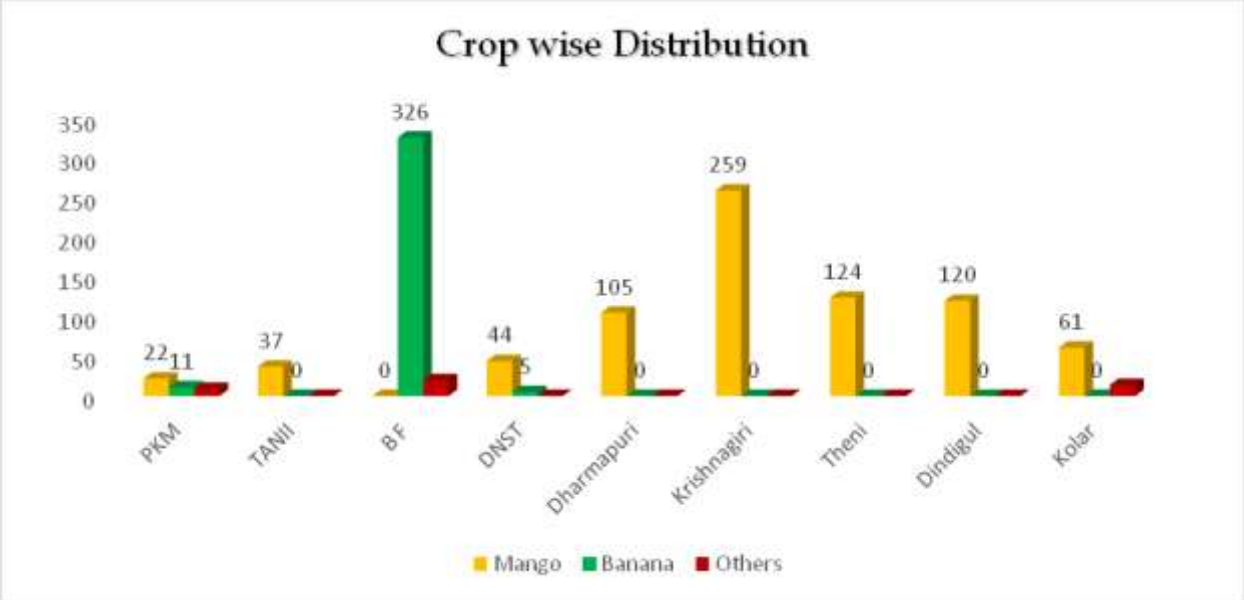
EFF Technology Delivery at the Farm Gate

Our survey suggested that the post-harvest losses of mango in Tamil Nadu is 35.3% from the point of production till it reaches the consumer. The losses were attributed to the improper harvesting, handling, grading, transportation, whole sale and retailing outlets besides lack of cold storage. In order to reduce post-production losses, the TNAU and University of Guelph, Canada, has jointly developed a technology "Enhanced Freshness Formulation (EFF)" which is known to reduce post-harvest losses when the EFF is applied as a pre-harvest spray or post-harvest dip. The present study was conducted in four major mango producing domains of Tamil Nadu *viz.,* Krishnagiri, Dharmapuri, Theni, Dindigul districts of Tamil Nadu and Kolar district of Karnataka where EFF technology was practiced by large number of farmers and acceptance behavior of EFF technology was assessed based on the feedback from the farmers who had adopted the technology. The EFF was distributed at free of cost to get the feedback of the technology from the users. About 3640 mango farmers had undertaken the EFF trial suring the Phase II (Dec. 2014 - March 2018) of the project. The sensitization and eventual distribution of EFF took place invarious locations and the number of farmers expressing interest are provided in parenthesis as follows: Periyakulam Mango Workshop (52), Tamil Nadu Innovative Initiative (TANII) meet at RRS, Paiyur (40), Banana Festival (347), Department of Nano Science and Technology, TNAU (68), Krishnagiri (752), Dharmapuri (519), Theni (500), Dindigul (441), Kolar (300), Farmers Day in TNAU (28) and Krishnagiri Packhouse Meet (281). After the season, feedback from the users were collected by personal interview. Collected data were classified and analyzed. The findings of the study revealed that post-harvest losses had been reduced by 10-12% in the EFF sprayed fields in comparison to control. Farmers got additional 24% income/acre directly due to increased retention (10%) and two weeks delay in harvest (14%). Our feedback survey suggested that 55% of the respondents got the premium price after EFF spray, 31% expressed that the ripening of mango fruits was delayed, and 6% indicated that there was no difference between treated and control. The data clearly suggests that more than 80% of the fruit growers benefitted from the EFF technology.

EFF Distribution List – IDRC Phase II

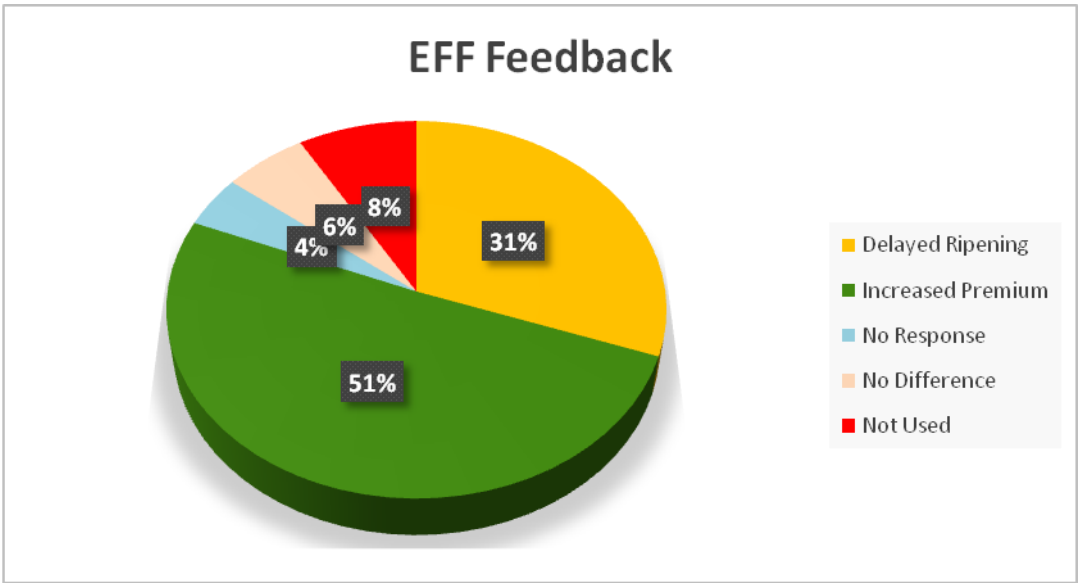
No	Date	Events	Beneficiaries
1	24.09.2016	Mango workshop at HC&RI, Periyakulam	52
2	28.03.2017	Tamil Nadu Innovative Initiative (TANII) meet at RRS, Paiyur	40
3	21st to 23rd July, 2017	National Banana Festival at AC&RI, Madurai	347
4	2015 – 2018	Department of Nano Science and Technology, TNAU, Coimbatore	68
5	May, 2017	MYRADA distribution in Dharmapuri	519
6	May – June, 2017	MYRADA distribution in Krishnagiri	752
7	July – August, 2017	MYRADA distribution in Theni	500
8	July, 2017	MYRADA distribution in Dindigul	441
9	May – June, 2017	MYRADA distribution in Kolar	612
10	9&10, Feb- 2018	Farmers Day at TNAU	28
11	12th March-2018	Packhouse meet at Krishnagiri	281
		Total	**3640**



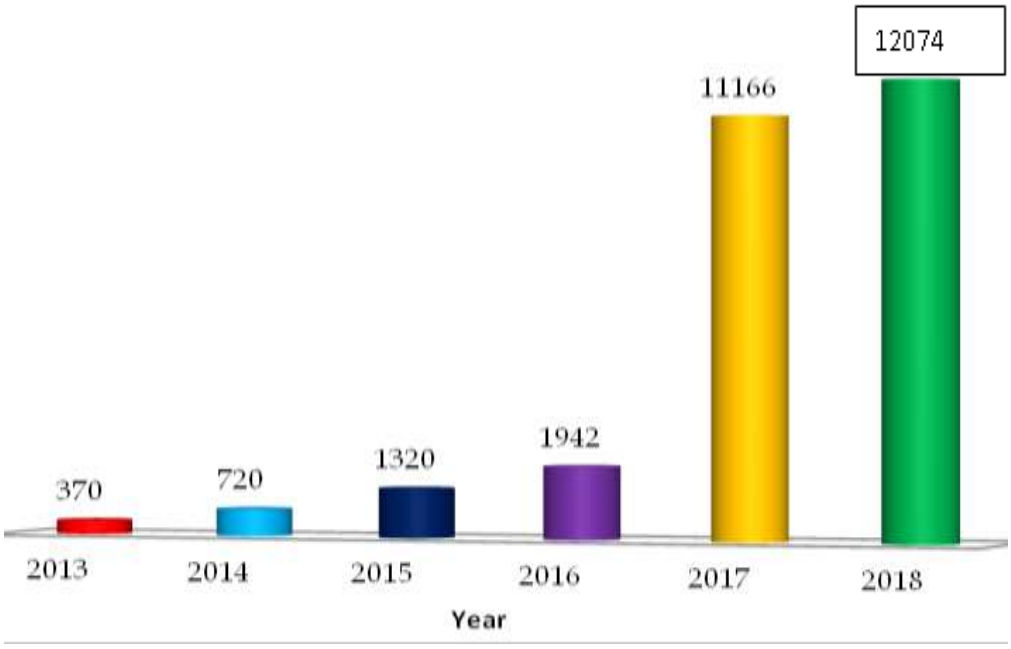


Farmers' remarks on using EFF

Impact of EFF	# Farmers
Delayed Ripening	350
Increased Premium	583
No Response	51
No Difference	68
Not Used	97



Progress in the use of EFF among Fruit Growers in TamilNadu, India as a result of IDRC-CIFSRF project on Enahnced Preservation of Fruits Using Nanotechnology between 2013-2018 (March)



A Cost Benefit Analysis of Hexanal Technology for Mango and Banana

A **cost benefit analysis (CBA)** is considered a systematic process for calculating and comparing benefits and costs of a decision, policy, or project. The very purpose of a CBA is to determine if an investment decision is feasible and the benefits outweigh the costs.

Pre-harvest spraying of hexanal formulation

Hexanal comes from plants and has been shown to extend the shelf life of perishable fruits when it is applied externally. It is available for application as *EFF* or *Enhanced Freshness Formulation*.

Experiments have shown that EFF use results in fruits staying on trees for 2-3 weeks longer, delayed ripening process, and keeps fruits fresh for at least 2 more weeks during normal storage (under ambient condition).

Pre-harvest spraying of mango

Two applications of EFF in mango orchards resulted in an extended period of fruit availability and it facilitated an increase in the farm gate price of the produce so growers realized additional income. Field-level experiments showed that EFF enhanced the preservation and appearance of the fruit and this lead to a premium price in the market.

Pre-harvest Spray of Hexanal in a Mango

Pre-harvest spraying of mango: cost and profit

Pre-harvest spraying extended the shelf life of fruits by two weeks and that helped the farmers to realize a 10-12% increase in price, a “premium price,” for the fruits earmarked for table purposes. This occurred because of the delay in harvesting. The cost of two applications of EFF spray works out to be Rs. 10,000 per acre. This can result in additional net fruit yield of 500 kg per acre which returns an additional Rs. 20,000 per acre and a net gain of roughly Rs. 10,000 per acre due to EFF spray. Further, since treated mangoes were available when mango was no longer available, the additional returns realised by the farmers in the project were estimated at Rs. 14000/acre depending on the prevailing

market price and season. The post-harvest losses were 5–7% less in the EFF-sprayed fruits meant for transport to distant markets like Mumbai, Pune, Delhi, and Calcutta.

Post-harvest dipping of mango

For some farmers, pre-harvest spraying of mango might be hard to do so post-harvest dipping of mango has been tried in pack houses to take advantage of the technology at large collection points instead of at farms. The EFF dip treatment retained the freshness of mango up to two weeks without any change in fruit quality so traders were able to reduce post-production losses by 7–10% depending on the distance and mode of transport.

Post-harvest dipping of mango: cost, advantages and profit

The cost of EFF dip treatment is as low as Re. 0.5 per kg. This technology is quite powerful as it is simple and bulk quantities can be treated in pack houses. Pack houses already do a series of sequential dipping in water, salt, and safe fungicides. The EFF dipping could easily fit into the pack house regime and help the farmers and traders to gain advantage of premium prices in the market. Such dip treatments are primarily performed by women with each pack house providing employment for 100 women. The women are happy to work in pack houses as they provide continuous employment. The net return obtained per tonne of mango fruit works out to Rs. 2,700.

Packaging of Mango Fruits in Carton Boxes

Post-harvest dipping of banana: cost and benefits

The EFF technology is very effective for banana as it extends the shelf life of fruits by 12–15 days. Major banana varieties such as Grand Naine, Poovan, Nendran, Red Banana, and Ney Poovan have been tested and the EFF technology found suitable for all of them. Industrialists have expressed their belief that the EFF technology is highly beneficial for green banana that is intended for chip-making. The EFF-dipped fruits were reported to remain fresh and maintain their quality throughout the storage period. The cost of EFF is Rs. 0.5 per kg. The benefit of using the technology can be huge if the fruit sold later in the marketing cycle in the domestic market as well as the increased opportunities with long- distance transport and export markets.

Nano-stickers for mango and banana

Nano-stickers have been developed using an electrospinning technology. These stickers entrap Hexanal and regulate its release while in the fruit storage boxes. Each box of fruits (2–3 kg) can be preserved

with a small (5 cm2) sticker. This technology is highly suitable for long distance transport of fruits. Results showed that mango and banana exposed to the nano-stickers had an extended shelf life of up to 3 weeks. This helped the farmers to get additional income at the retail level owing to reduction in post-harvest loss. Based on the retail prices of mango and banana at Rs. 30/kg, net additional return was Rs. 1,150/ton for mango and Rs. 390/ton for banana, respectively.

Nano-Stickers in Carton Boxes

A Hexanal Testimonial

Mr. Santha Kumar, an innovative mango grower in Krishnagiri in Tamil Nadu State, and on whose field the initial trials were conducted, has expressed that EFF-sprayed fields appeared well, that the trees were dark green in colour, and that the general crop stand was encouraging. He expressed that he was able to retain the fruits in the field for two weeks thereby his per acre return improved by Rs.10,000. He recommended the technology to the farmers living in the local area.

Summary of the cost benefit analysis of hexanal technology for mango and banana

In summary EFF can be used as pre-harvest spray or post-harvest dip for reducing post-harvest losses at all levels in the value chain – from the point of harvest to the consumer. The pre- harvest spray in the mango orchard led to a additional net return of 14000 per acre. The net return obtained per tonne of mango fruit due to EFF dip works out to Rs. 2,700. Based on the current prices of mango and banana at Rs. 30/kg, net additional return was Rs. 1,150/ton for mango and Rs. 390/ton for banana, respectively. The data clearly suggest that farmers can benefit a lot by introducing a simple hexanal- based nanotechnology. Hence this technology can be advocated in all the mango and banana growing areas to reduce post production losses and gain additional monetary returns.

Technology Sharing Experiences in Sri Lanka

The Industrial Technology Institute (ITI) project team selected three new technologies with the potential for commercial application. The technologies address the problem of post-harvest loss and included: a) the post-harvest application of wax formulations for maintaining fruit quality and extending storage life, b) a pre-harvest spray treatment for retention of fruits on trees to stretch the harvest season and extend the period of availability of mango, and c) a banana fiber-based fruit wrap to maintain quality of fruits during storage and transportation. The models adopted for commercialization of the technologies included interaction with both fruit producers and agro-input industries. The models were chosen to ensure that the benefits of the technologies would contribute to income generation, employment opportunities for women, better returns for farmers while improving food security in rural communities.

In Sri Lanka post-harvest losses remain high at 30–40% of the harvested crop. This loss has been valued at US $ 90 million. In addition to monetary losses, there is much to be desired with respect to nutrition for both urban and rural Sri Lankan populations as diets are well short of approved nutrition recommendations.

The international, collaborative project for "Enhancing the Preservation of Fruits Using Nano Technology" funded by the Canadian International Food Security Research Fund (CIFSRF), provided the opportunity to address these issues with help from other partnering countries. Commercialization is one of the primary mechanisms for implementing technological solutions but it offers several challenges and we discuss the process and success here.

THE TECHNOLOGIES

The project generated several technologies derived from the original hexanal-based Enhanced Freshness Formulation (EFF). Two of the hexanal-based technologies developed and subjected to laboratory-scale evaluation in Sri Lanka were selected for commercial application.

The Bio Wax formulations – of which one is incorporated with hexanal - showed that the storage life of mango could be extended for up to 21 days when fruits were stored at 13.5 °C ± 2 °C. This extended storage period would accommodate transport of the product to distant destinations, domestically as well as abroad.

The pre-harvest EFF spray treatment was tested on two, high value, Sri Lankan mango varieties. The spray treatment technology was observed to maintain the quality of fruits and to extend the harvest period of the tested mango varieties by up to 4 weeks.

Currently polyethylene sleeves are used to protect fruits from physical injury during transportation. Polyethylene is not biodegradable. Banana pseudo stem is a huge agricultural waste in Sri Lanka. Researchers were able to develop technology to use the banana pseudo stem and make biodegradable paper. This creates employment and income-generating opportunities for women in banana growing areas. The eco-friendly paper used as a fruit wrap can replace the imported polyethylene

THE EXPERIENCE OF TECHNOLOGY TRANSFER

Based on interactions with the industry, the prerequisites identified for commercialization of the technologies were:

- Obtaining National regulatory clearance for commercial application
- Identification of suitable industry partners for commercialization.
- Demonstration of the availability of a sustainable market for the technology.

The commercialization process took place in three stages. Once a suitable industry partner was identified, the technology was transferred, and implementation schedules were developed for the commercial production and adoption stages via a series of three interactive models: (1) a big company collaboration model, (2) a medium industry collaboration model and (3) a farmer association model.

Large Scale Industry Partner Collaboration Model: This model related to the commercialization of the modified EFF spray treatment technology and the two Bio-wax formulations. In this instance the ITI entered into

partnership with a large company, an agro-industry service provider, and a supplier and distributor of agro chemicals with business interests in Sri Lanka and overseas.

Commercial Production of Modified EFF and Bio-waxes:

- To convince the industry partner, trials were conducted with a large-scale grower. The grower will share his experiences with the company as well as with other growers.
- Since EFF spray and the Bio-wax were not classified as pesticides or growth regulators, we were able to obtain regulatory clearance from the Department of Agriculture without much delay. Further ground work done by TNAU on biosafety helped to accelerate the process.
- An agreement through which the technologies were licensed to the industry partner was reached, enabling transfer of the technologies for commercial production of the EFF and bio-wax formulations.

Adoption of Modified EFF and Bio-wax Technologies:

- In order to ensure adoption of the technologies, trials were conducted with prospective end users– including growers and farmer organizations.
- With the bio-wax formulations, commercial-scale trials and demonstrations were conducted in collaboration with exporters and supermarket chains.

Medium Industry Collaboration Model: This was adopted for commercialization of the banana fibre-based fruit wrap. The industry partner was based in a banana producing region and had already commenced fibre extraction from banana pseudo stem. The introduction of the fruit wrap added further value to the banana fibre extraction process.

Commercial Production of Fruit Wrap

After sufficient demonstrations, commercial-scale production was done in collaboration with the industry partner. Test market trials followed, and were conducted with an exporter and large-scale grower. It became apparent at this point that the industry partner required assistance to secure a market for his products which was provided by the research team. Food safety concerns were addressed by incorporating a procedure for microbial disinfestation into the production protocol.

Adoption of Fruit Wrap Technology

Collaborative, commercial-scale trials with end users were conducted. End users included a growers' collection and distribution centre, a large-scale mango grower, and a supermarket chain. Researchers engaged in follow-up action and assisted the industry partner to secure a market for the wrap.

Farmer Association Model

A farmer association model was also used for commercialization of the fruit wrap. The association was provided with infrastructure facilities for processing banana pseudo stem via a Canadian government-funded, United Nations Development Programme (UNDP) operating in the Northern Province of Sri Lanka.

Commercial Production of Fruit Wrap

The ITI research team assisted the farmer association with the selection of equipment and the planning of space requirements for the fibre processing plant. The farmer association benefited from test market trials conducted by the industry partner with the exporter and large-scale grower. Food safety issues were addressed by introducing necessary steps in the production protocol.

LESSONS LEARNED

Many lessons were learned including the following:

- Commercialization/commercial production of technology output is a means of promoting the adoption of new technology developed by researchers.
- Researchers had to ensure that the cost of production of the technology, conformed to purchase prices acceptable to industry partners and user markets.
- Market demand and market size were crucial to the sustainability of commercially viable, cost-effective manufacturing of products by industry partners.

- Ensuring credibility of food safety with use of the technology and technology-related products had to be established satisfactorily to meet consumer requirements.
- Shelf life and stability of products (Bio-waxes and EFF formulation) had to be conducive to the rate of movement of product from the production line to the shelf and to when purchased by consumers.
- The need for sustaining consistency in quality and quantity of products to meet market demands.
- Ensuring that opportunities are provided for men *and* women to benefit from employment and income generation.
- Implementing any spin-off technologies can benefit rural communities by providing employment and income-generating opportunities to women – opportunities that are usually in short supply in these regions.
- Availability of researchers to provide necessary support if required, to ensure sustained commercial production of the respective products particularly when working with financially vulnerable industries.

CONCLUSIONS

Commercialization of the new post-harvest technologies proved to be a challenging but rewarding experience. The adoption of the technologies by respective stakeholders will contribute toward increasing the income of targeted farmers and help minimize post-harvest loss. The experiences shared in this outcome story, across the four different models of taking research from the laboratory to adoption by stakeholders, indicate the need for careful attention to three important components of commercialization: product development, production, and adoption. Other critical elements of the process are the need for careful selection of compatible industry partners, close engagement with these partners throughout the commercialization process, aiding the industry partner when required, and the development of pathways for ensuring sustainability of the industry.

ACKNOWLEDGEMENTS

The ITI research team acknowledge with gratitude the financial support received from Global Affairs Canada through the International Development and Research Centre's (IDRC), Canadian International Food Security Research Fund (CIFSRF). We are indebted to all our international collaborative partners for sharing their research findings with us, particularly Prof. Gopinadhan Paliyath and Prof. Jayasankar Subramanian from the University of Guelph, Canada and Prof. K. S. Subramanian from Tamil Nadu Agricultural University, India.

Value Chain Actors and Enhanced Freshness Formulation (EFF) Technologies

Agricultural value chains refer to the activities involved in bringing a product from the field to the consumer (Miller & Jones 2010: xv). Commodity chain analysis can highlight economic insecurity, labour insecurity, and other inequalities among participants along the chain, particularly those closer to the production side (Kaplinsky 2001; Dolan 2004; Nakazibwe & Pelupessy 2014), and can also offer examples of how to support producers (Kamya 2015). How might Enhanced Freshness Formulation (EFF) technologies create opportunities for increased economic security for the less powerful participants in agro-commodity chains?

Case illustrations from two participant countries, Trinidad and Tobago and Sri Lanka, demonstrate possibilities for increased food and economic security, including for those who are more vulnerable in the agricultural and food sectors. They also highlight factors that can make these possibilities more or less sustainable.

Picture showing the stage in which green papaya are used in the processing for substitution of potato in the school meal program in Trinidad and Tobago

Green Papaya, School Meals, and Nutrition

Do EFF technologies have possibilities for improving national food security and nutrition? The use of green papaya in Trinidad and Tobago offers some possible approaches to answering this question.

The National School Nutrition Program in Trinidad and Tobago provides no-cost breakfast and lunch meals to over 820 pre-primary, primary, secondary, and special schools. This amounts to almost 140,000 meals served each school day.[1] Menus aim to provide 1/3 of the recommended daily nutrients through lunch, and 1/4 of these daily through breakfast.[2] In 2015, The National Agricultural Marketing and Development Corporation's Deputy CEO, Calvin James, noted the high food import bills of the country, and indicated that locally-produced fruits such as cassava, breadfruit, and green papaya could be substituted for white potatoes in the school nutrition program.[3] Trials of EFF spray for papaya demonstrate potential benefits for actors across the value chain, including small-scale producers, processors, caterers, and consumers, due to the potential to use green papaya as a potato substitute. EFF spray slows the ripening of the fruit, retaining the green colour that processors want to see when buying papaya to make value-added products.

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[1] Government of the Republic of Trinidad and Tobago. http://www.ttconnect.gov.tt/gortt/portal/ttconnect/!ut/p/a1/jdBNC4JAEAbgX-PVGZUN7ebBTA1C-9K9hMa2GuaKmvbzM29iWXOb4XnhZYBCCLSI24zHTSaKOH_vdHH2fBWJY2i49dFANXAUC4mn6Xu1B9EIBPaqBxZR1t5RQ8T_8vhlzJ_5HSvgBHSO2S6ZgGnNAcz0cIHyXCTDTyKzSDSdA63YlVWskh9Vf06bpqyXEkrYdZ3MheA5ky-xhJ8SqagbCEcQyvshfDo3krcb8wXuWoab/dl5/d5/L2dBISEvZ0FBIS9nQSEh/?WCM_GLOBAL_CONTEXT=/gortt/wcm/connect/GorTT+Web+Content/TTConnect/Citizen/Role/AParent/EducationandTraining/School+Nutrition+Programme

[2] Government of the Republic of Trinidad and Tobago. http://moe.edu.tt/services/administration/units/national-school-dietary-services/itemlist/category/74-national-schools-dietary-services

[3] De Souza, Janelle. 2015. Grow Food, Grow Jobs. Trinidad and Tobago Newsday. Available online: http://www.newsday.co.tt/news/0,221538.html

What kind of nutritional, economic and food sovereignty implications might this have?

For students, the replacement of potatoes with green papaya has the potential for reducing the intake of carbohydrates in the diet. In a country with a diabetes prevalence rate above 14%, developing a taste for green papaya early in life has immediate and long-term nutritional benefits. This would further recent national calls to make the school meals healthier. In addition, it could lower labour costs associated with the school nutrition program, without compromising on quality.

For producers, this widens the local market for green papaya, and allows for staggered production of the fruit. It also extends the shelf life for exporting to neighbouring islands like Barbados. Current export marketing arrangements are that farmers only get paid if the retailer receives an unspoiled product. These marketing arrangements systematically disempower farmers, and EFF therefore has the potential to help minimize these kinds of losses, and make income more predictable for producers.

For the country, the increased use of green papaya has the benefit of reducing dependence on imported goods and lowering national food bills, while supporting local farmers and their livelihoods.

Kaplinsky (2000: 131) argues that efficiency in value chains is in part dependent on scientific and educational innovation. As scientific innovations, EFF technology trials have demonstrated the potential for an overall gross reduction in post-harvest fruit losses, which can mean increased economic security for farmers, and the possibility of decreased food prices for consumers.

However, innovation does not have to rest solely within laboratories. Value chain members can also use new agricultural technologies to create localized innovations, approaches, and efficiencies.

Innovations in fiber and packaging

Picture showing the extraction of banana fibers and banana fiber based paper that are used in packaging industry in Sri Lanka. Over 70% of the workers in these operations are women

Can the application of EFF technologies allow for localized innovations using sustainable resources? While the project has focused on lab-based developments, innovators in Sri Lanka considered how banana plant waste could be processed to create a sustainable, biodegradable paper for extending storage life of fruits, for packaging export fruit, and for other purposes. This case shows the possibilities for increasing efficiencies through using plant materials previously considered 'waste' while at the same time highlighting the dilemma of an environmentally friendly product that remains 'on hold' due to various challenges faced by a small/medium-scale industry.

Have you ever noticed that in grocery stores, some high-valued fruits are wrapped in protective coverings? These are most often webbed polyethylene sleeves which are not biodegradable and that do not contribute towards a sustainable environment.

Banana fibre paper offers an alternative. It is made from the banana pseudostem, an agricultural waste product. Impregnating this paper with EFF technologies allows it to do double duty – protecting fruit from injury during transportation over long distances, and increasing shelf life. Results from simulated transportation and storage trials carried out at the Industrial Technology Institute (ITI), Sri Lanka, showed no significant difference in fruit quality between polyethylene sleeves and banana fibre-based wraps. Even better, this technology adds further value through employment and income-generating opportunities, especially for women who otherwise have limited employment options.

The processing factory that is part of this project is located in a major banana production region of the country, was already involved in fibre extraction, but had limited marketing options. The fruit wrap added another value-added product to this enterprise. ITI also introduced a microbial disinfestation procedure into the production protocol to increase food safety.

What makes these kinds of innovations possible? Technology development is important, but the right conditions are needed to implement them effectively. In this case, the banana industry partner was entrepreneurial, and also willing to take innovation risks and invest his own funds to experiment with new methods. He also knew of the need for employment for poor rural women and men. The factory now employs seven women and three men who are the breadwinners of their families and do not own land for cultivation. The women workers are aged between 50-60 and started working at this factory only after their husbands either passed away, became unable to work, or left the families.

Other factors are important as well, including markets and networking. The entrepreneur has yet to find a steady market for the banana fibre products and the factory continues to operate by producing banana chips. While they have mastered the technology and can produce good quality fibre, paper, and rope, workers are aware that the lack of a market for the fibre could mean that the business will run at a loss, ultimately impacting their economic security and well-being.

What can these case studies tell us about scaling up, knowledge transfer, and sustainability for EFF technologies?

The potentials for EFF technologies to address food and income inequalities, and foster local innovations are promising. In order to ensure benefits for small and marginal farmers and the most vulnerable participants in agro-commodity chains, these technologies must:

- Remain financially accessible to farmers of all income groups, once licensed for sale in different countries.
- Come in package sizes that suit the different needs, farm sizes, and incomes of farmer groups. Small and marginal farmers are unlikely to buy in large quantities and keep excess material for successive seasons. Access to smaller packages that are affordable, and that can be used over a short period of time, is critical.
- Go hand-in-hand with continued education and training on the uses and benefits of the technologies for all relevant actors across product value chains. This might include approaches to initial financing for farmers ('try before you buy') to test the technologies for themselves for a season. These hands-on, farm-level experiences may be more useful for technology uptake than documentation and advertisements.
- Connect with organizations with marketing networks and/or civil society organizations so that entrepreneurial innovations such as banana fibre production are supported, expanded, and become sustainable practices.

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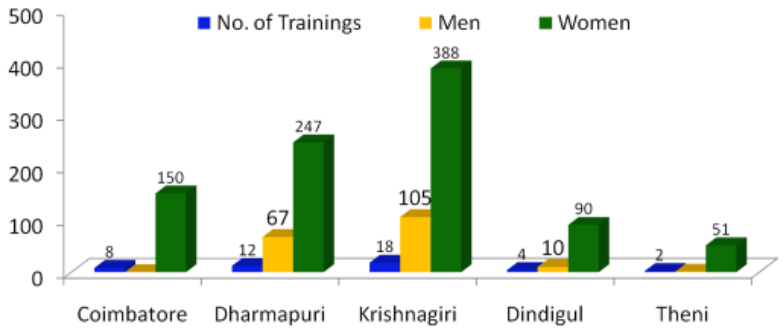
Value Addition Training

Mango is a seasonal fruit being produced and supplied between April and July. Some of the farmers produce off–season mangoes in October- November. In this scenario, there are plenty of mangoes dropped at the beginning of the season (Feb- Mar) which affects the price in the market. In order to use the wastage during the beginning of the season and rejected fruits from the main season, efforts were undertaken to train the women farmers on "Value Added Products of Mango". The Global affairs Canada and IDRC jointly funding the project to TNAU entitled "Enhanced Preservation of Fruits using Nano Technology" in which value addition training was an important component. This was done utilizing the services of MYRADA, an NGO that is associated with this project.

During the past five years' value addition trainings have been conducted regularly to benefit women farmers of Krishnagiri, Dharmapuri, Theni and Dindigul districts of Tamil Nadu. A total of 1109 farmers (926 Women and 183 Men) have been trained to make various value added products of mango (Mango pickle, Vaddu mangai, Mango Chips, Amuchur Powder, Mango Jam, RTS, Squash, Halwa and Mango Bar). The feedbacks about value addition training, acquisition of skills perceived from beneficiaries has been collected immediately after training. Technology adaptation of participants was estimated by conducting follow-up survey from the beneficiaries. Prior to the training, the participants had knowledge in pickle, thokku and vaddu mangai for household purpose only. On completion, they acquired knowledge on commercial production of value added products of mango. The study revealed that, 12.8% of beneficiaries are very much interested to adopt the commercial scale business through collaboration. The beneficiaries got awareness about hygienic and preservative measures during preparation of value added products. The trainees, expressed concerns on marketing the products. They also need financial support for investment, guidelines for product development, infrastructure facilities to execute macro-scale level entrepreneurship. The study recommends the policy should emphasize: increasing agricultural and post-harvest knowledge content in formal education, developing and manifesting a positive attitude and improving skills of potential producers, as well as improving producer's access to resources. The preparation of value added mango food products like pickle, jam, RTS/RTE, mango bar by the members of the Mango Producer's Group (MPGs) resulted in additional savings for the household expenditure. If they adopt pickle preparation as a commercial level per season, they earn Rs. 19,200 per ha as a profit by using mango wastage drawn from one hectare of farm. Overall, the study suggests that value addition training has helped the women farmers to become a successful entrepreneur.

S.No	Location	Trainings	Men	Women	Total
	IDRC Phase II				
1	Dharmapuri	12	67	247	314
2	Krishnagiri	18	105	388	493
3	Dindigul	4	10	90	100
4	Theni	2	1	51	52
Total		36	183	776	959

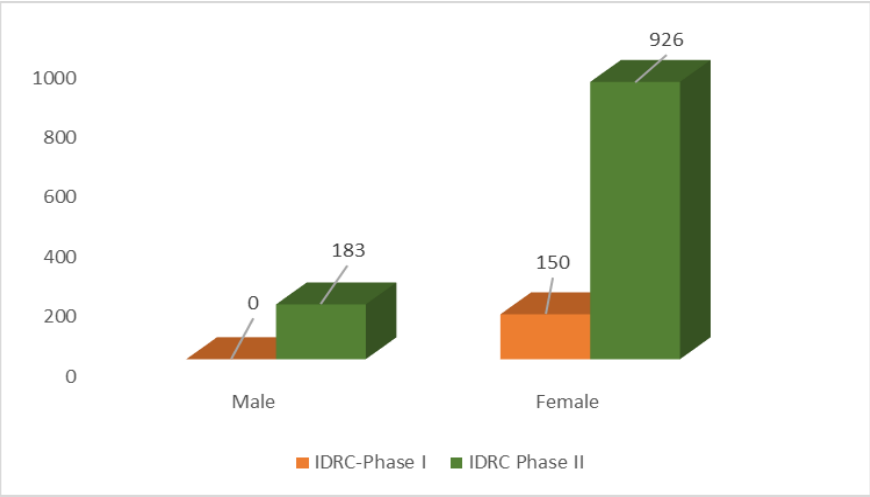
Value Addition Training and Participants



Gender in Mango Value Addition Trainings

S.No	Location	Men	Women
	IDRC I		
1	TNAU,CBE	-	150
	IDRC II		
2	Dharmapuri	67	247
3	Krishnagiri	105	388
4	Dindigul	10	90
5	Theni	1	51
Total		**183**	**926**

Gender disaggregated participation in Phase I & II Trainings



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CIFSRF-Enhancing Preservation of Fruits Using Nanotechnology

BIOSAFETY OF HEXANAL

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2015

EXECUTIVE SUMMARY

Hexanal is chemically hexanaldehyde, a six carbon compound, naturally produced by plants in trace quantities. When the plants are wounded or cut, there is a grassy odour which is chemically referred as “hexanal”. Apart from a wound response, hexanal is widely produced by fruits such as apple, apricot, banana, sweet and sour cherries, citrus peel oils and juices, berries, guava, tomato and much more, as a component of flavor volatiles produced during ripening. This naturally producing volatile compound has an effect on fruit ripening processes when it is externally sprayed.

The scientists (GopinadhanPaliyath and Jayasankar Subramanian) at the University of Guelph, Canada, have extensively studied the use of this compound in extending shelf-life of a wide array of fruits, vegetables and flowers etc. Hexanal inhibits the phospholipase – D enzyme in the skin of the fruits besides slowing down the evolution of ethylene during the ripening processes. These physiological mechanisms facilitate preservation of perishables under storage conditions. The scientists have found that hexanal based effective fresh formulations as a pre-harvest sprays or dip treatment before storage can extend the shelf-life of 2-3 weeks without loss fruit quality, flavor and colour. Even, the vapour of hexanal has a potential to alter the ripening of fruits that resulted in the extension of shelf-life. Such shelf-life extension will help the Asian countries where fruits and vegetables are produced aplenty but the per capita availability is just 50% of their daily requirement due to the post-harvest losses.

The Department of Foreign Affairs, Trade and Development (DFATD), Canada, and International Development Research Center (IDRC), Canada, have jointly funded a research program on “Enhanced Preservation of Fruits using Nanotechnology” under the Canadian International Food Security Research Fund (CIFSRF). Researchers at the University of Guelph, Canada, Tamil Nadu Agricultural University, India and Industrial Technology Institute, Sri Lanka, have collaboratively worked on various aspects of hexanal formulations and its effects on fruit orchards, its integration in packaging through bio-nano materials besides wax formulations.

During the past three years, Tamil Nadu Agricultural University, Coimbatore, has undertaken assiduous efforts to determine the safety of the hexanal and its formulations. The safety tests were done as stipulated by the OECD (Organization for Economic Cooperation and Development). In order to test the safety of the chemical, a set of protocols were adopted to examine the ill-effects against beneficial microorganisms, honey bees, natural enemies, earthworms, fish and human cell lines. In all the cases, a standard set of treatments were exercised. The data have unequivocally demonstrated that hexanal and its formulations had no ill-effects against all trophic levels even after three times recommended doses of hexanal formulations were tested. The orchards in which hexanal spray was taken up had least impact on the occurrence of honeybees or natural enemies. Interestingly, while beneficial organisms had no deterrence against hexanal or its formulations, the incidence of pests such as fruit fly and post-harvest disease causing pathogens. More research is requited to precisely predict the mechanism involved deterrence against pests while allowing natural enemies and honey bees freely move around in the sprayed orchards. The exposure to various types of human cell lines were tested at various concentrations at various types of cells such as cancer cells, liver cells and epithelial cells in order to ensure that the new chemical formulations is absolutely safer. This tests help to understand the safety to humans in the event of direct exposure to hexanal formulations.

The biosafety manual on hexanal is designed to deliver the readers about the biochemistry, mode of action, its interaction with insect ecology, soil fauna and flora besides humans. This is base document with a strong scientific support, we are well positioned to indicate the safety of hexanl against vast spectrum of organisms at all trophic levels. This will provide a data base for commercialization of the product.

CONTENTS

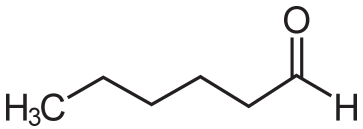
CHAPTER 1

HEXANAL

Hexanal a volatile C6 aldehyde (n-hexanal, hexenals) and its corresponding alcohols are produced in plant tissues upon wounding. Among these, hexanal possesses a “green”-grassy odour. When fruits or vegetables are cut or wounded, emanating odour is primarily due to the presence of volatile C6 aldehydes such as hexanal.. C18unsaturated fatty acids such as linoleic and linolenic acid are the biological precursors of hexanal (Drawert *et al.,* 1966). The enzymatic biosynthetic sequence of reactions that result in the formation of volatiles has been intensively investigated. Lipolytic acyl hydrolase, lipoxygenase, hydroperoxide lyase, (E,Z)-2,3-enal isomerase, and alcohol dehydrogenase are known to be involved in the production of hexanal (Phillips Galliard, 1978). Apart from a wound response, hexanal is widely produced by fruits such as apple, apricot, banana, sweet and sour cherry, citrus peel oils and juices, berries, guava, tomato and much more, as a component of flavor volatiles produced during ripening (Fenaroli, 1975).

1.1. Structure of Hexanal

Hexanal, or hexanaldehyde, is an alkyl aldehyde used in the flavor industry to produce fruity flavors. It has the odour of freshly cut grass. Molecular weight of hexanal is 100. The molecular structure of hexanal is given below:



1.2. Mode of action

Naturally occurring hexanal concentration in plant is extremely small or in traces. However, the exogenously synthesized hexanal application on fruits and vegetables have been shown to extend their shelf-life without associated ill-effects to the fruits or consumers. Fruits and vegetables are degraded during storage due to the action of a wide range of enzymes. One of the important enzymes produced during ripening and senescence of fruits that initiates and propagates membrane degradation is the phospholipase D (PLD). The activities get further aggravated through an autocatalytic cycle comprising several enzymes, alterations in cytosolic calcium and pH, membrane destabilization and inactivation of regulatory enzymes that control cytosolic calcium and pH (eg. ATPases) and enhanced by reactive oxygen species (ROS) produced during enzymatic processes and stress conditions (Paliyath and Droillard, 1992). The catalytic pathway of phospholipid catabolism is illustrated below.

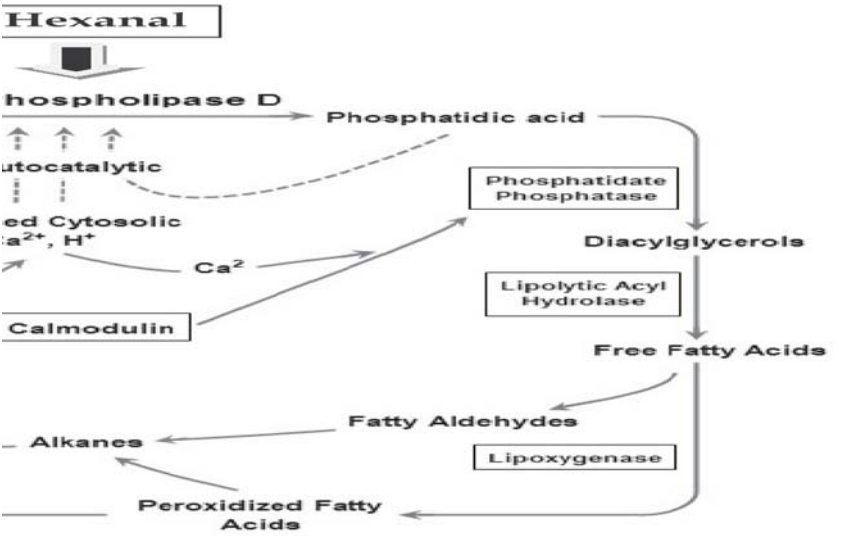


Fig. 1. A diagrammatic model describing the sequences of events that occur during membrane degradation cycle. Phospholipase D acts on phospholipids generating phosphatidic acid which undergo sequential catabolic breakdown downstream. None of these enzymes are able to act on phospholipase D directly. Therefore, once phospholipase D is inhibited, the whole cycle is slowed down. The site of action of hexanal is indicated by arrow.

Indirectly, inhibition of PLD by hexanal leads to a lesser demand for carbon substrates to replenish the phospholipids lost during ripening. Thus, the metabolic precursor Acetyl CoA can be channelled to the biosynthesis of quality enhancing components such as isoprenoids, carotenoids, anthocyanins, phenolic components, etc., as well, increased activity of pentose phosphate pathway leads to increased precursor levels for anthocyanins and NADPH for increased antioxidant enzyme function (Fig. 2).

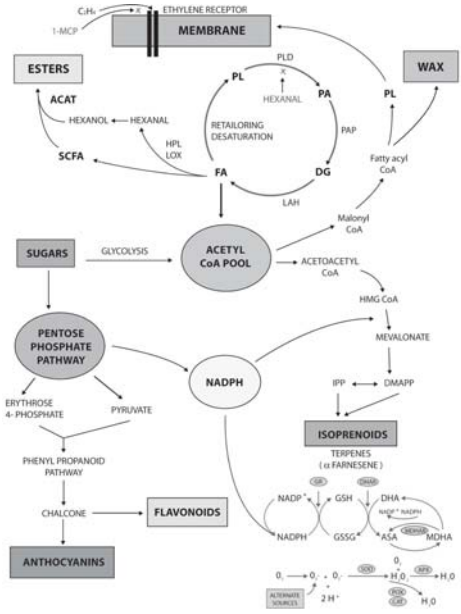


Fig. 2. A diagrammatic representation of
Phospholipase D Inhibition and Metabolite Channelling

1.3. Application of Hexanal for extending shelf-life of perishables

Hexanal has been observed to be a strong inhibitor of PLD action, and diverse technologies for its application for enhancing shelf life and quality of fruits, vegetables and flowers are currently under development (Paliyath *et al.,*1999, 2003; Paliyath and Murr, 2007; Paliyath and Subramanian,2008). But, it is widely recognized that hexanal application inhibits the phospholipase-D resulting in several physiological responses, thus slowing down ethylene stimulation of ripening processes.. These processes may eventually enhance sensory and nutritional qualities, as well as shelf life of fruits (Sharma *et al.,* 2008). Exogenous application of hexanal has been reported to improve the shelf-life of a wide array of fruits and vegetables.

1.3.1. Apple

Effects of hexanal as a component of packaging atmosphere on the shelf life and increase in naturally occurring microbial populations in fresh apple slices during storage at 4 and 15 °C were studied by Lanciotti *et al.* (1999). This volatile molecule significantly extended the shelf life. When added to a modified atmosphere (70% N_2 and 30% CO_2), hexanal was very effective in preventing browning reactions for at least 16 days at 15 °C.

Further, Jun *et al.* (1996) reported that hexanal was actively converted to aroma volatiles in 'Jonagold' and 'Golden Delicious' apple slices, with increases in hexanol and hexyl acetate production. Within 16 hours after treatment, no hexanal could be detected from treated fruit. Since, hexanal was metabolized to aroma-related volatiles by the fruit slices, the hexanal could be a residue-less antifungal agent.

Pre-harvest spray of apple fruits with a hexanal containing aqueous formulations has been shown to reduce premature drop in apples, reduce superficial scald, and increase firmness. Post harvest

dips of apples in a hexanal formulation have also been found to enhance shelf life and quality (Paliyath and Subramanian, 2008).

1.3.2. Cherry

Sharma *et al.* (2010) reported that sweet cherries subjected to pre-harvest spray with a hexanal formulation (enhanced freshness formulation, EFF) had better color, brightness and firmness than unsprayed cherries, even after 30 days of storage at 4 °C. These EFF-treated cherries also showed higher chroma values indicative of enhanced red color. Post-harvest application of hexanal vapour also enhanced the firmness of cherries. Results suggest that a pre-harvest application of EFF combined with post-harvest treatment of hexanal can enhance the quality and shelf life of sweet cherry.

1.3.3. Longan

Porntip *et al.* (2010) reported that hexanal vapour exposure of longan fruits for 2 h at 900 ìL L^{-1} before cold storage reduced the fruit decay, pericarp phenolic content, and increased polyphenoloxidase (PPO) and peroxidase (POD) activities. Overall, the use of hexanal vapour reduced post-harvest disease of longan fruit.

1.3.4. Mangoes

Pre-harvest spray of hexanal formulation (0.02% v/v of hexanal; 1.6 mM) twice at 30 and 15 days prior to harvest enhanced the retention fruits in the orchards for 3 weeks, thus reducing premature fruit drop and prolonging the harvest window , besides enhancing the shelf-life and quality of fruits for another three weeks under cold storage (12°C) conditions. The shelf-life extension of mango fruits was closely associated with reduced physiological loss in weight, respiration rate, and ethylene evolution. These physiological processes collectively contributed for the enhanced shelf-life of fruits (Anon, 2014; Subramanyan *et al.,* unpublished).

1.3.5. Tomato

The effectiveness of hexanal formulations have been conclusively demonstrated through basic and applied research. Post harvest dips of green house grown fresh tomato in hexanal formulation, extended the shelf life for over 45 days (Tiwari and Paliyath, 2011). Recently, Cheema *et al.* (2014) have shown that tomatoes subjected to pre-harvest spray with enhanced freshness formulation (EFF) containing 1 mM hexanal twice a week had better colour, and firmness than untreated fruit and hexanal formulation treated fruit. EFF treated tomatoes also showed low hue angle values indicative of enhanced red colour. Pre-harvest spray with 1 mM hexanal twice a week resulted in higher levels of ascorbic acid and soluble solids in fruit than those subjected to EFF treatment, and the control. Postharvest dip application of harvested tomatoes in 2 mM hexanal as EFF resulted in enhanced brightness and hue angle values, reduced red colour, increased fruit firmness and ascorbic acid content after 21 days of storage, indicative of better quality. The results suggest that hexanal has the potential to enhance shelf-life and quality of greenhouse tomatoes.

1,3.6. Strawberry

Recent studies have shown that preharvest application of hexanal formulation to strawberry fruits resulted In changes in volatile profile of field grown strawberries (Misran *et al.,* 2015). As well, similar treatments have also been shown to enhance fruit mass, and reduce pectinolytic enzymes resulting in increased firmness (Sharkawy, Subramanian, Sullivan, Unpublished). Independent work by Del Monte has also shown reduced fungal decay in strawberry fruits subjected to hexanal vapour exposure.

1.3.7. Guava

Independent investigations at Punjab Agricultural University (Karanbir Singh Gill, manuscript in review) has shown that hexanal

formulation (EFF) is very effective in extending shelf life and quality of guava fruits. Preharvest spray as well as post harvest dips weer beneficial. Shelf life extension up to 28 days were observed as a result of preharvest sprays. Enhancement of quality parameters (firmnesss, ascorbic acid content, consumer preference etc.) were also observed.

Despite the fact that the hexanal formulation is quite effective in a spectrum of fruits and vegetables, it is essential to determine the biosafety of the formulation prior to registration and commercialization. Further, the characterization of hexanal formulation suggests that it can be categorized as “nano-emulsion” which is more effective than the conventional form of treatment owing to uniform and extremely small droplet sizes, typically less than 100 nm range. In addition, high kinetic stability, low viscosity and optical transparency make them very attractive systems for product delivery (Lijuan *et al*., 2007). Biosafety studies have been undertaken in order to assess the impact of hexanal formulation at all trophic levels namely beneficial microorganisms, homey bees, predators and parasites, earthworms, fish and human cell models.

CHAPTER 2

GUIDELINES FOR BIOSAFETY STUDIES

Any new chemical that is to be commercialized should undergo biosafety testing as per the guidelines stipulated by the OECD (Organisation for Economic Co-operation and Development). This is to promote policies that will improve the economic and social well-being of people around the world. The OECD provides a forum in which governments can work together to share experiences and seek solutions to common problems. We work with governments to understand what drives economic, social and environmental change. They have developed a standard set of protocols that are to be followed in order to assess the safety of new chemical formulations.

"The OECD Guidelines for the Testing of Chemicals" are a unique tool for assessing the potential effects of chemicals on human health and the environment. Accepted internationally as standard methods for safety testing, the guidelines are used by professionals in industry, academia and government involved in the testing and assessment of chemicals (industrial chemicals, pesticides, agricultural inputs, etc.). These guidelines are regularly updated with the assistance of thousands of international experts from OECD member countries. OECD Test Guidelines are covered by the "Mutual Acceptance of Data", implying

that data generated in the testing of chemicals in an OECD member country, or a partner country having adhered to the “Decision, in accordance with OECD Test Guidelines and Principles of Good Laboratory Practice” (GLP), be accepted in other OECD countries and partner counties having adherence.

The OECD has set 150 standard operational protocols (SOPs) of the most relevant internationally agreed testing methods used by government, industry and independent laboratories to identify and characterise potential hazards of chemicals. They are a set of tools for professionals, used primarily in regulatory safety testing and subsequent chemical and chemical product notification, chemical registration and chemical evaluation. They can also be used for the selection and ranking of candidate chemicals during the development of new chemicals and products, and in toxicology research. This group of tests cover physico-chemical properties to the decision, for the purposes of assessment and other uses relating to the protection of human health and the environment.

Despite the fact that hexanal is naturally produced by plants in trace quantities, synthetically produced hexanal in combination with a set adjuvants and surfactants make this formulation very unique and warrants safety tests.

CHAPTER 3

HONEY BEES

Honey bees are widely recognized as the indicator biosafety of new chemicals or formulations. Bee activity is very much essential for agricultural production systems especially for cross-pollinated crops. Thus, testing of chemicals against safety of honey bees is a pre-requisite. In order to test the safety of hexanal and its formulations, two sets of experiments namely dry film toxicity and exposure to hexanal treated mango fruits were conducted.

Laboratory experiments

3.3. Preparation of Hexanal Formulation

Hexanal formulation was prepared by mixing 1 ml of hexanal, 10 ml ethanol and 10 ml Tween 20 and volume was made up to 100 ml with double distilled water. In the field experiment the concentrate was diluted 50 times and sprayed to the mango trees to provide complete coverage of fruits until dripping. From the concentrate, 2ml, 4ml, and 6ml volumes were taken in separate standard volumetric flask (100 ml) and made up to 100 ml to prepare 0.02, 0.04, and 0.06 per cent of nanoemulsion of hexanal respectively. Pure hexanal was used as a standard and different concentration prepared by taking 20 µl, 40 µl, 60 µl of pure hexanal in standard volumetric flask (100 ml), and were made

up to 100 ml using 70 percent ethanol, to prepare 0.02, 0.04, 0.06 percent concentration of pure hexanal. respectively. Absolute ethanol (99.9 %) 200 µl was taken and made up to 100 ml in standard 100 ml volumetric flask using distilled water to prepare 0.2 per cent of ethanol. Similarly, 200 µl of Tween 20 was taken and made up to 100 ml in a standard 100 ml volumetric flask using distilled water to prepare 0.2 per cent of Tween 20.

3.4. Dry film toxicity

A laboratory experiment was conducted to assess the toxicity of nanoemulsion of hexanal with eight treatments, along with control replicated three times against honey bees, as per the guidelines given by EPA, (1996). Honey bee, *Aphiscerana indica* F., was used as the test species. Bees were obtained from Apiary,Tamil Nadu Agricultural University, Coimbatore. Healthy bees were collected from the same hive. Immediately after collection, bees were anesthetized by keeping in refrigerator (10^{o}C) for no longer than 3 min. Experiment was conducted with 1 to 7 days old worker bees, and bees used in the test were assigned randomly to treatments and controls. Whatman 40 filter paper was impregnated with the respective concentration of hexanal and placed in a plastic container. To this plastic container, 20 honey bees were released and closed tightly and kept undisturbed for one hour. After contact period of one hour, the honey bees were transferred to fresh container and observation was taken at 1, 3, 6, 12, 24 and 48 hours after treatment, and per cent mortality was recorded. A 50 per cent sugar/water solution was provided ad libitum throughout the holding and testing periods. Experiment was conducted in control lighting, and other standard environmental variables. Temperature was maintained at 25 ± 2^{o}C, with relative humidity between 70 and 80 per cent. Test bees were maintained in the dark except during dosing and observations.

Dry film method

Table 3.1. Toxicity of Hexanal and its Formulations to honey bee (*Aphiscerana indica* F.) – Dry film toxicity

Treatments	Mortality (%)*					
	1 HAT	3 HAT	6 HAT	12 HAT	24 HAT	48 HAT
Hexanal Formulation @ 0.02%	0	0	0	0	0	
Hexanal Formulation @ 0.04%	0	0	0	0	0	
Hexanal Formulation @ 0.06%	0	0	0	0	0	
Pure hexanal @ 0.02%	0	0	0	0	0	
Pure hexanal @ 0.04%	0	0	0	0	0	
Pure hexanal @ 0.06%	0	0	0	0	0	
Ethanol @ 0.2%	0	0	0	0	0	
Tween 20 @ 0.2%	0	0	0	0	0	
Control	0	0	0	0	0	

*Mean of three replications; HAT – Hours after treatment.

The data on the toxicity of hexanal treatments to worker honey bees clearly indicated that either hexanal or its formulation at varying concentrations, or any adjuvants added, had absolutely no impact on the activities of honey bees even after 48 hrs of exposure. The results unequivocally demonstrated that hexanal formulation is safer to honey bees even at 3 times the recommended dose.

3.5. Exposure of honey bees to hexanal treated mango fruits

The contact effect of hexanal through the hexanal treated mango fruit varieties on honey bees were assessed using the fruits treated with hexanal at different intervals**.** Freshly harvested mango fruits of varieties *viz*., Neelum, Bangalora and Banganapalli were collected from Cumbum and stored at 15° C. Two fruits from each treatment were taken in a polythene cover and 10 honey bees were released into the polythene covers, and kept tightly closed. These werekept undisturbed and sufficient pin holes were made for aeration. Observation was taken at 1, 3, 6, 12, 24 and 48 h after treatment and mortality (%) was recorded.

Exposure of honey bees to hexanal treated fruits

Table 3.2. Toxicity of hexanal treated mangoes varieties to the honey bees *Aphis ceranaindica*F.

Treatments	Mortality (%)*																	
	Neelum (HAT)						Bangalora (HAT)						Banganapalli (HAT)					
	1	3	6	12	24	48	1	3	6	12	24	48	1	3	6	12	24	48
Hexanal spray 30 DBH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hexanal spray 30 & 15 DBH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hexanal spray 15 DBH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control (Unsprayed)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*****Mean of three replications; HAT – Hours after treatments.

The data vividly indicated that the honey bee workers had no mortality of bees as a result of their exposure to fruits that were sprayed with hexanal formulation even after 48 hrs. The data further reinforces that there is no deterrence of hexanal formulation against honey bees. The two set of experiments clearly have shown that the hexanal formulation had absolutely no impact on honey bee activities even at 3 times of recommended dose of spray.

CHAPTER 4

NATURAL ENEMIES

Predators and parasites are natural enemies of crop pests and they are referred to as beneficial insects in the agricultural or horticultural production systems. While evaluating the new molecule of pesticides or any other formulations, we need to ensure that predators and parasites are not disturbed. The hexanal formulation spray is often recognized by the farmers to have a strong odor that persists even after several hours of spray. This grassy odor may create a repellent action which may likely cause anecological imbalance. In order to evaluate if such disturbances occurred after hexanal formulation spray, the following study was conducted

1.3. Safety of Hexanal to Natural Enemies

Laboratory experiments were conducted with nine treatments (Hexanal formulations at three concentrations 0.02%, 0.04%, 0.06%; pure hexanal 0.02%, 0.04%, 0.06%; Tween 20 and Ethanol, along with control) replicated thrice in a CRD. The experiment was to assess the safety of hexanal nanoemulsion to the egg parasitoid *Trichogramma chilonis* Ishii (Prabhu, 1991) and an insect predator *Chrysoperla zastrowi arabica* (Esben – Petersen), (Shukla *et al.*, 1995 and Reddy and Divakar, 1998).

1.4. Methodology

1.4.7. Egg Parasitoid (*Trichogramma chilonis)*

A. Adult emergence

Trichogramma egg cards were purchased from the Department of Agricultural Entomology, TNAU. The cards were exposed to nine treatments, and were cut into bits of 1x1 cm^2. Treated egg cards were dried and put into separate test tubes (18 x 150 mm), and closed tightly with cotton. The number of wasps that emerged from each treatment was recorded after 48 hrs and the emergence (%) was worked out using the formula,

$$\text{Adult emergence (\%)} = \frac{\text{No. of wasps emerged (eggs with emergence slit)}}{\text{Total no. of eggs in 1 cm}^2} \times 100$$

The mortality data was categorized as follows (Kuttalam, 1999)

Mortality (%)	Category
d" 50	Harmless
50 – 70	Slightly harmful
80 – 99	Moderately harmful
> 99	Harmful

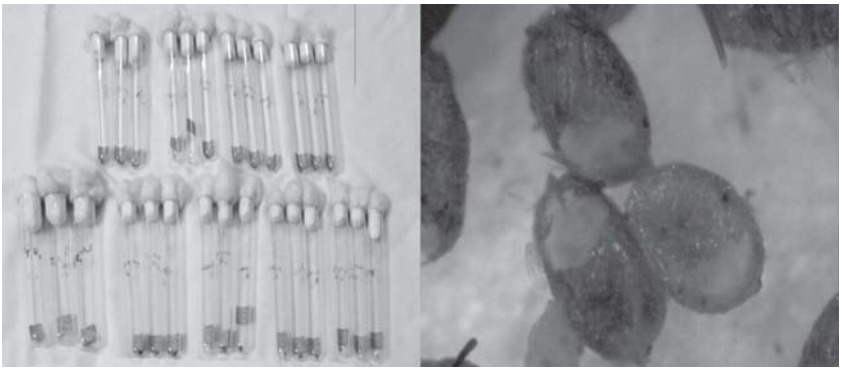
Table 4.1 Effect of hexanal on adult emergence of *Trichogramma chilonis*

Treatments	Adult emergence (%)*		
	Experiment I	Experiment II	Mean
Hexanal Formulation @ 0.02%	97.6 (81.12)[a]	96.7 (79.57)[a]	97.15
Hexanal Formulation @ 0.04%	97.21 (80.43)[a]	96.01 (78.51)[a]	96.61

Hexanal Formulation @ 0.06%	95.96 (78.45)[a]	95.6 (77.93)[a]	95.78
Pure hexanal @ 0.02%	95.34 (77.57)[a]	95.78 (78.18)[a]	95.56
Pure hexanal @ 0.04%	94.03 (75.89)[a]	95 (77.11)[a]	94.51
Pure hexanal @ 0.06%	94.83 (76.90)[a]	93.79 (75.60)[a]	94.31
Ethanol @ 0.2%	92.4 (74.03)[a]	93.7 (75.50)[a]	93.05
Tween 20 @ 0.2%	93.1 (74.80)[a]	94.98 (77.09)[a]	94.04
Control	99.25 (85.08)[a]	99.01 (84.33)[a]	99.13

*Mean of three replications; Figures in parentheses are *arc sine* transformed values; In a column, means followed by a common letter(s) are not *Mean of three replications; Figures in parentheses are *arc sine* transformed values; In a column, means followed by a common letter(s) are not significantly different by DMRT (P=0.05).

Emergence of egg parasitoid



The effect of hexanal treatments on the adult emergence of *T. chilonis*is is given in Table 4.1. The results had indicated that all the treatments exerted lesser impact on the emergence of adults. The mean adult emergence of two experiments ranged from 97.15 to 93.05 per cent in different treatments. Hexanal at the field recommended dose (0.02%) recorded 97.15 per cent adult emergence while higher dose (0.06%) registered 95.78 per cent. On the other hand, pure hexanal (0.06%) treated cards recorded 94.31 per cent adult emergence which is statistically at par with untreated check (99.1%). The data has clearly indicated that the adult emergence of egg parasitoids was not affected by hexanal formulation spray at the recommended dose of concentration (0.02%), but there was a slight reduction in adult emergence of 1-2% when the concentrations were increased by 2-3 times.

A. Parasitisation study

Corcyra egg cards were sprayed with hexanal alone or its formulation at various concentrations as explained earlier. The treated egg cards were cut into bits of 2 x 1 cm^2 and the *Trichogramma* parasitized egg cards into 1x1 cm^2. One bit of treated egg card and one bit of parasitized egg card were put into a test tube (18 x 150 mm) and the number of parasitized eggs (eggs appearing black and plumy) were recorded after 48 h using optical microscope. The parasitisation (%) was worked out using the formula,

$$\text{Parasitisation (\%)} = \frac{\text{No. of parasitised eggs}}{\text{Total no. of } \textit{Corcyra} \text{ egg}} \times 100$$

Parasitized Eggs

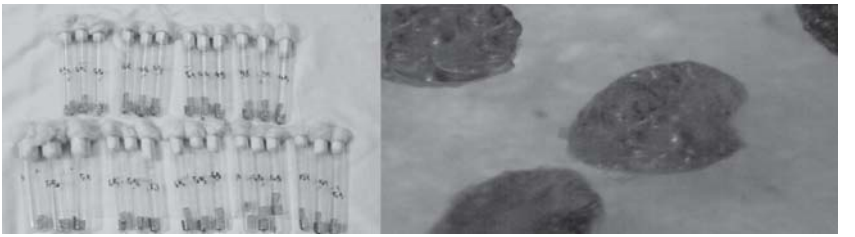


Table 4.2. Toxicity of nanoemulsion of hexanal to of *Trichogramma chilonis* Ishii. - Parasitization

Treatments	Parasitization (%)		
	Experiment I	Experiment II	Mean
Hexanal Formulation @ 0.02%	96.69 (79.55)[a]	97.2 (80.41)[a]	96.94
Hexanal Formulation @ 0.04%	96.49 (79.24)[a]	96.57 (79.36)[a]	96.53
Hexanal Formulation @ 0.06%	95.87 (78.32)[a]	95.31 (77.53)[a]	95.59
Pure hexanal @ 0.02%	95.74 (78.13)[a]	94.57 (76.56)[a]	95.15
Pure hexanal @ 0.04%	94.49 (76.46)[a]	93.99 (75.84)[a]	94.24
Pure hexanal @ 0.06%	94.7 (76.73)[a]	92.67 (74.32)[a]	93.68
Ethanol @ 0.2%	93.14 (74.85)[a]	92.57 (74.21)[a]	92.85
Tween 20 @ 0.2%	96.62 (79.44)[a]	95.11 (77.26)[a]	95.86
Control	98.86 (83.92)[a]	99.2 (84.91)[a]	99.03

*Mean of three replications; Numbers in parentheses are arc sine transformed values; In a column, means followed by a common letter(s) are not significantly different by DMRT (P=0.05).

The hexanal treatments had very little impact on the parasitization of *T. chilonis*. The mean parasitization in 0.02%, 0.04% and 0.06% of hexanal formulation treatments were 96.9, 96.5 and 95.6%, respectively. The pure hexanal spray had a similar trend of response, but slightly lower values of parasitization. Further, Ethanol (0.2%) or Tween 20 by itself had slightly inhibitory effects on parasitization.

4.2.2. Effect of Hexanal on Predator (*Chrysoperla zastrowi arabic)* (Esben – Petersen).

A. Grub emergence

Chrysoperla zastrowi arabic egg cards were purchased form Department of Agricultural Entomology, TNAU, Coimbatore. The cards were sprayed with different concentration of hexanal as indicated in the previous section and were cut into bits of 1 x 1 cm^2. Treated egg cards were dried under shade and put into separate test tubes (18 x 150 mm) and the number of adults that emerged from each treatment was recorded after 48 hrs using an optical microscope. The emergence (%) was calculated using the formula,

$$\text{Grub emergence (\%)} = \frac{\text{No. of wasps emerged (eggs with emergence slit)}}{\text{Total no. of eggs in 1 cm}^2} \times 100$$

Grub emergence

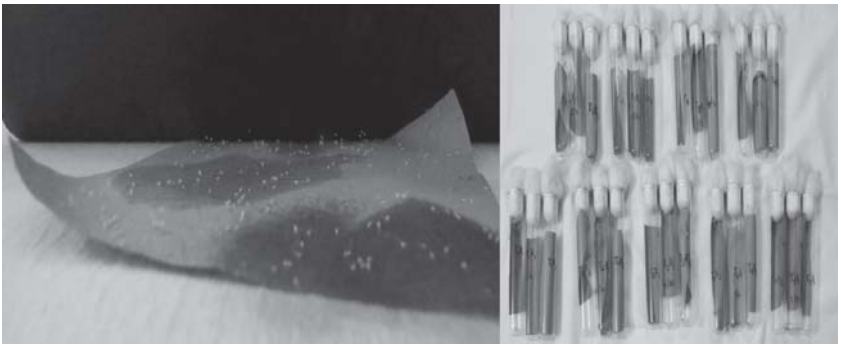


Table 4.3. Effect of hexanal on *Chrysoperla zastrowi arabica* (Esben – Petersen) – Grub emergence

Treatments	Grub emergence (%)		
	Experiment I	Experiment II	Mean
Hexanal Formulation @ 0.02%	90.71 (72.30)[a]	93.24 (74.96)[a]	91.97
Hexanal Formulation @ 0.04%	84.02 (66.47)[a]	86.09 (68.13)[a]	85.05
Hexanal Formulation @ 0.06%	81.43 (64.50)[a]	84.18 (66.59)[a]	82.80
Pure hexanal @ 0.02%	83.87 (66.35)[a]	85.88 (67.96)[a]	84.87
Pure hexanal @ 0.04%	81.42 (64.50)[a]	81.49 (64.55)[a]	81.45
Pure hexanal @ 0.06%	79.87 (63.37)[a]	80.27 (63.66)[a]	80.07
Ethanol @ 0.2%	79.43 (63.06)[a]	79.68 (63.23)[a]	79.55
Tween 20 @ 0.2%	82.71 (65.46)[a]	81.72 (64.72)[a]	82.21
Control	94.91 (77.00)[a]	95.7 (77.07)[a]	95.30

*Mean of three replications; Figures in parentheses are arc sine transformed values; In a column, means followed by a common letter(s) are not significantly different by DMRT (P=0.05).

The mean egg hatchability observed in untreated check was 95.30 per cent (Table 4.3). Among the treatments, recommended dose of hexanal formulation @ 0.02 per cent treated cards showed a grub emergence of 91.97 which is comparable to control. Even at higher concentrations of 0.06%, the grub emergence was lower but statistically comparable to control.

B. Grub Mortality

i. Larval feeding method

Eggs of *C. cephalonica* were exposed to UV radiation of 15 W for 15 min to kill the embryo. The UV killed *Corcyra* eggs were taken and sprayed with different treatments as mentioned. The treated eggs were shade dried for 15 min and then transferred to plastic tray (plates) with 24 wells @ 1 cc per well. Second instars *C. zastrowi arabica* grubs were transferred to these wells @ 1 grub per well. After the grubs completely fed the hexanal treated eggs, the grubs were provided with untreated *Corcyra* eggs till pupation. Observations were made on the grub mortality (12, 24 and 48 h after treatment), pupation and adult emergence.

$$\text{Grub mortality (\%)} = \frac{\text{No. of grubs dead}}{\text{Total number of grubs}} \times 100$$

Larval Feeding Method ***Direct Spray Method***

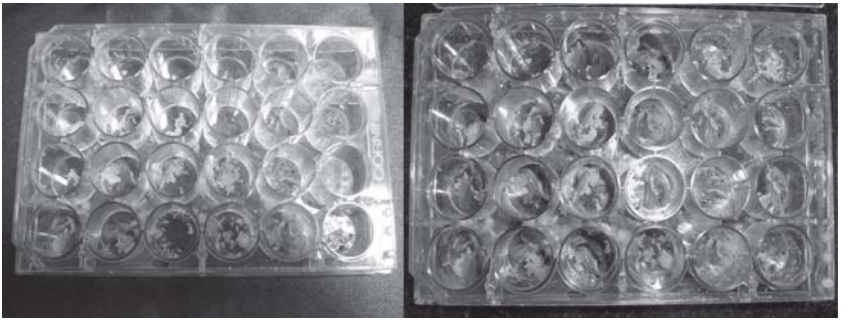


Table 4.4. Toxicity of nanoemulsion of hexanal to *Chrysoperla zastrowi arabica* (Esben – Petersen) - Larval feeding method

Treatments	Larval feeding			Pupation (%)	Adult emergence (%)
	Mortality of grubs (%)*				
	12 HAT	24 HAT	48 HAT		
Hexanal Formulation @ 0.02%	0	0	0	100.00	100.00
Hexanal Formulation @ 0.04%	0	0	0	100.00	100.00
Hexanal Formulation @ 0.06%	0	0	0	100.00	100.00
Pure hexanal @ 0.02%	0	0	0	100.00	100.00
Pure hexanal @ 0.04%	0	0	0	100.00	100.00
Pure hexanal @ 0.06%	0	0	0	100.00	100.00
Ethanol @ 0.2%	0	0	0	100.00	100.00
Tween 20 @ 0.2%	0	0	0	100.00	100.00
Control	0	0	0	100.00	100.00

*Mean of three replications, HAT – Hours after treatment

The data from diet contamination or larval feeding bioassay clearly indicated that hexanal and its formulation had no toxic effects either on pupation or adult emergence. Thus, hexanal formulation is absolutely safer to *C. zastrowi arabica* (Table 4.4).

i. Direct Spray Method

Another study, hexanal spray was done directly on the grubs using an atomizer. Eggs of *C. cephalonica* were exposed to UV radiation of 15 W for 15 min to kill the embryo. Second instar *C. zastrowi arabica* grubs were taken and sprayed with hexanal formulations, having concentrations of hexanal as indicated in 3.1.3. Hexanal treated grubs were left in the plastic tray (plates) containing 24 wells @ 1 grub per well and provided with 1cc of UV treated *Corcyra* eggs per well. Observations were made on the grub mortality at 12, 24 and 48 hrs after treatment and the pupation and adult emergence were noted.

Table 4.5. Toxicity of nanoemulsion of hexanal to *Chrysoperla zastrowi arabica* (Esben – Petersen) - Direct spray Method.

Treatments	Direct Spray			Pupation (%)	Adult emergence (%)
	Mortality of grubs (%)*				
	12 HAT	24 HAT	48 HAT		
Nano emulsion @ 0.02%	0	0	0	100.00	100.00
Nano emulsion @ 0.04%	0	0	0	100.00	100.00
Nano emulsion @ 0.06%	0	0	0	100.00	100.00
Pure hexanal @ 0.02%	0	0	0	100.00	100.00
Pure hexanal @ 0.04%	0	0	0	100.00	100.00
Pure hexanal @ 0.06%	0	0	0	100.00	100.00
Ethanol @ 0.2%	0	0	0	100.00	100.00
Tween 20 @ 0.2%	0	0	0	100.00	100.00
Control	0	0	0	100.00	100.00

*Mean of three replications, HAT – Hours after treatment.

Hexanal treatments to the *Chrysoperla* grubs was studied using direct spray method, and found no toxicity effects on the predators. There was no grub mortality in any of the treatments, and showed 100 per cent pupation and adult emergence (Table 4.5.). The data clearly indicated the safety of hexanal formulation to predator population.

Overall, the biosafety tests conducted for hexanal and its formulations against predators and parasites have demonstrated that there is absolutely no harmful effects either during any of the development stages such as grub emergence, pupation or adult emergence. Further, even at very high concentrations, hexanal had least impacts on the natural enemies which eventually preserved the natural environment.

CHAPTER 5

EARTHWORMS

Earthworms are widely believed as the “Farmers Friend” as its activities in soil improve the physical conditions of the soil which closely coincided with the microbial activities. Since, these macro-fauna are beneficial, new molecules of any pesticides or agri-inputs must not deter their activities. Most of the pesticides carrying Cu or Zn are known to have ill-effects on *Eudrilus eugeniae* (Reinecke *et al*., 1997). Reddy and Reddy (1992). Application of endosulfan at 3 ml l^{-1} recorded complete mortality of the population of *Drawida willisi* Mich. and *Lampito mauritii* Kinberg. The effects of sub-lethal concentrations of lead nitrate on the growth and reproduction of the African composting earthworm species, *Eudrilus eugeniae*, was studied by exposing worms in an organic substrate to lead-nitrate-contaminated food over a period of 76 days. The results revealed that growth was initially affected negatively by the presence of lead, while the maturation rate and cocoon production were not affected (Maboeta *et al.*, 1999). Carbaryl, imidacloprid and cyfluthrin had a larger negative effects on earthworms (Magdel *et al.,* 2002). Considering the ill-effects of pesticides on earthworms, efforts were undertaken to determine whether there is an impact of hexanal formulation on earthworms.

The effect of hexanal on earthworm *E. eugeniae* was tested using artificial soil test method proposed by Edwards and Bohlen (1992). The

culture of *E. eugeniae* was obtained from a vermicompost unit at Udumalpet (Vermi Gold Organics, Erisanampatti, Udumalpet). Garden soil and FYM (mixed in the ratio (2:1)) were taken in the tubular plastic tubs (12 x 4 cm) and treated with different treatments as indicated in earlier chapters. A number of 20 earthworms washed cleanly in water, were placed on the top of the substrate. The set up was kept under shade and covered with kada cloth. After (every 120 hrs) 5 days, 50 g of FYM was mixed inside the container and water lost by evaporation was replaced daily. The numbers of live earthworms were counted and the weight of the worms were recorded before release and after experimental period of 30 days. Earthworms were considered dead if they did not respond to a gentle mechanical stimulus.

Earthworms before and after the exposure to hexanal formulation

The study of the toxicity of hexanal treatments on earthworms had indicated no ill-effects (Table 5.1). Incremental concentrations of hexanal formulations at 0.02%, 0.04% and 0.06% had survival of earthworms at 100%, 98.3% and 96.7%, respectively. Indeed, the average weight of earthworms had progressively increased by three to four times regardless of concentrations of hexanal formulation (Table 5.1).

Table 5.1. Effect of Nanoemulsion of hexanal on the weight of the earthworms *Eudrillus eugeniea* (Kinberg)

Treatment	Weight of 20 earthworms (g)			% of Survival
	Initial weight	15th day	30th day	
Nano emulsion @ 0.02%	1.22 (1.10)[a]	3.27 (1.80)[a]	5.08 (2.25)[a]	100.00 (90.04)[a]
Nano emulsion @ 0.04%	1.42 (1.19)[a]	3.11 (1.76)[a]	5.23 (2.28)[a]	98.33 (82.61)[a]
Nano emulsion @ 0.06%	1.36 (1.16)[a]	3.21 (1.79)[a]	5.17 (2.27)[a]	96.66 (79.51)[a]
Pure hexanal @ 0.02%	1.36 (1.16)[a]	3.25 (1.80)[a]	5.21 (2.28)[a]	100.00 (90.04)[a]
Pure hexanal @ 0.04%	1.22 (1.10)[a]	3.12 (1.76)[a]	4.99 (2.23)[a]	98.33 (82.61)[a]
Pure hexanal @ 0.06%	1.36 (1.16)[a]	3.20 (1.78)[a]	5.12 (2.26)[a]	98.33 (82.61)[a]
Ethanol @ 0.2%	1.23 (1.11)[a]	3.27 (1.80)[a]	5.06 (2.25)[a]	100.00 (90.04)[a]
Tween 20 @ 0.2%	1.36 (1.16)[a]	3.26 (1.80)[a]	5.13 (2.26)[a]	100.00 (90.04)[a]
Control	1.56 (1.25)[a]	3.40 (1.84)[a]	5.36 (2.31)[a]	100.00 (90.04)[a]

*Mean of three replications; Figures in parentheses are square root transformed values; In a column, means followed by a common letter(s) are not significantly different by DMRT (P=0.05).

The biosafety studies on earthworms are also shown no toxicity or deterrence effects caused by either hexanal or its formulations even at 2-3 times of recommended dose of pre-harvest sprays.

CHAPTER 6

BENEFICIAL MICROORGANISMS

Soil organisms are essential for the bio -geocycling of plant nutrients. These are known to be indicators of soil health. Any introduction of organic/ inorganic chemicals such as pesticides, growth regulators etc., may have an effect on the beneficial microorganisms. Hexanal was taken as a model molecule to study the relationship between the antifungal activity of a volatile compound and its vapor pressure. The different indices taken into consideration evidenced the importance of the hexanal vapor pressure for its bioactivity against the fungal mould. Moreover, the temperature increase enhanced the hexanal antifungal activity, due to its effect on vapor pressure (Gardini *et al.,* 1997). According to Lamba (2007) trypticase Soy agar plates were pre-inoculated with *Salmonella typhimurium,* and the plates were exposed to 50µL hexanal, with exposure time interval (30, 45, 60, 75, 90 and 120 min) to detect the antimicrobial activity of hexanal. There was no inhibition in 30 min time in both control and hexanal exposed cultures, while no growth was found at 90th min in hexanal exposed culture, and no inhibition was observed in control. One per cent emulsion produced effective bactericidal activity against *Bacillus cereus, Bacillus subtilis, Haemophilus influenzae, Neisseria gonorrhoeae, Streptococcus pneumoniae,* and *Vibrio cholerae* in 15 minutes while most enteric gram-negative bacteria were resistant (Hamouda *et al.,* 2010).

T**oxicity Studies**

6.1. Against fungal cultures (*Trichoderma harzianum* Rifai and *Trichoderma viride* Pers)

Laboratory experiments were conducted to assess the safety of hexanal against the fungal cultures used in biocontrol. Microbial Type Culture Collection (MTCC) fungal cultures *Trichoderma viride*, *Trichoderma harzianum* were obtained from Institute of Microbial Technology (IMTECH), Chandigarh. Agar well method (Saba *et al.*, 2012; Kavitha *et al.,* 2011) and paper disc method were carried out to study the toxicity of hexanal to the fungi.

6.1.1. Culture inoculation

Rose bengal broths were prepared and autoclaved at 121^{o} C (15 lbs) for 20 minutes. To the rose bengal broths MTCC mother culture of *T. viride* and *T. harzianum* were added and inoculated separately in the laminar air flow chamber and incubated at 32 ± 2°C in an incubator for two days. The rose bengal agar medium was poured into the sterilized petri plates under aseptic condition and allowed to solidify. The fungal spores from the broth were placed on the petri plates and incubated at 32 ± 2°C in an incubator for further studies.

a. Paper disc method

Rose bengal agar medium was prepared and autoclaved at 121^{o} C (15 lbs) for 20 minutes and poured into the petri plates and allowed to solidify. Experiments were conducted with eight treatments along with control and three replications. 8 mm circular disc of 7 days old *T. viride*, *T*. *harzianum* was transferred to the appropriate solidified plates at the centre using 8 mm dia cork borer under aseptic condition. Then the paper discs made from nitrocellulose paper impregnated with respective treatments (filtered using PTFE (0.22µm) syringe filter) were placed on the surface of the plates at four places and were incubated at 32 ± 2°C in an incubator. The radial growth of *T. viride*, *T. harzianum* and the zone

of inhibition was observed and measured after 24, 48 and 72 h after inoculation.

b. Agar well method

Culturing of fungus was done similar to earlier paragraphs. Wells were cut at four places using cork borer in each of the plates and 20 µl of respective treatments (filtered using PTFE (0.22µm) syringe filter) were added into the well in the respective plate. The plates were incubated at 32 ±2°C in an incubator. The radial growth of *T. viride*, *T. harzianum* and the zone of inhibition was observed and measured after 24, 48 and 72 h after inoculation.

Fungal culture T. viride in (a) Paper disc (b) Agar well method

Fungal culture** T. harzianum **in (a) Paper disc (b) Agar well method

Table 6.1. Toxicity of nanoemulsion of hexanal to MTCC fungal cultures -*Trichoderma viride* Pers and *T. harzianum* Rifai

Treatment	*Trichoderma viride*		*Trichoderma harzianum*	
	Paper disc	Agar well	Paper disc	Agar well
Nano emulsion @ 0.02%	0.00	0.00	0.00	0.00
Nano emulsion @ 0.04%	0.00	0.00	0.00	0.00
Nano emulsion @ 0.06%	0.00	0.00	0.00	0.00
Pure hexanal @ 0.02%	0.00	0.00	0.00	0.00
Pure hexanal @ 0.04%	0.00	0.00	0.00	0.00
Pure hexanal @ 0.06%	0.00	0.00	0.00	0.00
Ethanol @ 0.2%	0.00	0.00	0.00	0.00
Tween 20 @ 0.2%	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00

*Mean of three replications

The data on MTCC (Microbial Typical Culture Collection) fungal cultures exhibited no inhibition to hexanal or its formulation regardless of concentrations (Table 6.1)

6.2. Toxicity against bacterial cultures (*Pseudomonas fluorescens* and *Bacillus subtilis*)

Laboratory experiments were conducted to assess the safety of hexanal against the bacterial cultures. MTCC bacterial cultures *Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from Institute of Microbial Technology (IMTECH), Chandigarh. Agar well method and paper disc method were used to study the toxicity of hexanal.

6.2.1. Culture inoculation

Nutrient broths were prepared and autoclaved at 121^{o} C (15 lbs) for 20 mins . To the nutrient broths MTCC mother culture of *P.*

fluorescens, B. subtilis were inoculated in a laminar air flow chamber and kept with shaking @ 60 rpm for 24 hrs.

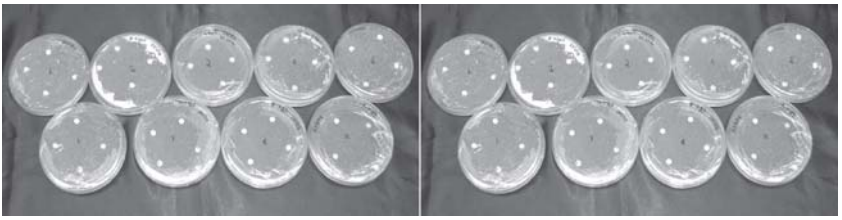
6.2.2. Paper disc method

Nutrient agar medium was prepared and autoclaved at 121° C (15 lbs) for 20 minutes. To a warm medium, 3ml of grown *P. fluorescens, B. subtilis* culture from the broth was added. After adding the culture the medium was poured into the petri plates and allowed to solidify under aseptic conditions. Paper discs made from nitrocellulose paper impregnated with respective hexanal formulations and controls(filtered using PTFE (0.22µm) syringe filter) as referred in 3.1.3 were placed on the surface of the medium at four place sand were incubated at 32 ±2°C in an incubator. Growth of *P. fluorescens, B. subtilis* and the zone of inhibition were observed and the diameter of growth zone measured after 24, 48 and 72 h after inoculation.

6.2.3. Agar well method

Nutrient agar medium was prepared and autoclaved at 121° C (15 lbs) for 20 minutes. Experiments were conducted with eight treatments and the respective controls along with three replications each. To a luke warm medium, 3ml of grown *P. fluorescens,* and *B. subtilis* culture from the broth was added. The culture added medium was poured into the petri plates and made to solidify under aseptic condition. Wells were cut at four places using cork borer of 8m dia in each of the plates and 20 µl of the respective treatments (filtered using PTFE (0.22µm) syringe filter) as referred in 3.1.3 were added to the well in the respective plate. The plates were incubated at 32 ±2°C in an incubator. Growth of *P. fluorescens, B. subtilis* and the zone of inhibition was observed and measured with scale after 24, 48 and 72 h after inoculation.

Bacterial culture P. fluorescens under (a) Paper disc (b) Agar well methods



Bacterial culture** B. subtilis **under (a) Paper disc (b) Agar well methods

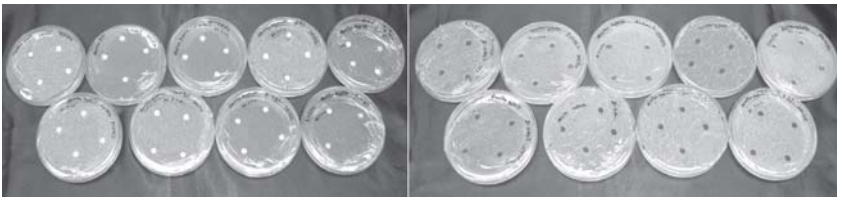


Table 6.2. Toxicity of nanoemulsion of hexanal to MTCC bacterial cultures

Treatment	*Pseudomonas fluorescens*		*Bacillus subtilis*	
	Paper disc	Agar well	Paper disc	Agar well
Nano emulsion @ 0.02%	0.00	0.00	0.00	0.00
Nano emulsion @ 0.04%	0.00	0.00	0.00	0.00
Nano emulsion @ 0.06%	0.00	0.00	0.00	0.00
Pure hexanal @ 0.02%	0.00	0.00	0.00	0.00
Pure hexanal @ 0.04%	0.00	0.00	0.00	0.00
Pure hexanal @ 0.06%	0.00	0.00	0.00	0.00
Ethanol @ 0.2%	0.00	0.00	0.00	0.00
Tween 20 @ 0.2%	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00

*Mean of three replications

The data on MTCC (Microbial Typical Culture Collection) bacterial cultures also exhibited no inhibitory effects to hexanal or its formulation regardless of concentrations (Table 6.2)

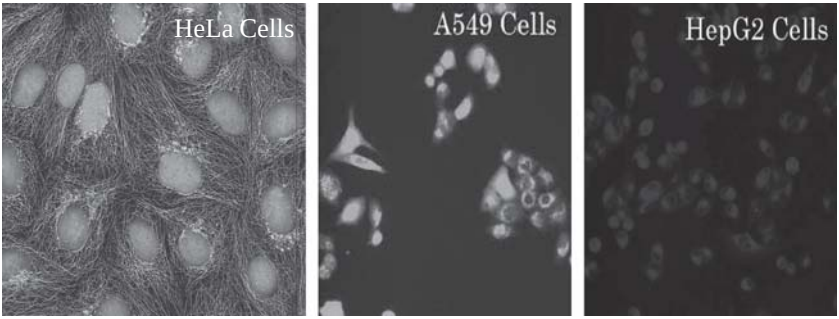
CHAPTER 7

HUMAN CELL LINES

One of the requirements to test the safety of the new chemical formulations is the biosafety against mammalian cells. Cell culture can be used to screen for toxicity both by estimation of the basal functions of the cell (i.e. those processes common to all types of cells) or by tests on specialized cell functions. General toxicity tests, aimed mainly at detection of the biological activity of test substances, can be carried out on many cell types (e.g. fibroblasts, HeLa and hepatoma cells). A number of parameters including vital staining, cytosolic enzyme release, cell growth and cloning efficiency are used as end-points to measure toxicity. These tests will help us gain insights of possible interactions in human systems.

7.1. HeLa cells, A549 cells, HepG2 cells

HeLa cells derived from cervical cancer cells were used *invitro* for cancer studies, A549 cells are adenocarcinomic human alveolar basal epithelial cells, and HepG2 is a perpetual cell line which was derived from the liver tissue. These three human cell lines were used for the toxicity study of nanoemulsion of hexanal. The studies were conducted at Chromus Biotech lab, Bengaluru.



7.1.1. LDH assay

Lactate dehydrogenase (LDH), which is a soluble cytosolic enzyme present in most eukaryotic cells, was released into culture medium upon cell death due to damage of plasma membrane. Increase in LDH activity in culture supernatant is proportional to the number of lysed cells. LDH cytotoxicity assay kit provided a colorimetric method to measure LDH activity using reaction cocktails containing lactate, NAD^+, diaphrose and INT (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride). LDH catalyses the reduction of NAD^+ to NADH in the presence of L-lactate, while the formation of NADH is measured in a coupled reaction in which the tetrazolium salt INT isreduced to a red formazan product. The amount of the highly coloured and soluble formazan was measured at 490 nm spectrophotometrically.

Accurately, 200 µl of cells were seeded on to 96 well plate (1x105 ml^{-1}) 24 hr prior to treatment with test sample. Cells were treated with different concentration of hexanal (30000, 10000, 3333, 1111, 370 and 123 ppm) and incubated for 72 hrs. LDH standards of 200mU, 100mU, 50mU, 25mU, 12.5mU were made. One hundred µl of each of the standards prepared were transferred into the appropriate wells in a new 96 well plate. One hundred µl of each supernatant was transferred from each well of the cultured cells to corresponding wells on the new plate. One hundred µl of Reaction Solution (Mix of INT, NAD, LDH assay buffer, PMS, Lactate) were added to each well and incubated at 37°C for 10

minutes. Absorbance was read at 490 nm. Sample concentration was determined using the following formula,

LDH activity (mU) = (A 490 nm – Y intercept)/ slope (C)

$$\text{Total LDH Activity (mU/ml) in sample} = \frac{\text{Value from LDH activity (mU)}}{\text{sample volume assayed (usually 0.1 ml)}} X$$

where,
mU – milli Unit,
ml - milli litre.

7.1.1. MTT assay

The MTT assay is a colorimetric assay for measuring the activity of cellular enzymes that reduce the tetrazolium dye, MTT, to its insoluble formazan, giving a purple color. Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. The absorbance of this coloured solution was quantified by measuring at a wavelength of 570nm by a spectrophotometer. The absorption maximum is dependent on the solvent employed. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore, conversion is directly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced through a dose-response curve. Mitochondrial dehydrogenases of viable cells cleaves the tetrazolium ring, yielding purple MTT formazan crystals which is insoluble in aqueous solutions. The crystals could be dissolved in acidified isopropanol. The resulting purple solution was spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed, and an increase in absorbance.

Accurately, 200 µ l of cells were seeded on to 96 well plate ($1x10^5 ml^{-1}$). Cells were treated with different concentration of hexanal (30000, 10000, 3333, 1111, 370 and 123 ppm) and incubated for 72 hrs. Media from the wells were removed and replaced with 100 µ l of fresh media. Ten µ l of MTT reagent was added to each well and incubated for 4 hrs. After an incubation period, formazan crystals were formed which was dissolved by adding equal amount of detergent. Absorbance was measured at a wavelength of 570 nm. Background absorbance of multi-well plates was measured at 690 nm and subtracted from the 570 nm measurement.

7.2. Cytotoxicity and genotoxicity studies

7.2.1. Cytotoxicity studies of hexanal on human cell lines using Lactate hydrogenase (LDH) assay

The results from LDH assay based cell cytotoxicity test is shown in table 7.1. HeLa, A549 and HepG2 cells were used for the studies. Hexanal at different concentrations (30000, 10000, 3333, 1111, 370, and 123 ppm were tested for LDH activity. Increase in LDH activity in culture supernatant is proportional to the number of lysed cells. From the absorbance @ 490 nm for the standards Y intercept (0.1547) and slope (0.0053) was determined and total LDH activity was calculated. High units of LDH was found in 30000 ppm hexanal treated cells which was1643.919 mU/ml in HeLa cells, 1716.475 mU/ml in A549 cells and 1698.702 mU/ml in HepG2 cells. These results show that hexanal at such high concentration is toxic to cells. Concentrations below 2000ppm were found found to be safe for use (**Chromous Biotech Pvt. Ltd., Bengaluru**). . These results confirm that hexanal, at recommended field spray concentration of 0.04 per cent (400 ppm) is found to be non toxic to human cell lines.

Table 7.1. Effect of Hexanal on Cytotoxicity of Human Cell Lines as per LDH Activity

Hexanal (ppm)	HeLa Cells*	A 549 Cells	HepG2 cells
30000	1643.919	1716.475	1698.702
10000	1554.279	1474	1534.458
3333	1501.843	1291.902	1226.947
1111	191.7302	313.334	284.4528
370	175.5566	193.5377	152.5792
123	86.81321	54.29623	75.66038

* mU / ml – mili Unit per milli litre;

Limit >300 mU/ml

7.1.1. Tetrazolium (MTT) assay studies

MTT assay is a colorimetric assay for measuring the activity of cellular enzymes that reduce the tetrazolium dye (MTT), to its insoluble formazan, giving a purple color. The results of MTT assay oncell viability is shown in Figurexx . HeLa, A549 and HepG2 cells were used for the studies. Concentrations of hexanal as high as 30000 ppm are toxic to cells. At 30000 ppm, Hela cells, A549 and HepG2 showed zero per cent viability. For HeLa cells CC_{50} (50% Cytotoxic concentration) was 3228 ppm of hexanal, for A549 cells CC_{50} was at 5128 ppm hexanal, and for HepG2 cellsthe CC_{50} value was 501 ppm. (Table 7.2.). Concentrations below 2000ppm was found to be safe for use (**Chromous Biotech Pvt. Ltd., Bengaluru**). The assay also confirms that recommended field spray concentration of 0.04 per cent (400 ppm) is found to be non-toxic to human cell lines.

CHAPTER 8

FISH TOXICITY

A laboratory experiment was conducted to assess the toxicity of nanoemulsion of hexanal against zebra fishes as per the guidelines given by Organization for Economic Co-operation and Development (OECD, 1992).

Experiment

Tests were conducted to assess the toxicity of nanoemulsion of hexanal against zebra fishes *Danio rerio* with eight treatments along with control replicated two times as mentioned. Juvenile fishes with length 2.0 ± 1.0 cm were acquired from Beena aquarium, Papanaikenpalayam, Coimbatore. Fishes were kept for acclimation period of 14 days to adapt to the test environment. Feeding of fishes was stopped before 48 hrs of initiation of the test. Aquarium tank of size 1.5'x 0.75'x 1' (LxBxH) were used for the study. Hexanal formulations to provide appropriate test levels of hexanalwere added to the respective tanks, and fishes were introduced within 30 min after the addition of treatments. 15 fishes per tank were used for the study. Twelve to 16 hours of photoperiod was maintained daily. Gentle aeration was provided using aerator to maintain 80 per cent aeration. Acute testing was performed for a minimum of 96 h. Fishes were not fed during the test period.

Results

Hexanal treatments had no impact on the behavior of the fishes during the entire test period. There was no mortality in any of the treatments and replication throughout the test period. The result of the study is given in the following table.

Treatment	Mortality (%)*					
	12 hrs	24 hrs	36 hrs	48 hrs	96 hrs	108 hrs
Nano emulsion @ 0.02%	**0**	**0**	**0**	**0**	**0**	**0**
Nano emulsion @ 0.04%	**0**	**0**	**0**	**0**	**0**	**0**
Nano emulsion @ 0.06%	**0**	**0**	**0**	**0**	**0**	**0**
Pure hexanal @ 0.02%	**0**	**0**	**0**	**0**	**0**	**0**
Pure hexanal @ 0.04%	**0**	**0**	**0**	**0**	**0**	**0**
Pure hexanal @ 0.06%	**0**	**0**	**0**	**0**	**0**	**0**
Ethanol @ 0.2%	**0**	**0**	**0**	**0**	**0**	**0**
Tween 20 @ 0.2%	**0**	**0**	**0**	**0**	**0**	**0**
Control	**0**	**0**	**0**	**0**	**0**	**0**

CONCLUSION

Hexanal formulations is found be very effective in extending shelf-life of fruits, vegetables and flowers when it is applied externally. The scientists have found that hexanal based effective fresh formulations as a pre-harvest sprays or dip treatment before storage can extend the shelf-life of 2-3 weeks without loss fruit quality, flavor and colour. Even, the vapour of hexanal has a potential to alter the ripening of fruits that resulted in the extension of shelf-life. Such shelf-life extension will help the Asian countries where fruits and vegetables are produced aplenty but the per capita availability is just 50% of their daily requirement due to the post-harvest losses.

During the past three years, Tamil Nadu Agricultural University, Coimbatore, has undertaken studies to determine the safety of the hexanal and its formulations against beneficial microorganisms, honey bees, natural enemies, earthworms, fish and human cell lines using standard operational protocols stipulated by the OECD (Organization for Economic Cooperation and Development). The studies have concluded the following :

- Hexanalhad no effects on adult emergence and parasitizationof the egg parasitoid *Trichogramma chilonis* Ishii indicating the safety of the formulation. Further, hexanal formulation had absolutely no adverse effects on the pupation and adult emergence of predator*Chrysoperla zastrowi arabica* (Esben – Petersen). The data suggest safety of hexanal formulation to natural enemies.

- There was no deterrence of hexanalformulation spray against honey bees even at higher concentrations than the recommended dose of 0.02%. Honey bees are widely believed as an indicator of chemical toxicity
- Earthworm activities in the soil were not affected when hexanal formulation was sprayed at various concentrations. The data clearly indicated hexanal was non toxic to the earthworms.

- Nanoemulsion of hexanal toxicity studies carried out against bacterial cultures *Pseudomonas fluorescens, Bacillus subtilis* and fungal cultures *Trichoderma viride* Pers*, Trichoderma harzianum* Rifai by agar well and paper disc method revealed that there was no inhibition in both bacterial and fungal cultures in both the methods.

- HeLa, A549 and HepG2 cells were used for assessing the cell cytotoxicity studies by LDH assay and cell viability by MTT assay. Hexanal at 200, 400, 600 ppm used for the biosafety studies indicated the safety to cell lines. Result further revealed that hexanal was not toxic upto 2000 ppm and such a concentration could not be used in the field. Hence,hexanal at recommended field dose (200 ppm) was found safe for human beings.

- Hexanal formulation against aquatic animals was tested using zebra fish. There no mortality was observed.

Biosafety studies have unequivocally demonstrated that hexanal formulations is safer to honey bees, natural enemies, earthworms, microbial cultures in mango eco system besides humans. Hence, it can be inferred that hexanal field spraying at 200 ppm (0.02 %) may not affect non target organisms in mango eco system and human being.

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APPENDIX – I

SIGMA-ALDRICH sigma-aldrich.com

Material Safety Data Sheet

Version 4.3
Revision Date 08/05/2011
Print Date 07/04/2012

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	: Hexanal			
Product Number	: W255718			
Brand	: Aldrich			
Product Use	: For laboratory research purposes.			
Supplier	: Sigma-Aldrich Canada, Ltd 2149 Winston Park Drive OAKVILLE ON L6H 6J8 CANADA	Manufacturer	:	Sigma-Aldrich Corporation 3050 Spruce St. St. Louis, Missouri 63103 USA
Telephone	: +1 9058299500			
Fax	: +1 9058299292			
Emergency Phone # (For both supplier and manufacturer)	: 1-800-424-9300			
Preparation Information	: Sigma-Aldrich Corporation Product Safety - Americas Region 1-800-521-8956			

2. HAZARDS IDENTIFICATION

Emergency Overview

WHMIS Classification

B2 Flammable liquid — Flammable liquid

GHS Classification

Flammable liquids (Category 3)
Acute toxicity, Oral (Category 5)
Skin irritation (Category 3)
Eye irritation (Category 2B)

GHS Label elements, including precautionary statements

Pictogram

Signal word: Warning

Hazard statement(s)

H226	Flammable liquid and vapour.
H303	May be harmful if swallowed.
H316	Causes mild skin irritation.
H320	Causes eye irritation.

Precautionary statement(s)

P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

HMIS Classification

Health hazard:	1
Flammability:	3
Physical hazards:	0

Potential Health Effects

Inhalation — May be harmful if inhaled. May cause respiratory tract irritation.

Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Caproaldehyde
Aldehyde C_6
Hexyl aldehyde

Formula : $C_6H_{12}O$
Molecular Weight : 100.16 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
Hexanal			
66-25-1	200-624-5	-	-

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Conditions of flammability
Flammable in the presence of a source of ignition when the temperature is above the flash point. Keep away from heat/sparks/open flame/hot surface. No smoking.

Suitable extinguishing media
For small (incipient) fires, use media such as "alcohol" foam, dry chemical, or carbon dioxide. For large fires, apply water from as far as possible. Use very large quantities (flooding) of water applied as a mist or spray; solid streams of water may be ineffective. Cool all affected containers with flooding quantities of water.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

Further information
Use water spray to cool unopened containers.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

Methods and materials for containment and cleaning up
Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations (see section 13).

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.
Keep away from sources of ignition - No smoking. Take measures to prevent the build up of electrostatic charge.

Conditions for safe storage
Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Recommended storage temperature: 2 - 8 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
impervious clothing, Flame retardant antistatic protective clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Specific engineering controls
Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	clear, liquid
Colour	colourless

Safety data

pH	no data available
Melting	Melting point/range: < -20 °C (< -4 °F)

point/freezing point	
Boiling point	130 - 131 °C (266 - 268 °F) - lit.
Flash point	25 °C (77 °F) - closed cup
Ignition temperature	204 °C (399 °F)
Autoignition temperature	no data available
Lower explosion limit	1 %(V)
Upper explosion limit	7.5 %(V)
Vapour pressure	13 hPa (10 mmHg) at 25 °C (77 °F)
Density	0.815 g/cm3 at 25 °C (77 °F) 0.815 g/cm3 at 25 °C (77 °F)
Water solubility	no data available
Partition coefficient: n-octanol/water	log Pow: 1.78
Relative vapour density	3.46 - (Air = 1.0)
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions

Vapours may form explosive mixture with air.

Conditions to avoid
Heat, flames and sparks.

Materials to avoid
Oxidizing agents, Strong bases, Strong reducing agents, Do not store near acids.

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides
Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
LD50 Oral - rat - 4,890 mg/kg

Inhalation LC50
no data available

Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
Skin - rabbit - Mild skin irritation - 24 h

Serious eye damage/eye irritation
Eyes - rabbit - Mild eye irritation - 24 h

Respiratory or skin sensitization
no data available

Germ cell mutagenicity

Genotoxicity in vitro - Hamster - Lungs
Mutation in mammalian somatic cells.

Genotoxicity in vitro - rat - Liver
Unscheduled DNA synthesis

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Signs and Symptoms of Exposure
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Synergistic effects
no data available

Additional Information
RTECS: MN7175000

12. ECOLOGICAL INFORMATION

Toxicity

Toxicity to fish	LC0 - Pimephales promelas (fathead minnow) - 22 mg/l - 96 h

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

Do not empty into drains.

no data available

13. DISPOSAL CONSIDERATIONS

Product

Burn in a chemical incinerator equipped with an afterburner and scrubber but exert extra care in igniting as this material is highly flammable. Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging

Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)

UN number: 1207 Class: 3 Packing group: III
Proper shipping name: Hexaldehyde
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG

UN number: 1207 Class: 3 Packing group: III EMS-No: F-E, S-D
Proper shipping name: HEXALDEHYDE
Marine pollutant: No

IATA

UN number: 1207 Class: 3 Packing group: III
Proper shipping name: Hexaldehyde

15. REGULATORY INFORMATION

WHMIS Classification

B2 Flammable liquid Flammable liquid

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

16. OTHER INFORMATION

Further information

Copyright 2011 Sigma-Aldrich Co. License granted to make unlimited paper copies for internal use only.
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

APPENDIX – II

SIGMA-ALDRICH sigma-aldrich.com

Material Safety Data Sheet

Version 4.5
Revision Date 04/10/2012
Print Date 07/04/2012

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Hexanal		
Product Number	:	115606		
Brand	:	Aldrich		
Product Use	:	For laboratory research purposes.		
Supplier	:	Sigma-Aldrich Canada, Ltd 2149 Winston Park Drive OAKVILLE ON L6H 6J8 CANADA	Manufacturer :	Sigma-Aldrich Corporation 3050 Spruce St. St. Louis, Missouri 63103 USA
Telephone	:	+1 9058299500		
Fax	:	+1 9058299292		
Emergency Phone # (For both supplier and manufacturer)	:	1-800-424-9300		
Preparation Information	:	Sigma-Aldrich Corporation Product Safety - Americas Region 1-800-521-8956		

2. HAZARDS IDENTIFICATION

Emergency Overview

WHMIS Classification

B2 Flammable liquid — Flammable liquid

GHS Classification

Flammable liquids (Category 3)
Acute toxicity, Oral (Category 5)
Skin irritation (Category 3)
Eye irritation (Category 2B)

GHS Label elements, including precautionary statements

Pictogram

Signal word: Warning

Hazard statement(s)

H226	Flammable liquid and vapour.
H303	May be harmful if swallowed.
H316	Causes mild skin irritation.
H320	Causes eye irritation.

Precautionary statement(s)

P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

HMIS Classification

Health hazard:	1
Flammability:	3
Physical hazards:	0

Potential Health Effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Caproaldehyde
Aldehyde C_6
Hexyl aldehyde

Formula : $C_6H_{12}O$
Molecular Weight : 100.16 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
Hexanal			
66-25-1	200-624-5	-	-

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIREFIGHTING MEASURES

Conditions of flammability
Flammable in the presence of a source of ignition when the temperature is above the flash point. Keep away from heat/sparks/open flame/hot surface. No smoking.

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

Further information
Use water spray to cool unopened containers.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

Methods and materials for containment and cleaning up
Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations (see section 13).

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.
Keep away from sources of ignition - No smoking. Take measures to prevent the build up of electrostatic charge.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Recommended storage temperature: 2 - 8 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Splash protection
Material: butyl-rubber
Minimum layer thickness: 0.3 mm
Break through time: > 30 min
Material tested:Butoject® (Aldrich Z677647, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 873000, e-mail sales@kcl.de, test method: EN374
If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an Industrial Hygienist familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Eye protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
impervious clothing, Flame retardant antistatic protective clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Specific engineering controls
Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	clear, liquid
Colour	colourless

Safety data

pH	no data available
Melting point/freezing point	Melting point/range: < -20 °C (< -4 °F)
Boiling point	130 - 131 °C (266 - 268 °F) - lit.
Flash point	25 °C (77 °F) - closed cup
Ignition temperature	204 °C (399 °F)
Autoignition temperature	no data available
Lower explosion limit	1 %(V)
Upper explosion limit	7.5 %(V)
Vapour pressure	13 hPa (10 mmHg) at 25 °C (77 °F)
Density	0.814 g/cm3
Water solubility	no data available
Partition coefficient: n-octanol/water	log Pow: 1.78
Relative vapour density	3.46 - (Air = 1.0)
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions

Vapours may form explosive mixture with air.

Conditions to avoid
Heat, flames and sparks.

Materials to avoid
Oxidizing agents, Strong bases, Strong reducing agents, Do not store near acids.

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides
Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
LD50 Oral - rat - 4,890 mg/kg

Inhalation LC50
no data available

Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
Skin - rabbit - Mild skin irritation - 24 h

Serious eye damage/eye irritation
Eyes - rabbit - Mild eye irritation - 24 h

Respiratory or skin sensitization
no data available

Germ cell mutagenicity

Genotoxicity in vitro - Hamster - Lungs
Mutation in mammalian somatic cells.

Genotoxicity in vitro - rat - Liver
Unscheduled DNA synthesis

Carcinogenicity

IARC:	No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Signs and Symptoms of Exposure
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Synergistic effects
no data available

Additional Information
RTECS: MN7175000

12. ECOLOGICAL INFORMATION

Toxicity

Toxicity to fish	LC0 - Pimephales promelas (fathead minnow) - 22 mg/l - 96 h

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

Do not empty into drains.

no data available

13. DISPOSAL CONSIDERATIONS

Product
Burn in a chemical incinerator equipped with an afterburner and scrubber but exert extra care in igniting as this material is highly flammable. Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN number: 1207 Class: 3 Packing group: III
Proper shipping name: Hexaldehyde
Reportable Quantity (RQ):
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
UN number: 1207 Class: 3 Packing group: III EMS-No: F-E, S-D
Proper shipping name: HEXALDEHYDE
Marine pollutant: No

IATA
UN number: 1207 Class: 3 Packing group: III
Proper shipping name: Hexaldehyde

15. REGULATORY INFORMATION

WHMIS Classification

B2	Flammable liquid	Flammable liquid

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

16. OTHER INFORMATION

Further information
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APPENDIX – III

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

WORLD HEALTH ORGANIZATION

SAFETY EVALUATION OF CERTAIN
FOOD ADDITIVES AND CONTAMINANTS

WHO FOOD ADDITIVES SERIES 40

Prepared by:
The forty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

World Health Organization, Geneva 1998

SATURATED ALIPHATIC ACYCLIC LINEAR PRIMARY ALCOHOLS, ALDEHYDES, AND ACIDS

First draft prepared by
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1. EVALUATION

1.1 Introduction

The Committee evaluated a group of 38 flavouring agents that includes selected saturated aliphatic acyclic linear primary alcohols, aldehydes and acids of chain length C_{1-18} using the Procedure for the Safety Evaluation of Flavouring Agents (the "Procedure") (see Figure 1 in the Introduction to the section on Substances Evaluated Using the

Procedure for the Safety Evaluation of Flavouring Agents and Table 1 in this section).

Several substances in the group had been evaluated previously by the Committee. At the seventeenth meeting a group ADI "not limited" was allocated to acetic acid and its potassium and sodium salts, an ADI "not limited" was allocated to propionic acid, and a group ADI of 0-3 mg/kg bw was allocated to formic acid and ethyl formate (Annex 1, reference 32). A group ADI of 0-0.1 mg/kg bw was established for ocatanal and nonanal, singly or in combination, at the twenty-eighth meeting (Annex 1, reference 66). At the twenty-ninth meeting, ADIs "not specified" were allocated to the aluminium, ammonium, calcium, magnesium, potassium, and sodium salts of lauric, myristic, palmitic, and stearic acids (Annex 1, reference 70). At that meeting, the Committee did not establish ADIs for myristic, palmitic or stearic acids owing to lack of information on the manufacture or use of the food-grade material, but noted that these substances are normal constituents of coconut oil, butter and other edible oils. ADIs have not been allocated to butyl alcohol, decanal or propyl alcohol because the data were considered to be inadequate (Annex 1, references 38, 14, and 56, respectively).

One substance structurally related to the group, ethyl alcohol, was evaluated as a flavouring agent at the forty-sixth meeting of the Committee (reference Annex 122). The Committee determined that ethyl alcohol posed no safety concern at its current level of intake when used as a flavouring agent.

1.2 Estimated daily *per capita* intake

The total annual production volume of the 38 substances from their use as flavouring substances is approximately 2100 tonnes in the USA (NAS, 1987). In the USA, approximately 90% of the total volume (NAS, 1987) is accounted for by acetic acid, which includes uses (aciduvant or solvent) in food other than flavour use. Data are not available on the specific flavour use of acetic acid in Europe. Disregarding the annual volume of acetic acid, the total reported annual volume of the remaining 37 aliphatic substances is approximately 200 tonnes from use as flavouring substances in the USA (NAS, 1987) and 300 tonnes in Europe (IOFI, 1995). In the unlikely event that all of the substances in this group were simultaneously consumed on a daily basis, the estimated daily *per capita* intakes in Europe and the USA would be approximately 40 mg per day and 30 mg per day, respectively (excluding acetic acid and propionic acid which have ADIs "not limited"). According to the European and USA production statistics and derived intakes, acetaldehyde, butyl alcohol and butyric acid are the major flavouring substances in this group. Acetaldehyde and butyl alcohol constitute about 46% of the daily *per capita* intake of flavouring agents in this group in the USA and acetaldehyde and butyric acid constitute about 50% of the daily intake in Europe. Other flavouring agents in this group that are used at higher intake levels (i.e., >1800 µg per day) include butyric acid, propionic acid, propyl alcohol and stearic acid in the USA and octanoic acid, hexanoic acid, valeraldehyde, butyl alcohol and hexyl alcohol in Europe (Table 1).

Linear saturated aliphatic alcohols, aldehydes and acids are ubiquitous in nature. Low molecular weight alcohols and acids have been detected in almost every known fruit and vegetable (CIVO-TNO, 1996). However, there are relatively few reports for the natural occurrence of the corresponding aldehydes. In the USA, the available quantitative data indicate that the dietary consumption of saturated linear aliphatic alcohols, aldehydes and acids from naturally occurring sources exceeds the consumption from their use as flavouring substances (Stofberg & Kirschman, 1985; Stofberg & Grundschober, 1987).

1.3 Absorption, metabolism and elimination

Linear aliphatic acyclic alcohols (Lington & Bevan, 1994), aldehydes (Brabec, 1993) and carboxylic acids (von Oettingen, 1960; Dawson *et al.*, 1964; Katz & Guest, 1994) are absorbed through the gastrointestinal tract. Plasma half-lives are difficult to measure since many low molecular weight alcohols (e.g., ethanol), aldehydes and carboxylic acids (e.g., acetate and propionate) are endogenous in humans (Lington & Bevan, 1994). Acetaldehyde has been detected in whole blood (<0.2 mg/litre) and acetate is a blood buffer (Tietz, 1986).

The flavouring agents in this group of selected saturated aliphatic linear alcohols, aldehydes and acids are all metabolized via fatty acid and tricarboxylic acid pathways. Additional information can be found in introduction to this chapter on flavouring agents.

1.4 Application of the procedure for the safety evaluation of flavouring agents

Step 1. All of the flavouring agents in this group were classified in structural class I (Cramer *et al.*, 1978).

Step 2. All of the flavouring agents in this group are known or can be readily predicted to be efficiently metabolized to substances

harmless to humans at the estimated intakes of the flavouring agents.

Step A3. Twenty-seven substances in this group fall below the human intake threshold for class I (i.e., 1800 µg per day) at their current levels of intake; therefore, these substances were determined to be of no safety concern on the basis of their structural class and low levels of estimated intake.

Step A4. Eleven substances in this group exceeded the human intake threshold for class I. In all cases, the substances can be predicted to undergo complete metabolism to endogenous products via the fatty acid and tricarboxylic acid pathways. In the opinion of the Committee the endogenous levels of metabolites from these substances would not give rise to perturbations outside the physiological range. Therefore, these 11 substances were also determined to be of no safety concern based on their structural class and known metabolism.

Table 1 summarizes the evaluation of the 38 saturated aliphatic, acyclic linear primary alcohols, aldehydes and acids using the Procedure.

Table 1. Summary of results of safety evaluations of saturated aliphatic acyclic linear primary alcohols, aldehydes and acids.

Step 1: All of the substances in the group are in structural class I, the human intake threshold of which is 1800 µg per dayay.
Step 2: All of the substances in this group are metabolized to innocuous products.

Substance	Step A3 Does intake exceed the human intake threshold?[1] Intake estimates (µg per person per day)	Step A4 Endogenous or metabolized to endogenous substances?	Comments	Conclusion based on current levels of intake
Formic acid	No USA: 160 Europe: 800	N/R	Formic acid is produced endogenously and it is a normal component of intermediate metabolism.	No safety concern
Acetaldehyde	Yes USA: 9 700 Europe: 11 000	Yes	Acetaldehyde is oxidized to acetate which is metabolized via the citric acid cycle; acetaldehyde can also be reduced to ethanol.	No safety concern
Acetic acid	Yes USA: 360 000 Europe: N/D[2]	Yes	Acetic acid is metabolized to CO_2; it acetylates amines and can be incorporated into proteins.	No safety concern
Propyl alcohol	Yes USA: 2700 Europe: 420	Yes	Propyl alcohol is oxidized to propionaldehyde which yields propionate; propionate undergoes metabolism in the citric acid cycle.	No safety concern
Propionaldehyde	No USA: 140 Europe: 33	N/R	See propyl alcohol.	No safety concern
Propionic acid	Yes USA: 5200 Europe: 1100	Yes	See propyl alcohol.	No safety concern
Butyl alcohol	Yes USA: 8100 Europe: 1900	Yes	Butyl alcohol is oxidized to its corresponding aldehyde, which is oxidized to the acid; metabolism via fatty acid and tricarboxylic acid pathways.	No safety concern

Table 1. Continued...

Substance	Step A3 Does intake exceed the human intake threshold?[1] Intake estimates (µg per person per day)	Step A4 Endogenous or metabolized to endogenous substances?	Comments	Conclusion based on current levels of intake
Butyraldehyde	No USA: 17 Europe: 26	N/R	See butyl alcohol.	No safety concern
Butyric acid	Yes USA: 5 900 Europe: 10 000	Yes	See butyl alcohol.	No safety concern
Amyl alcohol	No USA: 44 Europe: 97	N/R	Amyl alcohol is oxidized to its corresponding aldehyde, which is rapidly oxidized to the acid; metabolism via fatty acid and tricarboxylic acid pathways.	No safety concern
Valeraldehyde	Yes USA: 8.8 Europe: 3000	Yes	See amyl alcohol.	No safety concern
Valeric acid	No	N/R	See amyl alcohol.	No safety concern

	USA: 850 Europe: 140				
Hexyl alcohol	Yes USA: 800 Europe: 1900		Yes	Hexyl alcohol is oxidized to its corresponding aldehyde, which is rapidly oxidized to the acid; metabolism via fatty acid and tricarboxylic acid pathways.	No safety concern
Hexanal	No USA: 260 Europe: 780		N/R	See hexyl alcohol.	No safety concern
Hexanoic acid	Yes USA: 1300 Europe: 3500		Yes	See hexyl alcohol.	No safety concern

Table 1. Continued...

Substance	Step A3 Does intake exceed the human intake threshold?[1] Intake estimates (µg per person per day)	Step A4 Endogenous or metabolized to endogenous substances?	Comments	Conclusion based on current levels of intake
Heptyl alcohol	No USA: 7 Europe: 12	N/R	Heptyl alcohol is oxidized to its corresponding aldehyde, which is rapidly oxidized to the acid; metabolism via fatty acid and tricarboxylic acid pathways.	No safety concern
Heptanal	No USA: 3.2 Europe: 200	N/R	See heptyl alcohol.	No safety concern
Heptanoic acid	No USA: 5.3 Europe: 170	N/R	See heptyl alcohol.	No safety concern
1-Octanol	No USA: 32 Europe: 230	N/R	1-Octanol is oxidized to its corresponding aldehyde, which is rapidly oxidized to the acid; metabolism via fatty acid and tricarboxylic acid pathways.	No safety concern
Octanal	No USA: 90 Europe: 170	N/R	See 1-octanol.	No safety concern
Octanoic acid	Yes USA: 650 Europe: 3800	Yes	See 1-octanol.	No safety concern
Nonyl alcohol	No USA: 2.1 Europe: 8.1	N/R	Nonyl alcohol is oxidized to its corresponding aldehyde, which is rapidly oxidized to the acid; metabolism via fatty acid and tricarboxylic acid pathways.	No safety concern

Table 1. Continued...

Substance	Step A3 Does intake exceed the human intake threshold?[1] Intake estimates (µg per person per day)	Step A4 Endogenous or metabolized to endogenous substances?	Comments	Conclusion based on current levels of intake
Nonanal	No USA: 17 Europe: 130	N/R	See nonyl alcohol.	No safety concern
Nonanoic acid	No USA: 63 Europe: 64	N/R	See nonyl alcohol.	No safety concern
1-Decanol	No USA: 7 Europe: 290	N/R	1-Decanol is oxidized to its corresponding aldehyde, which is rapidly oxidized to the acid; metabolism via fatty acid pathways and tricarboxylic acid pathways.	No safety
Decanal	No USA: 61 Europe: 288	N/R	See 1-decanol.	No safety concern
Decanoic acid	No USA: 980 Europe: 1400	Yes	See 1-decanol; at high concentrations, decanoic acid undergoes omega-oxidation.	No safety concern
Undecyl alcohol	No USA: 11 Europe: 0.9	N/R	Undecyl alcohol is oxidized to its corresponding aldehyde, which is rapidly oxidized to the acid; metabolism via fatty acid and tricarboxylic acid pathways.	No safety concern

Undecanal	No USA: 1.5 Europe: 480	N/R	See undecyl alcohol.	No safety concern

Table 1. Continued...

Substance	Step A3 Does intake exceed the human intake threshold?[1] Intake estimates (µg per person per day)	Step A4 Endogenous or metabolized to endogenous substances?	Comments	Conclusion based on current levels of intake
Undecanoic acid	No USA: 8.8 Europe: 4.6	N/R	See undecyl alcohol.	No safety concern
Lauryl alcohol	No USA: 80 Europe: 170	N/R	Lauryl alcohol is oxidized to its corresponding aldehyde, which is rapidly oxidized to the acid; metabolism via fatty acid and tricarboxylic acid pathways.	No safety concern
Lauric aldehyde	No USA: 21 Europe: 52	N/R	See lauryl alcohol.	No safety concern
Lauric acid	No USA: 1200 Europe: 590	N/R	See lauryl alcohol.	No safety concern
Myristaldehyde	No USA: 25 Europe: 9.4	N/R	Myristaldehyde is rapidly oxidized to its corresponding acid; metabolism via fatty acid and tricarboxylic acid pathways.	No safety concern
Myristic acid	No USA: 72 Europe: 160	N/R	See myristaldehyde.	No safety concern
1-Hexadecanol	No USA: 0.2 Europe: 3.6	Yes	1-Hexadecanol is oxidized to its corresponding aldehyde, which is rapidly oxidized to the acid; metabolism via fatty acid and tricarboxylic acid pathways.	No safety concern

Table 1. Continued...

Substance	Step A3 Does intake exceed the human intake threshold?[1] Intake estimates (µg per person per day)	Step A4 Endogenous or metabolized to endogenous substances?	Comments	Conclusion based on current levels of intake
Palmitic acid	No USA: 234 Europe: 89	N/R	beta-Oxidation of palmitic acid yields 2-carbon units that enter the tricarboxylic acid cycle.	No safety concern
Stearic acid	Yes USA: 1900 Europe: 58	Yes	beta-Oxidation of stearic acid yields 2-carbon units that enter the tricarboxylic acid cycle.	No safety concern

[1] N/R: Not required for evaluation because consumption of the substance was determined to be of no safety concern at Step A3 of the Procedure.
[2] N/D: No intake data reported.

1.5 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all of the substances in this group of flavouring agents were simultaneously consumed on a daily basis, the estimated daily *per capita* intake in Europe and the USA would exceed the human intake threshold for substances in class I. All of the substances in this group and their metabolites are innocuous and endogenous, and their combined intake was judged by the Committee not to give rise to perturbations outside the physiological range.

1.6 Conclusions

The Committee concluded that the substances in this group would not present safety concerns at the current levels of intake.

No toxicity data were required for the application of the Procedure. The Committee noted that the available toxicity data were consistent with the results of the safety evaluation using the Procedure. In cases where ADIs were previously established, these ADIs were maintained at the present meeting.

2. RELEVANT BACKGROUND INFORMATION

2.1 Intake data

The most recent data on the annual production volumes of the flavouring agents in this group in the USA and in Europe are given in

Table 2. The estimates of intake were calculated assuming under-reporting of the production data and consumption by 10% of the population, as indicated in the footnote to Table 2.

Table 2. Annual production and estimated per capita intake of saturated aliphatic acyclic linear primary alcohols, aldehydes and acids in the USA and Europe

Substance	Most recent annual production volume[1] tonnes	Daily Per Capita Intake[2] ("eaters only")	
		µg/day	µg/kg bw/day
1. Formic acid			
USA	0.84	160	2.7
Europe	5.6	800	13
2. Acetaldehyde			
USA	51	9700	160
Europe	78	11 000	180

Table 2. Continued...

Substance	Most recent annual production volume[1] tonnes	Daily Per Capita Intake[2] ("eaters only")	
		µg/day	µg/kg bw/day
3. Acetic acid			
USA[3]	1910	360 000	6000
Europe	0	0	0
4. Propyl alcohol			
USA	14	2700	45
Europe	2.9	420	6.9
5. Propionaldehyde			
USA	0.72	140	2.3
Europe	2.29	330	5.5
6. Propionic acid			
USA	27	5200	86
Europe	8.0	1100	19
7. Butyl alcohol			
USA	43	8100	140
Europe	13	1900	32
8. Butyraldehyde			
USA	0.09	17	0.29
Europe	0.19	26	0.44
9. Butyric acid			
USA	31	5900	98
Europe	73	10 000	170
10. Amyl alcohol			
USA	0.23	43	0.73
Europe	0.68	96	1.6
11. Valeraldhyde			
USA	0.046	8.7	0.15
Europe	21	3000	50
12. Valeric acid			
USA	4.4	850	14
Europe	0.97	140	2.3
13. Hexyl alcohol			
USA	4.3	810	14
Europe	13	1900	31

Table 2. Continued...

Substance	Most recent annual production volume[1] tonnes	Daily Per Capita Intake[2] ("eaters only")	
		µg/day	µg/kg bw/day
14. Hexanal			
USA	1.4	260	4.3
Europe	5.4	780	13

15. Hexanoic acid			
USA	6.8	1300	22
Europe	25	3500	59
16. Heptyl alcohol			
USA	0.037	7.0	0.12
Europe	0.081	11	0.19
17. Heptanal			
USA	0.017	3.2	0.05
Europe	1.5	210	3.5
18. Heptanoic acid			
USA	0.028	5.3	0.09
Europe	1.2	170	2.9
19. 1-Octanol			
USA	0.17	32	0.54
Europe	1.6	230	3.9
20. Octanal			
USA	0.47	90	1.5
Europe	1.2	170	2.8
21. Octanoic acid			
USA	3.43	650	11
Europe	27	3800	63
22. Nonyl alcohol			
USA	0.011	2.1	0.03
Europe	0.057	8.1	0.14
23. Nonanal			
USA	0.09	17	0.29
Europe	0.91	130	2.2
24. Nonanoic acid			
USA	0.33	63	1.0
Europe	0.45	64	1.1

Table 2. Continued...

Substance	Most recent annual production volume[1] tonnes	Daily Per Capita Intake[2] ("eaters only")	
		µg/day	µg/kg bw/day
25. 1-Decanol			
USA	0.037	7.0	0.12
Europe	0.2	28	0.48
26. Decanal			
USA	0.32	61	1.0
Europe	2.0	290	4.9
27. Decanoic acid			
USA	5.1	980	16
Europe	9.9	1400	24
28. Undecyl alcohol			
USA	0.06	11	0.19
Europe	0.006	0.86	0.01
29. Undecanal			
USA	0.008	1.5	0.03
Europe	3.4	480	8.0
30. Undecanoic acid			
USA	0.046	8.7	0.15
Europe	0.032	4.6	0.08
31. Lauryl alcohol			
USA	0.42	80	1.3
Europe	1.2	170	2.8
32. Lauric aldehyde			
USA	0.011	21	0.35
Europe	0.36	52	0.86
33. Lauric acid			
USA	6.5	1200	21
Europe	4.2	590	9.9
34. Myristaldehyde			
USA	0.13	25	0.41
Europe	0.066	9.4	0.16
35. Myristic acid			
USA	0.38	72	1.2

Europe	1.1	150	2.6

Table 2. Continued...

Substance	Most recent annual production volume[1] tonnes	Daily Per Capita Intake[2] ("eaters only")	
		µg/day	µg/kg bw/day
36. 1-Hexadecanol			
USA	0.0009	0.17	0.003
Europe	0.025	3.6	0.06
37. Palmitic acid			
USA	1.2	230	3.9
Europe	0.63	89	1.5
38. Stearic acid			
USA	9.9	1900	31
Europe	0.41	58	0.97
Totals			
USA	2110	400 000	6700
Europe	300	43 000	720
Total excluding acetic acid			
USA	200	38 000	640

[1] USA: National Academy of Science (NAS, 1987) Evaluating the safety of food chemicals. Washington, DC. Europe: International Organization of the Flavour Industry (IOFI, 1995) European inquiry on volume of use. Private communication to FEMA.

[2] Intake calculated as follows: [[(annual volume, kg) x (1 x 109 µg/kg)]/ [population x 0.6 x 365 days]], where population (10%, "eaters only") = 24 x 106 for the USA and 32 x 106 for Europe; 0.6 represents the assumption that only 60% of the flavour volume was reported in the survey [NAS, 1987; IOFI, 1995]. Intake (µg/kg bw/day) calculated as follows: [µg/day/body weight], where body weight = 60 kg. Slight variations may occur from rounding off.

[3] The USA production volume reported for acetic acid includes use of acetic acid as a solvent by the flavour and food industries.

2.2 Toxicological studies

2.2.1 Acute toxicity

Linear aliphatic alcohols, aldehydes and carboxylic acids exhibit low acute toxicity. For this group of saturated, aliphatic, acyclic, linear primary alcohols, aldehydes and acids used as flavouring agents, studies in rodents indicate LD_{50} values typically > 1 g/kg bw for 36 of the 38 substances. Generally, LD_{50} values of aldehydes and carboxylic acids having a carbon chain length greater than 3 are >2500 mg/kg bw. LD_{50} values were not available for undecanoic acid and palmitic acid. The acute toxicity studies that were available are summarized in Table 3.

Table 3. Acute toxicity studies for Saturated Aliphatic Acyclic Linear Primary Alcohols, Aldehydes and Acids

Substance	Species	Sex[1]	Route	LD_{50} (mg/kg bw)	Reference
Formic acid	mouse	NR	oral	1100	Malorny, 1969
Acetic acid	mouse	NR	gavage	4960	Woodard et al., 1941
	rat	NR	gavage	3310	Woodard et al., 1941
	rat	NR	oral	3530	Smyth et al., 1951
Propionic acid	rat	male	gavage	4290	Smyth et al., 1962
Butyric acid	rat	male & female	oral	8790	Smyth et al., 1954
	rat	NR	oral	2940	Smyth et al., 1951
Valeric acid	rat	NR	oral	1844	Smyth et al., 1969a
Hexanoic acid	rat	male	gavage	6440	Smyth et al., 1962
	rat	male	gavage	3000	Lewis, 1989
Heptanoic acid	rat	NR	oral	7000	Guest et al., 1982
Octanoic acid	rat	male	gavage	1283	Smyth et al., 1962
	rat	male & female	gavage	10 080	Jenner et al., 1964
Nonanoic acid	rat	NR	oral	3200	Fassett, 1963
Decanoic acid	rat	male	gavage	3301	Smyth et al., 1962
Lauric acid	mouse	NR	oral	1238	Schafer & Bowles, 1985
Myristic acid	rat	NR	oral	>5000	Moreno, 1977
Stearic acid	rat	NR	oral	>5000	Moreno, 1977
Acetaldehyde	rat	NR	oral	1930	Smyth et al., 1951
Propionaldehyde	rat	NR	oral	1110	Smyth et al., 1951

Butyraldehyde	rat	NR	oral	5890	Smyth et al., 1951
Valeraldehyde	rat	male	gavage	3000-6400	Smyth et al., 1962, 1969a
Hexanal	rat	male	gavage	7740	Smyth et al., 1962
	rat	male & female	oral	4890	Smyth et al., 1954
Heptanal	rat	NR	oral	>5000	Moreno, 1974
Octanal	rat	male	gavage	4600	Smyth et al., 1962
Nonanal	rat	male & female	gavage	>5000	Shelanski & Moldovan, 1971
Decanal	mouse	NR	gavage	>4175	Jenner et al., 1964
	rat	male & female	gavage	>3332	Jenner et al., 1964
Undecanal	rat	male & female	gavage	>5000	Shelanski & Moldovan, 1971
Lauric aldehyde	rat	male & female	gavage	>23 100	Calandra, 1971

Table 3. Continued...

Substance	Species	Sex[1]	Route	LD_{50} (mg/kg bw)	Reference
Myristaldehyde	rat	male & female	gavage	>4000	Lynch, 1971
	rat	NR	oral	4500	Smyth et al., 1962
Propyl alcohol	rat	male & female	gavage	6500	Jenner et al., 1964
	rat	male & female	gavage	6500	Taylor et al., 1964
	rat	NR	oral	5000	Levenstein, 1976
	rat	male & female	oral	1870	Smyth et al., 1954
	rat	NR	oral	5400	Rinehart et al., 1967
Butyl alcohol	rat	male & female	gavage	2510	Jenner et al., 1964
	rat	male & female	gavage	790 (female); 2020 (male)	Purchase, 1969
	rat	NR	oral	4360	Smyth et al., 1951
Amyl alcohol	rat	male & female	gavage	3030	Jenner et al., 1964
	rat	NR	oral	5730	Carpanini et al., 1973
Hexyl alcohol	rat	male & female	gavage	720 (female); 1800 (male)	Purchase, 1969
	rat	NR	oral	4590	Smyth et al., 1954
Heptyl	mouse	NR	oral	4300	Yegorov & Adrianov, 1961a
1-Octanol	rat	NR	oral	4135	Levenstein & Wolven, 1972
Nonyl alcohol	mouse	NR	oral	19 000	Yegorov & Adrainov, 1961a
1-Decanol	rat	NR	oral	9800	Smyth et al., 1951
Undecyl alcohol	rat	male	gavage	3000	Smyth & Carpenter, 1944
Lauryl alcohol	rat	male & female	oral	1280	Lewis, 1989
1-Hexadecanol	rat	NR	oral	8400	Coopersmith & Rutowski, 1965

[1] NR = not reported.

2.2.2 Short-term and long-term toxicity and carcinogenicity

Although toxicity studies were not required to apply the Procedure to this group of flavouring agents, multiple dose toxicity studies lasting more than 21 days in were available for approximately half of the 38 substances in the group (see Table 4). The lowest NOELs derived from these studies were 50-60 mg/kg bw per day, reported for heptyl alcohol and propyl alcohol. Few multiple dose studies are available for aldehydes due to their volatility and reactivity. Not all of these studies were designed to provide comprehensive toxicological assessments of the substances tested; however, consideration of these studies did not raise concerns regarding the safe use of the substances in this group as flavouring agents.

Several studies were conducted to evaluate the irritant effects of alcohols and acids on the forestomach of the rat. There are several substances in this group for which data indicate that high doses given to rats cause lesions of the forestomach. These effects are not considered to be relevant to the human ingestion of these substances as flavouring agents in foods.

A brief summary of the available data on substances not previously evaluated by the Committee is given below.

2.2.2.1 Acetaldehyde

A NOEL of 125 mg/kg bw per day was reported for acetaldehyde added to the drinking-water of male and female rats for 4 weeks at level of 0, 25, 125 or 625 mg/kg bw per day (Til *et al.*, 1988); the only treatment-related effect was hyperkeratosis of the forestomach at 625 mg/kg bw per day. No adverse effects were seen when acetaldehyde in drinking-water at a daily intake level of 0.5 mg/kg bw was given to rats (Amirkanova & Latypova, 1967).

2.2.2.2 Propyl alcohol

No adverse effects on the liver were observed when male rats were give 1 or 2 M solutions of propyl alcohol (approximately 60 or 120 mg/kg bw per day) as a drinking-water substitute for 6 or 2 months, respectively. Mallory bodies were reported in some animals (Hillbom *et al.*, 1974a). In groups of rats given a 1 M solution of propyl alcohol as their sole source of drinking-water for 4 months, a lower

ratio of weight gain to caloric intake compared to controls was observed, but there were no effects on the liver. A NOEL of 60 mg/kg bw per day was determined in this study (Hillbom *et al.*, 1974b).

In a study of the factors affecting the distribution of propionic acid in the forestomach of rats, no adverse effects on the forestomach mucosa were reported when male rats were fed a pellet diet containing 0 or 2-3% propionic acid (about 3800-5800 mg/kg bw per day) for 12 weeks (Bueld & Netter, 1993).

Table 4. Short-term and long-term toxicity studies for saturated aliphatic acyclic linear primary alcohols, aldehydes and acids

Substance	Species, sex	Route	Time	NOEL[1] (mg/kg/bw per day)	Reference
Formic acid	rat, male & female	oral	2 years	>400	Malorny, 1969
Acetic acid	rat, male	oral	63 days	350	Pardoe, 1952
Propionic acid	rat, male	oral	24 weeks	3800	Bueld & Netter, 1993
Butyric acid	rat	oral	up to 500 days	500	Mori, 1953
Hexanoic acid	rat, male	diet	3 weeks	2000	Moody & Reddy, 1978
Decanoic acid	rat	diet	150 days	>5000	Mori, 1953
Lauric acid	rat, male	diet	18 weeks	>6000	Fitzhugh et al., 1960
10-Undecenoic acid[2]	rat	gavage	6-9 months	>400	Tislow et al., 1950
Palmitic acid	rats	diet	150 days	>5000	Mori, 1953
Stearic acid	mice	oral	3 weeks	>15 000	Tove, 1964
Acetaldehyde	rats, male & female	oral	4 weeks	125	Til et al., 1988
Hexanal	rat, male & female	oral	28 days	>125	Komsta et al., 1988
Myristaldehyde	mice	diet	130 days	>166	Galea et al., 1965
Propyl alcohol	rat, male	oral	4 months	60	Hillbom et al., 1974b
Butyl alcohol	rat, male	oral	28 days	940	Bio-Fax, 1969
Amyl alcohol	rat, male & female	oral	13 weeks	>1000	Butterworth et al., 1978
Hexyl alcohol	dog, male & female	oral	13 weeks	230	Eibert, 1992
Heptyl alcohol	rabbit	gavage	6 months	>50	Voskovofnikova, 1966
1-Octanol	mice	gavage	one month	>179	Voskovofnikova, 1966
Nonyl alcohol	rabbit	diet	67 days	>148	Treon, 1963
1-Hexadecanol	rat, male & female	diet	13 weeks	577	Eibert, 1992

[1] A NOEL (no-observed-effect level) reported in this table as "greater than" (>) indicates that no adverse effects were observed at the highest dose level in the study, and therefore an actual NOEL was not obtained.
[2] A structurally related substance.

2.2.2.3 Butyl alcohol

No adverse effects were observed when 6.9% butyl alcohol and 25% sucrose (about 5.6 mg/kg bw per day butyl alcohol) were added to the drinking-water of male rats for 13 weeks (Wakabayashi *et al.*, 1984).

In rats given control diets or diets with 0.69, 1.38, 2.75 or 5.5% butyl alcohol (equivalent to 690-5500 mg/kg bw), a statistically significant increase in the ratio of liver-to-body weight was reported in males at all but the lowest dose tested and in females only at the highest dose (PPG, 1991a).

In a 28-day study on male rats fed diets containing 0, 1000, 3500 or 10 000 mg butyl alcohol kg feed (about 90-940 mg/kg bw per day) in 2% corn oil, no deaths, gross lesions at necropsy or differences in liver and kidney weights were reported; there was a statistically significant increase in the ratio of adrenals-to-body weight at all doses compared to controls (Bio-Fax, 1969).

2.2.2.4 Butyric acid

In a study of the development of gastric lesions with diets containing fatty acids, rats fed a rice diet with 1% butyric acid (equivalent to 500 mg/kg bw per day) that was gradually increase to 10% (equivalent to 5000 mg/kg bw per day) over a period of 500 days had forestomach lesions with prominent keratin cysts after being fed the diet for more than 50 days. No lesions were observed in the glandular stomach (Mori, 1953).

2.2.2.5 Amyl alcohol

Amyl alcohol given to rats by gavage for 13 weeks at a dose level of 1000 mg/kg bw per day produced no effects on body weight gain, food or water consumption, haematological values, serum and urine analyses, renal function, organ weight or histopathology (Butterworth *et al.*, 1978).

2.2.2.6 Valeric acid

Rats fed 5% valeric acid (about 2500 mg/kg bw per day) in a rice diet for 115-150 days had papillomatous growths in the forestomach (Mori, 1953).

2.2.2.7 Hexyl alcohol

Two groups of male and female rats were fed hexyl alcohol at dietary levels of 0.25 and 0.50% for 13 weeks; a third group was fed 1% (reported to be equivalent to 577 mg/kg bw per day) for weeks 1-10, then 2, 4 and 6% for weeks 11, 12 and 13, respectively. Food consumption was decreased in the high-dose females, but no significant haematological changes, differences in urine analyses or histopathological effects were observed (Eibert, 1992).

In a 13-week study, hexyl alcohol at levels of 0.5 and 1% in the diet, or at a dose level of 1000 mg/kg bw per day in gelatin capsules, was given to dogs. At a dose of 1000 mg/kg bw per day, 4 out of 5 dogs died. Haematology, serum chemistry and urine analyses revealed no differences in treated dogs relative to controls. There was gastrointestinal inflammation in the mid- and high-dose groups. Congestion of the viscera and testicular atrophy were observed at the high dose. A NOAEL of 1%, which corresponds to a daily intake of 230-695 mg/kg bw, was determined from this study (Eibert, 1992).

2.2.2.8 Hexanal

No adverse effects were reported when hexanal was given to rats in drinking-water at concentrations of 1, 10, 100 and 1000 mg/litre (calculated to provide doses of about 0.1, 1.2, 12.6 and 124.7 mg/kg bw per day) for 4 weeks (Komsta *et al.*, 1988).

2.2.2.9 Hexanoic acid

No effects on hepatic peroxisomes or peroxisomal enzymes were induced in male rats fed hexanoic acid in the diet at a level of 2% for 3 weeks (Moody & Reddy, 1978).

In rats fed 10% (about 5000 mg/kg bw per day) hexanoic acid for 150 days, no changes in the glandular stomach or forestomach were observed (Mori, 1953).

2.2.2.10 Heptyl alcohol

Heptyl alcohol, administered intragastrically to mice, in the form of a solution or suspension over a one-month period, showed no cumulative effects at a dose of 150 mg/kg bw per day (Voskoboinikova, 1966). The NOEL in rabbits given 0, 1.4, 14 or 50 mg/kg bw per day heptyl alcohol by gavage in sunflower oil for 6 months was 50 mg/kg bw per day (Voskoboinikova, 1966).

2.2.2.11 1-Octanol

No cumulative effects were observed in a study in which 1-octanol was administered intragastrically to mice in the form of a solution or suspension over a one-month period at a dose of 180 mg/kg bw per day (Voskoboinikova, 1966).

2.2.2.12 Octanoic acid

Rats gavaged on gestation days 6-15 with octanoic acid in corn oil at dose levels of 0, 1125 or 1500 mg/kg bw per day exhibited maternal toxicity and maternal mortality. There was an decrease in the number of live pups on post-gestational day 6, but no developmental toxicity was reported (Narotsky *et al.*, 1994).

2.2.2.13 Nonyl alcohol

No adverse effects were reported when an isomeric mixture of nonyl alcohol, 2-methyl-1-octanol and 3-methyl-1-octanol, calculated to provide a daily intake level of 148 mg/kg bw, was added to the diet of rabbits for 67 days of an 83-day period (Treon, 1963).

2.2.2.14 Decanoic acid

In a study of gastric lesions, 10% decanoic acid (about 5000 mg/kg bw per day) in the diet of rats for 150 days resulted in no observable changes in the forestomach or glandular stomach (Mori, 1953).

2.2.2.15 Undecanoic acid

In a study with undecanoic acid, there was a marked inhibitory effect on growth in rats give 2.5% (about 1250 mg/kg bw per day) for 8 weeks (Newell *et al.*, 1949).

2.2.2.16 Myristaldehyde

No adverse effects on mortality and body and organ weights were reported when myristaldehyde was fed to mice at a level of 166 mg/kg bw per day for 130 days (Galea *et al.*, 1965).

2.2.2.17 1-Hexadecanol

Two groups of male and female rats were fed 1-hexadecanol for 13 weeks at dietary levels of 1 or 2.5%; a third group was fed 5% for weeks 1-10, 7.5% for week 11, and 10% for weeks 12 and 13. Decreased food consumption (in females at the intermediate and high dose) and/or body weights (in males and females at the high dose and in females only at the intermediate dose) were observed at various times in rats in the intermediate and high-dose groups. No significant

haematological findings, changes in urinalyses or pathological effects were reported between control and treated animals. A NOAEL of 1% (equal to 577 mg/kg bw per day) was determined from this study (Eibert, 1992).

In a 13-week study in dogs, at levels of 0, 0.5, 1 or 3%, no effects on body weight, organ weight or food consumption were reported. No significant haematological findings, changes in urinalyses or gross pathological effects were reported between control and treated animals; however, serum glutamate oxaloacetate transaminase levels were elevated at all three doses. A NOAEL of 3% (equal to 807 mg/kg bw per day) was determined from this study (Eibert, 1992).

In addition to the multiple dose studies described above, the Committee was aware of the results of a long-term inhalation study in which hamsters that were administered acetaldehyde developed an excess of upper respiratory tract tumours (Kruysse et al., 1975). Respiratory lesions were also observed in 2-week and 13-week whole body

inhalation studies on formic acid (National Toxicology Program, 1992). No systemic effects resulted, but the NTP recommended caution in extrapolating the results of these studies to man because humans do not metabolize formate to CO_2 as rapidly as rodents. The Committee considered that, under conditions of use of acetaldehyde and formic acid as flavouring agents, these observations were not predictive of a response in humans because these substances are endogenous and oral ingestion from their use as flavouring agents is low.

2.2.3 Genotoxicity

In vitro and *in vivo* genotoxicity studies for the flavouring agents in this group are listed in Tables 5 and 6. Saturated aliphatic acyclic linear primary alcohols, aldehydes, and carboxylic acids generally exhibited consistent negative results in the Ames assay, the unscheduled DNA synthesis test, and the *in vitro* or *in vivo* mouse micronucleus test. However, genotoxic activity has been reported for some low molecular weight alcohols, carboxylic acids and aldehydes in varied assays, including the sister chromatid exchange (SCE) assay, the chromosomal aberration test and the forward mutation assays with mouse lymphoma and Chinese hamster lung cells.

The positive results in *in vitro* genotoxicity assays for aliphatic aldehydes is not surprising in light of the recognized reactivity of the aldehyde functional group. Acetaldehyde induced an increase in SCE in adult human lymphocytes (He & Lambert, 1985) and human peripheral lymphocytes (Helander & Lindahl-Kiessling, 1991). Acetaldehyde and propionaldehyde induced an increase in SCE in Chinese hamster embryonic diploid cells (Furnus *et al.*, 1990). However, aldehydes exhibit a short plasma half-life and are efficiently oxidized to the corresponding acids, which are metabolized in the fatty acid or citric acid pathways. These are important *in vivo* conditions that are difficult to establish in the above-mentioned *in vitro* assays. In one *in vivo* test, there was no evidence of an increase in micronucleated polychromatic erythrocytes in the bone marrow cells of $B6C3F_1$ mice given a single intraperitoneal injection of 95 or 100 mg acetaldehyde/kg bw. Dose levels of 190 mg/kg bw (approximately 50% of the subcutaneous LD_{50} value) or more did increase the number of mouse micronuclei (Ozawa *et al.*, 1994). Administration via intraperitoneal injection, however, bypasses the liver, where 80% of the acetaldehyde from the portal circulation is converted to acetate.

Table 5. In vitro mutagenicity/genotoxicity studies for saturated aliphatic acyclic linear primary alcohols, aldehydes and acids

Substance name	Test system	Test cells	Concentration	Results	Reference
Formic acid	modified Ames test (preincubation method)	S. typhmiurium TA97, TA98, TA100, TA1535, Chinese hamster ovary cells	10-3333 µg/plate	negative[1]	Zeiger et al, 1992
	Chromosomal aberration test	(CHO) K1	8-14 mM	positive[1]	Morita et al, 1990
	Chromosomal aberration test	Chinese hamster ovary cells (CHO) K1	12-14 mM	negative[1]	Morita et al, 1990
Acetic acid	modified Ames test (preincubation method)	S. typhmiurium strains TA97, TA98, T100, TA1535, Chinese hamster	100-1000 µg/plate	negative[1]	Zeiger et al, 1992
	Chromosomal aberration test		10-14 mM	negative[1]	Morita et al, 1990
	Chromosomal aberration test	Chinese hamster ovary K1 cells	4-10 mM	positive[1]	Morita et al, 1990
	Sister chromatid exchange	Adult human lymphocytes	2.5-10 mM	positive	Sipi et al., 1992
Propionic acid	Modified Ames test (preincubation method)	S. typhmiurium TA97, TA98, TA1535, and TA1537	100-10 000 µg/plate	negative[1]	Zeiger et al, 1992
	DNA repair test (spot test)	E. coli strains WP2, WP67, polA-, uvrA-, CM871	125 µl/plate	positive	Basler et al., 198

	SOS chromotest	E. coli PQ37	0.01-10 mM	negative	Basler et al., 1987
	Ames test	S. typhmiurium TA98, TA100, TA1535, TA1537	0.01-10 µl/plate	negative	Basler et al., 1987
	Sister chromatid exchange	Adult human lymphocyte cells	2.5 mM	positive[4]	Sipi et al., 1992
	Sister chromatid exchange	Chinese hamster V79 cells	0.1-33.3 mM	negative[1]	Basler et al, 1987
Butyric acid	Chromosomal aberration test	Chinese hamster fibroblast cells	up to 1 mg/ml	negative[1]	Ishidate et al., 1984

Table 5. Continued...

Substance name	Test system	Test cells	Concentration	Results	Reference
	Ames test	S. typhmiurium TA92, TA1535, TA100, TA1537, TA94, TA98	up to 10 mg/plate	negative[1]	Ishidate et al., 1984
Hexanoic acid	Mouse lymphoma assay	mouse lymphoma L5178Y TK+/-	700 µg/ml 1000 µg/ml	positive[3] negative[2]	Heck et al., 1989
	Unscheduled DNA synthesis Ames test (plate	Rat hepatocytes	1000 nl/ml	negative	Heck et al., 1989
	incorporation assay)	S. typhmiurium TA98, TA100, TA1538, TA1535 and TA1537	75 mg/plate	negative[1]	Heck et al., 1989
Heptanoic acid	Mouse lymphoma assay	Mouse lymphoma L5178Y TK +/-	900 µg/ml 600 µg/ml	negative[2] positive[3]	Heck et al., 1989
	Unscheduled DNA synthesis assay	Rat hepatocytes	1000 nl/ml	negative	Heck et al., 1989
	Ames test (plate incorporation assay)	S. typhmiurium TA98, TA100, TA1538, TA1535 and TA1537	150 mg/plate	negative[1]	Heck et al., 1989
	Modified Ames test (preincubation method)	S. typhmiurium TA97, TA98, TA100, TA104, TA1535 and TA1537	10 mg/plate	negative	Zeiger et al., 1992
Octanoic acid	Plate and suspension assays	S. typhmiurium TA1535, TA1537 and TA1538	at 0.0000625-0.00025%	negative[1]	FDA, 1976
	Nonactivation suspension test	Saccharomyces cerevisiae D4	0.000325-0.001300%	negative	FDA, 1976
	Unscheduled DNA synthesis	Rat hepatocytes	300 nl/ml	negative	Heck et al., 1989
	Ames test (plate incorporation assay)	S. typhmiurium TA98, TA100, TA1538, TA1535 and TA1537	50 mg/plate	negative[1]	Heck et al., 1989

Table 5. Continued...

Substance name	Test system	Test cells	Concentration	Results	Reference
Decanoic acid	Rec assay	B. subtilis strains H17 and M45	18 µg/disk	negative	Oda et al., 1978
	Modified Ames test (preincubation method)	S. typhimurium TA98, TA100, TA1535, TA97 and TA1537	up to 666 µg/plate	negative[1]	Zeiger et al., 1988
Lauric acid	Modified Ames test (preincubation method)	S. typhimurium TA98, TA100, TA1535, TA97 and TA1537	up to 666 µg/plate	negative[1]	Zeiger et al, 1988
Myristic acid	Cell mutagenesis assay	Mouse lymphoma L5178Y TK+/-	62.5 µg/ml 125 µg/ml	negative[2] negative[3]	Heck et al., 1989
	Ames test (plate incorporation assay)	S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538	10 mg/plate	negative[1]	Heck et al., 1989
	Modified Ames test (preincubation method)	S. typhmiurium TA97, TA98, TA100, TA1535 and TA1537	up to 3333 µg/plate	negative	Zeiger et al., 1988
Stearic acid	Modified Ames test (preincubation method)	S. typhmiurium TA98, TA100, TA1535, TA1537 and TA1538	1-1000 µg/plate	negative[1]	Shimizu et al., 1985
	Ames test	S. typhmiurium TA98, TA100, TA1535, TA1537 and TA1538	50 µg/plate	negative[1]	Blevins & Taylor, 1982
Acetaldehyde	Sister chromatid exchange	Adult human lymphocytes	0.1-2.4 mM	positive	He & Lambert, 1985
	Forward mutation assay	L5178y mouse lymphoma TK+/-	0.004-0.008 mol/litre	positive[2]	Wangenheim & Bolcsfoldi, 1988
	Ames test	S. typhmiurium TA100, TA102 and TA104	Not reported	negative[1]	Dillon et al, 1992
	Chromosomal aberration test	Chinese hamster embryonic diploid cells	0.002%	positive	Furnus et al., 1990

	Sister chromatid exchange	Adult human peripheral lymphocytes	100-400 µM	positive	Helander & Lindahl-Kiessling, 1991

Table 5. Continued...

Substance name	Test system	Test cells	Concentration	Results	Reference
Propionaldehyde	Ames test	S. typhmiurium TA98, TA100 and TA102	0.13 nmol to 0.13 mmol/plate	negative[1]	Aeschbacher et al., 1989
	Forward mutation assay	V79 Chinese hamster lung cells	1-90 mM	positive[2]	Brambrilla et al, 1989
	Ames test	S. typhmiurium TA100, TA102 and TA104	not reported	negative[3]	Dillon et al, 1992
	Unscheduled DNA synthesis assay	Adult human hepatocytes	10-100 mM	negative	Martelli et al., 1994
	Chromosomal aberration test	Chinese hamster embryonic diploid cells	0.0005-0.002%	positive	Furnus et al., 1990
Butyraldehyde	Ames test	S. typhmiurium TA100, TA102 and TA104	not reported	negative[1]	Dillon et al, 1992
	Unscheduled DNA synthesis assay	Adult human hepatocytes	10-30 mM	negative	Martelli et al., 1994
	Forward mutation assay	V79 Chinese hamster lung cells	1-30 mM	positive[2]	Brambrilla et al., 1989
	Sister chromatid exchange	Chinese hamster ovary cells	9-90 µg/ml	positive[1]	Galloway et al., 1987
	Chromosome aberration test	Chinese hamster ovary cells	59-135 µg/ml	negative[1]	Galloway et al., 1987
	Sister chromatid exchange	Adult human lymphocytes	0.002%	negative	Obe & Beek, 1979
Valeraldehyde	Forward mutation assay	V79 Chinese hamster lung cells	3-30 mM	positive[2]	Brambilla et al., 1989
	Unscheduled DNA synthesis assay	Adult human and rat hepatocytes	3-100 mM	negative	Martelli et al., 1994
	Rec assay	B. subtilis strains H17 and M45	0.6 ml/plate	negative[1]	Matsui et al, 1989

Table 5. Continued...

Substance name	Test system	Test cells	Concentration	Results	Reference
Hexanal	Forward mutation assay	V79 Chinese hamster lung cells	3-30 mM	positive[2]	Brambrilla et al., 1989
	Unscheduled DNA synthesis assay	Adult human and rat hepatocytes	3-100 mM	negative	Martelli et al., 1994
	Ames test	S. typhmiurium TA102 and TA104	up to 1 mg/plate	negative	Marnett et al., 1985
	Ames test (spot test)	S. typhmiurium TA98, TA100, TA1535 and TA1537	3 µmol/plate	negative[1]	Florin et al, 1980
Heptanal	Ames test (spot test)	S. typhmiurium TA98, TA100, TA1535 and TA1537	3 µmol/plate	negative[1]	Florin et al, 1980
	Ames test	S. typhmiurium TA97, TA98, TA100, TA1535 and TA1537	1-3333 µg/plate	negative[1]	Zeiger et al, 1992
Octanal	Ames test (spot test)	S. typhmiurium TA98, TA100, TA1535 and TA1537	3 µmol/plate	negative[1]	Florin et al, 1980
Nonanal	Sister chromatid exchange	Rat (female Fischer 344 animals) hepatocytes	0.1-100 µM	positive	Eckl et al., 1993
	Unscheduled DNA synthesis	Adult human and rat hepatocytes assay	3-100 mM	negative	Martelli et al., 1994
	Forward mutation assay	V79 Chinese hamster lung cells	0.1-0.3 mM	positive[2]	Brambrilla et al., 1989
	Modified Ames test (preincubation method)	S. typhmiurium TA98, TA100 and TA1535	1-666 µg/plate	negative[1]	Mortelmans et al., 1986
	Ames test	S. typhmiurium TA102 and TA104	up to 1 mg/plate	negative	Marnett et al., 1985
	Chromosomal aberration test	Rat hepatocytes	0.4 µg/ml	negative	Eckl et al., 1993

Table 5. Continued...

Substance name	Test system	Test cells	Concentration	Results	Reference

Decanal	Rec assay	B. subtilis strains H17 and M45	5 µl per disk	positive	Yoo, 1986
	Rec assay	E. coli WP2, uvrA	0.005-0.04 mg/plate	negative	Yoo, 1986
	Chromosomal aberration test	Chinese hamster fibroblast cells	0.125 mg/ml	negative	Ishidate et al., 1984
	Ames test	S. typhmiurium TA92, TA1535, TA100, TA1537, TA94 and TA98	up to 1 mg/plate	negative[1]	Ishidate et al., 1984
Undecanal	Ames test (spot test)	S. typhmiurium TA98, TA100, TA1535 and 1537	3 µmol/plate	negative[1]	Florin et al, 1980
Propyl alcohol	Ames test	S. typhmiurium TA100	up to 100 µmol/plate	negative[1]	Stolzenberg & Hine, 1979
	Sister chromatid exchange	V79 Chinese hamster lung fibroblasts	3.3-100 mM	negative[2]	Von der Hude et al., 1987
	Sister chromatid exchange	Chinese hamster ovary cells	0.01%	negative	Obe & Ristow, 1977
	Micronucleus test	Chinese hamster lung fibroblast cells	50 µl/ml	negative[1]	Lasne et al., 1984
Butyl alcohol	Ames test	S. typhimurium TA102	up to 5000 µg/plate	negative[1]	Muller et al, 1993
	Sister chromatid exchange	Chinese hamster ovary cells	0.01%	negative	Obe & Ristow, 1977
	Forward mutation assay	Chinese hamster ovary cells	0.2-1.6 µl/ml	positive	PPG, 1991b
1-Octanol	Cell mutagenesis assay	Mouse lymphoma L5178Y TK+/-	100 µg/ml	negative[1]	Heck et al., 1989
	Ames test	S. typhmiurium TA1535, TA1537, TA1538, TA98 and TA100	2000 nl/plate	negative[1]	Heck et al., 1989
1-Decanol	Rec assay	B. subtilis strains H17 and M45	17 µg/disk	negative	Oda et al., 1978

Table 5. Continued...

Substance name	Test system	Test cells	Concentration	Results	Reference
Undecyl alcohol	Rec assay	B. subtilis strains H17 and M45	20 µg/disk	positive	Yoo, 1986
	Rec assay	E. coli WP2 uvrA	0.005-0.04 mg/plate	negative	Yoo, 1986
Lauryl alcohol	Modified Ames test (preincubation method)	S. typhmiurium TA98, TA100, TA1535, TA1537 and TA1538	0.01-0.50 µg/plate	negative[1]	Shimizu et al., 1985
1-Hexadecanol	Ames test	S. typhmiurium TA98, TA100, TA1535, TA1537 and TA1538	50 µg/plate	negative[1]	Blevins & Taylor, 1982

[1] Both with and without metabolic activation.
[2] Without metabolic activation
[3] With metabolic activation
[4] Positive only at middle dose (2.5 mM), negative at lower (1.25 mM) and higher doses (5 mM); no dose-response relationship

Table 6. Mutagenicity/genotoxicity studies for saturated aliphatic acyclic linear primary alcohols, aldehydes and acids

Substance name	Test system	Test organism	Concentration	Results	Reference
Propionic acid	Micronucleus test, intraperitoneal injection	Chinese hamster cells	5 ml/kg bw	negative	Basler et al., 1987
Acetaldehyde	Mouse bone marrow micronucleus test, intraperitoneal injection	Mouse	95-400 mg/kg bw	positive	Ozawa et al., 1994

In mutation assays with mammalian cell lines, hexanoic acid and heptanoic acid exhibited an increase in the frequency of mutations in mouse lymphoma L5178Y cells with S9 metabolic activation at concentrations greater than 600 µg/ml. The authors noted that culture conditions of low pH and high osmolality, which may occur upon incubation with acidic substances, have been shown to produce false-positive results in this and other assays (Heck *et al.*, 1989). Therefore, these results must be cautiously interpreted. Formic acid and acetic acid, which initially gave an increase in SCE in Chinese hamster ovary cells, were later shown to be negative when tested at physiological pH (Morita *et al.*, 1990). In a forward mutation assay, butyl alcohol tested at concentrations of 0.2 to 1.6 µl/ml was mutagenic when incubated with Chinese hamster ovary cells (PPG, 1991b). This result is also probably due to perturbations in the pH of the test medium.

2.2.4 Reproductive and developmental toxicity

Reproductive and developmental toxicity studies on low molecular
ight aliphatic alcohols (propyl alcohol and butyl alcohol) via
alation at high concentrations have been associated with
velopmental effects in the presence of maternal toxicity (Nelson
al., 1990). When butyric acid, valeric acid and octanoic acid
e given daily by tracheal intubation on days 6 to 15 of gestation,
y fetotoxicity was reported at the highest dose level (1500 mg/kg
per day) with octanoic acid; no other evidence of fetotoxicity,
velopmental toxicity or teratogenicity associated with these three
boxylic acids was observed (Narotsky *et al.*, 1994). There is no
idence to conclude that, when ingested as flavouring substances,
take of any of the substances in the group of linear saturated
iphatic substances would be associated with reproductive or
velopmental toxicity.

2.4.1 Propionic Acid

Fetal abnormalities or effects on survival were not observed when
calcium salt of propionic acid was fed to pregnant rodents (up to
mg/kg bw per day for 10 days, hamsters (up to 400 mg/kg bw per day
5 days) and rabbits (up to 400 mg/kg bw per day for 13 days)
RL, 1972).

2.4.2 Butyric Acid

Maternal weight loss and respiratory effects were observed in
ale rats given 100 or 133 mg/kg bw per day of butyric acid by
acheal intubation on days 6 to 15 of gestation (Narotsky *et al.*,
4). In dams with peripartum respiratory symptoms, reduced pup
ight and decreased progeny viability were reported, but no signs of
gnificant development toxicity were reported at either dose. Gastric
ritation was noted at necropsy.

2.4.3 Valeric Acid

Female rats given 0, 75 or 100 mg/kg bw per day valeric acid by
vage on days 6 to 15 of gestation exhibited signs of maternal
xicity including respiratory effects and decreased body weight, but
significant developmental toxicity at either dose. In Segment II of
is study, valeric acid was associated with maternal toxicity and
duced fetal weights at dose levels from 50 to 200 mg/kg bw per day.
fetal skeletal malformations were reported, except for sternebrae
riations. Gastric irritation was noted at necropsy (Narotsky
al., 1994).

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See Also:
Toxicological Abbreviations

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&
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Nanotechnology makes mango farming more profitable in Krishnagiri

By Anupam Srivastava,IDRC Regional Office, New Delhi

Krishnagiri (Tamil Nadu): It was a typical mango season a year ago for Varadarajan, a plantation-owner in Krishnagiri, the "mango district of Tamil Nadu". Varadrajan was doing his best to get a good harvest yet there were certain problems that looked so inherent to mango that he never expected a solution. As the mango season arrived, he lost some of the mangoes before they turned ripe. "They would simply drop off for no good reason," he says. At the same time, he could not think of sending his mangoes far due to the low shelf life of the fruit.

The loss of fruit due to various factors is as high as 20 to 50 per cent in the world, while in Asia it is around 30 per cent, says Prof. K.S. Subramanian from the Tamil Nadu Agricultural University (TNAU), Coimbatore in India, who is at the forefront of research on use of nanotechnology in keeping fruit fresh.

While these factors affected profitability, another issue is that the mango season is short. "When mangoes ripen, there are heaps of mangoes in the market, creating such an abundance and oversupply that one cannot get a good price. And then all of a sudden they are gone. If there was a way one could sell mangoes late in the season, one would earn a much better profit," says Varadarajan. As a result, his mangoes got him modest prices in the peak season.

One evening, at a farmers' meeting in the nearby town, Varadarajan heard about the experience of his farmer-friend, Santa Kumar. Kumar told a gathering of farmers that he knew of a natural spray called hexanal that could prolong the shelf life of mangoes. "It was too good to be true, but Santa Kumar had already tried it out and was talking about the results," says Varadarajan.

Santa Kumar said that he had used a hexanal, treated with nanotechnology, which could delay the ripening of fruit by at least two weeks. All one needed to do was mix it with water in recommended proportions and spray twice on the trees during the season. That prolonged the shelf life of mangoes by more than two weeks. There were other benefits such as fewer mangoes dropping off trees, and of course, the possibility of a longer mango season and sending one's fruit to far-off markets.

Varadarajan had a few queries which were answered at a team of academics from the Tamil Nadu Agriculture at Coimbatore. He learnt of not only the hexanal-based spray, but other nanotechnology-based products which could keep mangoes fresh and firm much longer than ever before.

Convinced that hexanal was worth a try, Varadarajan asked the team if he could have his plantation covered under the program. Last year, Varadarajan used hexanal on a part of his large farm, leaving out some of his trees as "control" for the sake of comparison. The field partners of TNAU, Myrada, a non-governmental organisation, worked with him to teach him how to mix and spray hexanal. They also introduced him to other methods of fruit preservation such as post-harvest dip, use of nano-technology stickers and films maximise the shelf-life of mangoes.

Vardarajan reaped the results last year and has expanded his business. "I got a season longer by three to four weeks while fruit retention on the trees are improved drastically," he says. Every tree treated with hexanal is yielding around 5 kilos more than the 'control' trees. With techniques such as post-harvest dip (briefly dipping fruit in hexanal and water mix), and use of stickers that release nano-particles in packaging, he found that the fruit, even after being plucked, stay firm and fresh for weeks. Varadarajan also has used nanotechnology to become an exporter of fruit, as these fruits remain firm and fresh after such a long distance. "Not only do I export my fruit, I also buy fruit and export it," he says. That has made business much more profitable for him. "Earlier I could not dream of sending my fruit far, but now nanotechnology keeps it fresh so I can," he says.Another farmer, Madhavan, grows three varieties of mango on 11 acres of land. He, too, reports his mangoes stay fresh longer and help him earn a better profit.

Increasingly, farmers in Krishnagiri are getting to learn more about nanotechnology and hexanal and are adopting these with great results. TNAU's community contact initiative with Myryada reaching out to Mango Producers' Groups (MPGs) has helped farmers and their spouses understand the benefits of nanotechnology while the MPGs also serve as points of sale for hexanal-based products which the farmers need.

Prof. Subramanyan hopes in the near future nanotechnology-based products will be produced commercially so that more farmers can benefit. "We are working on getting the permissions to make it possible," he says.

Pre-harvest Treatment of mango *(Mangifera indica)* var. TJC and var. Karthakolombaan with Enhanced Freshness Formulation (EFF)

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Introduction

Pre-harvest treatment methods are practiced in commercial mango plantations for disease control, finduction of flowering and plant growth regulation etc. However, limited information is available on methods adopted to retain fruits on trees for extending the harvesting season. This novel approach uses pre-harvest spray treatments of hexanal incorporated Enhanced Freshness Formulation (EFF) which has proved effective in reducing the membrane degradation by the inhibition of Phospholipase D enzyme (University of Guelph, Canada) .

Methodology

The spray trials were conducted at Ellawala Horticulture Mango Farm, Galkiriyagama. Mango trees suitable for the trial were selected and tagged from the same block of land/field but far enough from each other so that the treatments would not interfere. The field experiment was designed as 3 trees per treatment. The trees were sprayed with 2% EFF till dripping. The spaying pattern was modified according to fruit variety and fruit setting time. Experiments were carried out for both TJC and Karthakolomban (KK) mango varieties in 2 & 3 time frequency.

PREHARVEST SPRAY FOR TJC

S^0: Fruits sprayed 3 times with 2% EFF 20, 40, & 60 days before harvest.

S: Fruits sprayed 2 times with 2% EFF 20 & 40 days before harvest.

(Note: TJC – Fruit setting to Harvesting 100-120 days)

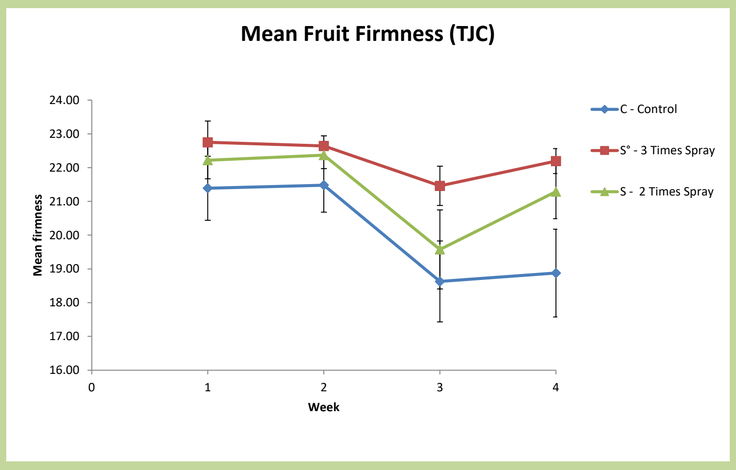
PREHARVEST SPRAY FOR KK

S^0: Fruits sprayed 3 times with 2% EFF; 15, 30 & 45 days before harvest.

S: Fruits sprayed 2 times with 2% EFF, 15 , 30 day before harvest.

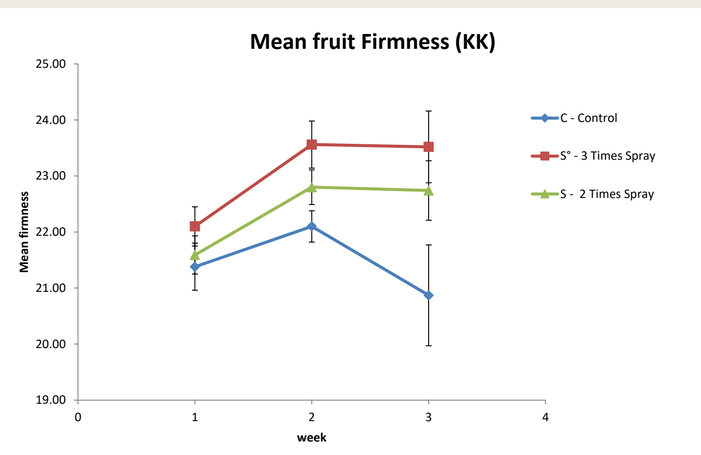
(Note: KK – Fruit setting to Harvesting 80-90 days)

Results:



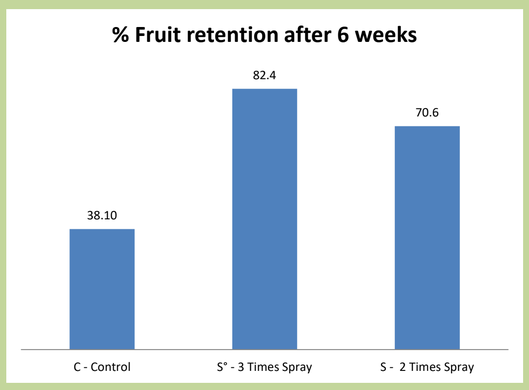
Control 4st Week

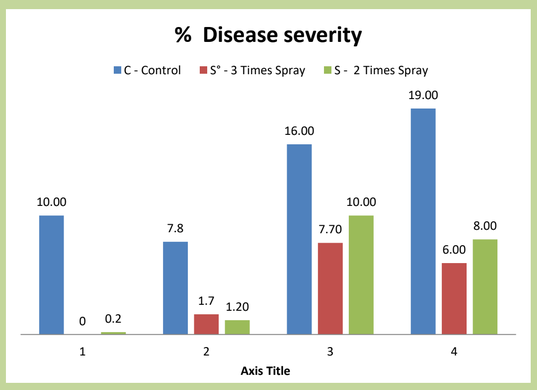
3 times EFF spray 4th Week

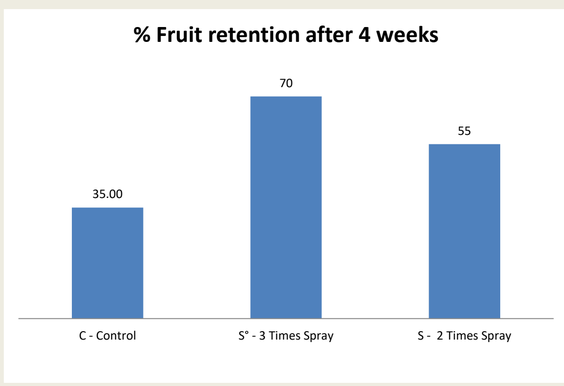


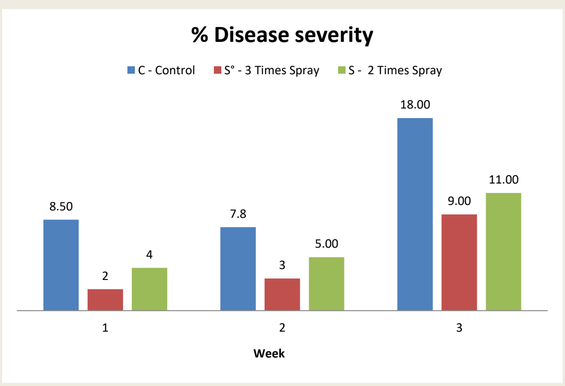
Control 3rd Week

3 times EFF spray 3rd Week









* No significance were observed With respect to pH, TSS, acidity, flesh & peel color for both TJC & KK varieties of mangoes for 7th day to 28th day harvesting period comparing to Control fruits.

Conclusion

The fruits sprayed with EFF were able to retain consistent firmness throughout the harvesting period of 4 weeks. EFF has proven to be effective in maintaining the fruit skin firmness of TJC mango with increased number of spraying times. Harvesting season of KK variety of mango and TJC variety of mango could be extended up to 4 weeks and 6 weeks respectively using 3 time EFF spray treatment.

Financial support provided by the Government of Canada & IDRC, Canada are herewith gratefully acknowledged.

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APPLICATION OF ESSENTIAL OIL INCORPORATED EDIBLE WAX FOR SHELF LIFE EXTENSION OF MANGO AND PAPAYA

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Introduction

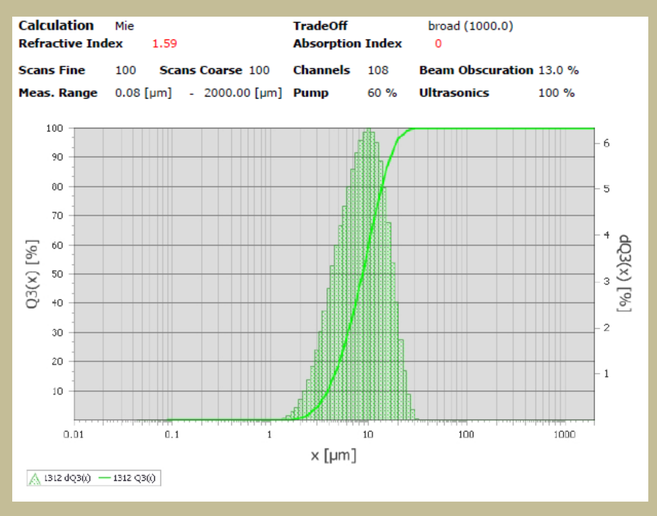
In Sri Lanka, postharvest loss of fruits is estimated to be around 30-40 %. This study aims at developing natural product based wax emulsions for use as protective coatings to slow down natural ripening, minimize moisture loss & pathogen infection. Reducing loss of produce and increasing the productivity of cultivations will increase farmer income and the availability of fruits to consumers. The slow release mechanism of the active ingredients in the bio-wax plays a key role in preserving the shelf life by minimizing loss due to disease.

Methodology

The Bio-wax formulation is a water based emulsion made of bees wax, cinnamon bark oil and other ingredients (Patent Application no 18030). The efficacy of wax application on storage life extension was tested on TJC & Karthakolommbaan variety mangoes and Red lady papaya variety. Randomly selected replicate fruits were dipped in wax for 30 sec., allowed to dry and stored at 13.5^{o}C. Fruit quality parameters were assed after 7, 14 and 21 days storage.

Wax Formulation

Preparation



Characterization

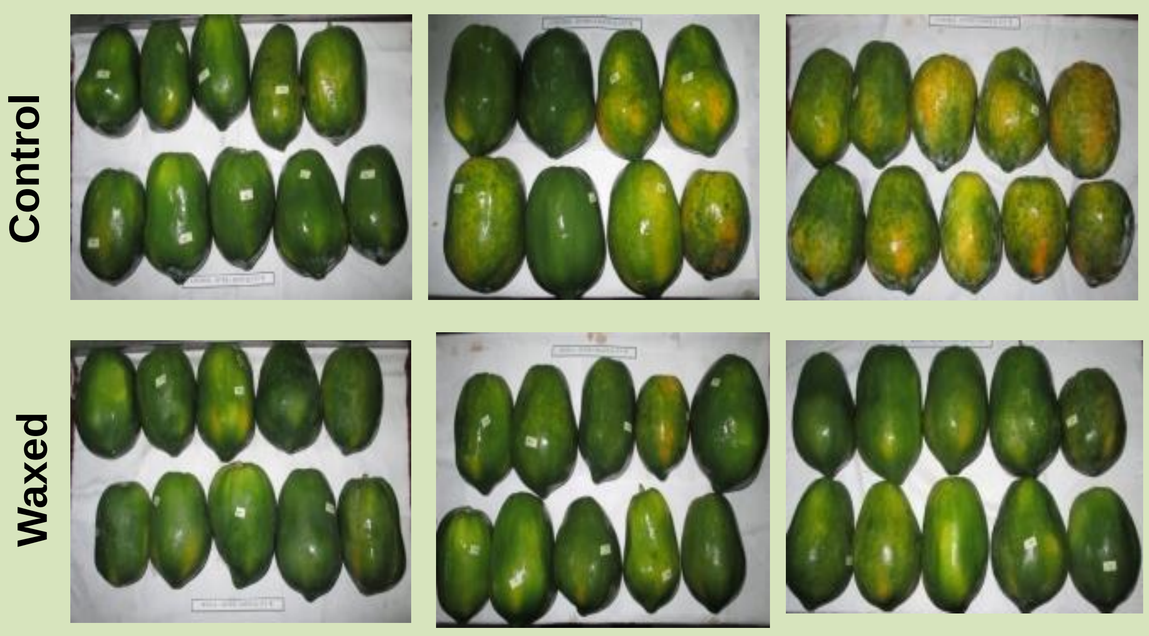
Lab scale Fruit trials

Scaling up

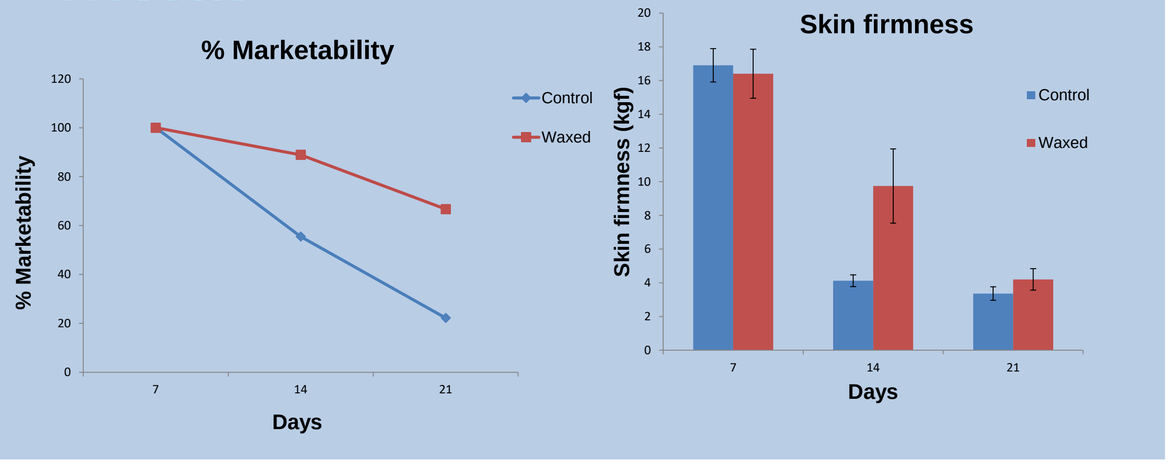
Laboratary scale fruit trials

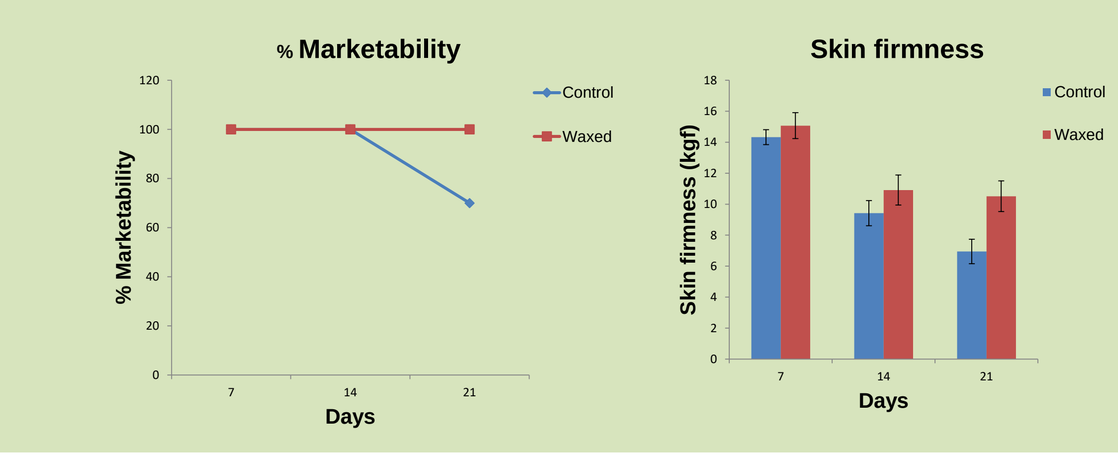
MANGO

PAPAYA



Results





- No significant difference was observed with respect to pH, TSS, acidity, flesh & peel color for both TJC & KK varieties of mangoes after 7, 14t & 21 days storage compared to Control fruits.
- Wax treated papaya showed significant improvement with respect to pH, TSS, acidity, flesh & peel color compared to controls after 14 & 21 days.

Conclusion:

Both mango and papaya varieties treated with wax showed better fruit quality compared to control fruits. Results indicate that storage life of bio-wax treated papaya could be extended for more than 21 days.

Financial support provided by the Government of Canada & IDRC, Canada are herewith gratefully acknowledged.

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Hexanal incorporated fibre-polymer composite board and its efficacy on shelf life of mango

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Introduction

Post harvest loss of fruit and vegetable in Sri Lanka could be close to 40%. Loss is due to poor management of produce after harvest resulting in physical injury, moisture loss and disease. Further to research conducted at the the University of Guelph, Canada it is known that hexanal is a Phospholipase D inhibitor, which prevents fruit membrane degradation. In this study hexanal was incorporated into a fiber polymer composite material, for use in fruit packs for reducing post harvest loss by slow release of hexanal vapour into the fruit pack during storage and transportation. .

Methodology

The fiber polymer composite board developed consisted of Banana fiber (9.73%), Polymeric materials (2.54%) and Bio polymers (17.34%). Hexanal was incorporated into the matrix by mixing 12.69% of final weight. The mixture was filled into a polythene case wrapped with aluminum foil and subjected to a hot press for 1 minute at 130°C. The aluminum foil pack was opened for use and pasted on the inside lid of the fruit box. Six randomly selected TJC mangoes were used in trials for efficacy testing. Boxes were placed in a cold room held at 13.5°C, and tested at 7 days intervals over a period of 28th days.

Testing Protocol – TJC mango

Harvest fruits → Sort → Pack in boxes → Cold storage 13.5°C → Test the fruits in 7 days interval

Parameters tested:

- Fruit firmness (kg)
- Flesh firmness (kg)
- °Brix
- Acidity (% Citric acid)
- pH
- % Physiological weight loss
- Colour of fruit & flesh

Results

Parameter	Storage period (Days)	Control	Composite	Statistical Difference
Fruit firmness (kg)	7	5.93 ± 0.29	10.28 ± 0.74	Significant
	14	2.79 ± 0.19	4.32 ± 0.39	Significant
Flesh firmness (kg)	7	1.00± 0.11	2.60± 0.39	Significant
	14	0.53± 0.06	0.95± 0.09	Significant
°Brix	7	13.0 ± 0.0	12.3 ± 0.7	Not significant
	14	15.67 ± 0.33	15.33 ± 0.33	Not significant
pH	7	3.59 ± 0.03	3.47 ± 0.02	Not significant
	14	4.37 ± 0.05	3.65± 0.03	Significant
Acidity (% Citric acid)	7	1.09 ± 0.05	1.15 ± 0.04	Not significant
	14	0.65 ± 0.07	0.81 ± 0.08	Not significant

Note: Control fruits were not in marketable condition at 21st day and thereafter

SOCIO-ECONOMIC IMPACT

HEXANAL INCORPORATED COMPOSITE MATERIAL HELPS EXTEND STORAGE LIFE OF MANGOES AND REDUCES LOSS. THIS ENABLES EXPORT OF LARGER VOLUMES OF FRUIT PURCHASED FROM FARMERS AND TRANSPORTED AT LOWER BY SEA FREIGHT.

PRODUCT DEVELOPED BASED ON…

- UTILIZATION OF BANANA PSEUDO STEM
- NONTOXIC INGREDIENTS
- HEXANAL AS ACTIVE INGREDIENT (REACTIVE & PHOTOOXIDATION)
- HEXANAL RELEASE PATTERN (EVOPARATION/ OXIDATION)
- STABILIZATION OF HEXANAL IN MATRIX

Conclusion: Mango fruits subjected to storage with hexanal incorporated composite for 21 days at 13.5°C showed good marketability traits with significantly higher fruit firmness & flesh firmness.

Financial support provided by the Government of Canada & IDRC, Canada are herewith gratefully acknowledged.

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