ENCAPSULATION AND REUSE OF ENZYMES FROM GUT MICRO BIOME OF PESTS PRESENT IN FRUITS AND VEGETABLES FOR REMOVAL OF PESTICIDES

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Introduction:

Pesticides play a crucial role in modern agriculture, safeguarding crops from destructive pests and ensuring food security for the population. However, current indiscriminate use of pesticide has led to risks associated with public health and food safety. The exorbitant amount of pesticides sprayed in agricultural fields leave residues in the vegetables and fruits that can be risky for consumption, and may give rise to serious health issues in the future. A major percentage (75-80%) of the pesticide residues are removed by simple washing with cold water. However, residual pesticide content in vegetables and fruits can be detrimental, giving rise to potential endocrine health disruption and certain neurological disorders.

Apart from direct consumption, pesticides also contaminate soil and water bodies. The bioaccumulation of these pesticide residues can travel higher up in the food chain, which eventually can hurt human health when animals that are higher up in the food chain are consumed. Recently, there has arisen a need to bring down the chronic exposure to low levels of pesticides out of public health concern, especially for children. This project aims to address and alleviate these concerns regarding pesticide residue in agricultural produce.

Pests infesting agricultural crops often stay alive even after the use of multiple rounds of pesticides. This is enough to conclude that the pests are able to sustain so well because their gut contains the necessary enzymes that can break down the pesticide formulations and help them survive in the vegetables for longer. The main focus of this project is to isolate and culture the gut micro biome of the pests' in-vitro for enzyme formation that can be ultimately used to get rid of pesticide residue of different formulations from fruits and vegetables.

Objectives:

The prime objectives of the project include:

- Identifying and isolating pesticide resistant pests that are found in fruits and vegetables
- Dissecting the gut and culturing the gut microbiome in a nutrient media.
- Checking the enzymatic action of the bacteria against different pesticide formulations and culturing the most potent pesticide degrading enzyme producing bacteria.
- Encapsulation of these enzymes for further future use for pesticide degradation and removal of residue.
- Developing a sustainable and cost-effective enzyme-based solution for degradation of pesticide residue on vegetables.

Methodology:

Phase 1 - Collection of pesticide resistant pest and insect species

Pesticides used against pests in agriculture often leave behind residues on the vegetables that are hard to get rid of. The continuous consumption of vegetables laden with pesticide can lead to toxic accumulation in the gut and body over time. It is crucial for removal of such pesticide residues from vegetables, so as to lessen the chances of toxin accumulation.

Some agricultural pests survive the pesticide application during production, as a result of enzymatic persistence. These enzymes are persistent against a certain formulation of pesticides, and might not persist at a higher level of concentration. The enzymatic action against the residual pesticide of specific formulation is the main focus for this study.

Phase 2 - Pesticide formulation for different crop species

Food crops like tomatoes, cauliflowers and cabbages are some of the most important agricultural crops in India. The production of these agricultural crops requires around 90- 160 days from sowing to harvest. At periodic intervals, dosages of pesticides like Chlorantraniliprole, Emamectin Benzoate Cypermethrin are sprayed to prevent pest infestation. The dosage and formulation of these pesticides and insecticides differ for different vegetables.

Pesticide Formulation	**Pest Control**
Chlorantraniniprole (18.5 %)	Broad range lepidopteran pests (Borers and Caterpillars)
Emamectin Benzoate 5 sg	Diamondback Moth in cabbage
Fipronil S 80%	Sucking pests
Cypermethrin 25% EC	Borer, bollworm, sucking pests

Phase 3 - Isolation and encapsulation of enzymes from the gut microbiome of pests

Gut microbiome contains many different enzyme producing microorganisms; in case of agricultural pests, the gut microbiome is a gateway to understanding the pesticide resistance of an organism. The isolation and encapsulation of the enzymes produced in the gut is the key in creating a low cost efficient solution to reduce the pesticide residue. Encapsulation using nanoparticles will enable to reuse the enzymes in a convenient manner.

Phase 4 - Preparation of a consortium of the enzymes derived from gut microbiome of the pests

The enzymes present in the gut of the microbes living in the digestive system of pests helps them to break down organic matter aiding for digestion. A consortium of these enzymes will help us develop biological control methods to target pesticide residues in vegetables. The individual action of the enzymes in contrast with the enzymatic action of the consortium will be the binding factor that brings the experiment to fruition. The reduction in the concentration of the pesticides will be measured using chromatographic techniques.

Conclusion:

Phase 1:

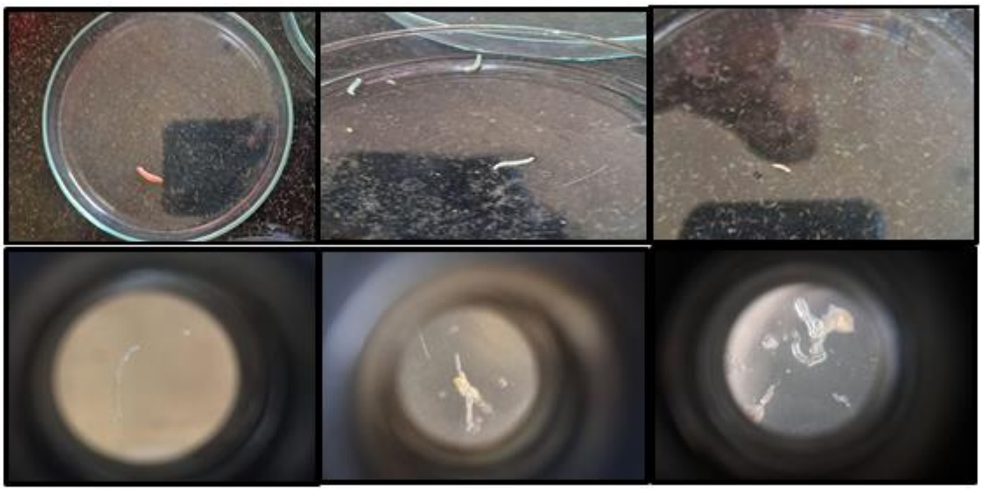
Vegetables and fruits available in the market were checked for the presence of pests, and the ones containing them were chosen for the project. While some vegetables showed signs of pest infestation, there were no signs of the actual pest presence; however, there were other vegetables that contained pests that were chosen for the study. The sample basically consisted of:

Name of the vegetable	Common name of the pest	Scientific name of the pest
Cauliflower	Diamondback moth	Plutella xysotella
Brinjal	Eggplant borer	Leucinodes orbonalis guenee

Collection of Vegetables infested by pests

Dissection of Pesticide Resistant Pests:

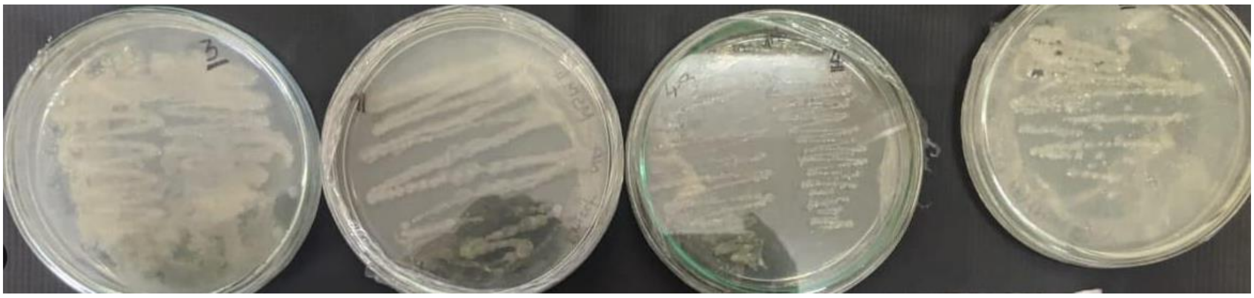
The pests were then carefully extricated from the vegetables, sterilized in a petri plate, and then proceeded with for dissection. The resultant dissected gut then was further processed for culturing of microbes.



Dissection of pests for gut microbes

Phase 2:

The inoculation of the gut into a nutrient medium for optimal results was performed, where the growth will be studied and tracked alongside the action of pesticides of different formulations to register the extent of enzymatic action by the bacteria. Later the initial culture of the gut bacteria will be transferred into a broth culture, and subjected to different pesticide formulations (normally used in agriculture fields) to study the survival rate.



The inoculation of pests gut into NA media

Phase 3:

The cultures that survive this treatment do so because of the action of certain enzymes present in the gut. As such, these become the bacteria of interest. Ultimately, this is the bacterial culture that will be further cultured, and tested for the enzymatic removal of the pesticide residue in vegetables and fruits.

Phase 4:

Encapsulation of these enzymes ensures a sustainable and environment-friendly way to counter the risks posed by continuous but chronic exposure to low quantities of pesticides. Encapsulation will be done by sodium alginate immobilization, where a calcium chloride solution can be used for cross-linking the alginate, leading to gel bead formation, thus encapsulating the enzyme.

In conclusion, this multi-phase study represents a comprehensive approach to addressing the issue of pesticide resistance in agricultural pests while also mitigating the potential risks associated with pesticide residues in fruits and vegetables.

By selectively isolating pests from infested produce and dissecting their guts to culture pesticide-resistant bacteria, this research lays the groundwork for understanding the enzymatic mechanisms underlying resistance. Through subsequent phases, the study investigates the efficacy of different pesticide formulations on these bacteria, identifying those with the highest survival rates and enzymatic activity.

The culmination of this work lies in the development of a sustainable solution: encapsulating these enzymes within sodium alginate beads. This encapsulation not only ensures the stability and longevity of the enzymes but also offers an environmentally friendly approach to pesticide residue removal.

Overall, this research presents a promising avenue for both pest management and food safety, offering insights into novel strategies for combating pesticide resistance and reducing pesticide residues in agricultural produce.

Scope for future work:

Any future research regarding the idea of this project may be to focus on identifying and characterizing specific enzymes responsible for pesticide degradation.

- Advanced metagenomic and proteomic techniques will facilitate the discovery of novel enzymes with unique catalytic properties. By employing genetic engineering and synthetic biology, these enzymes can be optimized for higher efficiency and broader substrate specificity.
- The enrichment of specific strains which favour the growth of bacteria and prioritise optimal enzyme production can be amplified in culture, and then the enzymes can be extracted and purified.
- These purified enzymes can be formulated into a bioremediation solution. This solution, when applied to fruits and vegetables, could potentially degrade any residual pesticides on the surface.They develop a formulation that ensures the enzymes remain stable and active during storage.
- Encapsulation techniques, such as Nano-encapsulation, microencapsulation, and immobilization on biodegradable polymers, can be refined to protect enzymes from environmental stressors while ensuring their sustained release and activity. These advancements will enable the development of enzyme-based formulations that can be easily applied to contaminated fruits and vegetables.
- The reuse of these encapsulated enzymes in agriculture offers a sustainable and eco-friendly alternative to conventional chemical treatments. It reduces the

reliance on synthetic pesticides, minimizing their adverse environmental impact and enhancing food safety.

- Further exploration of symbiotic relationships between pests and their gut microbiome will uncover additional beneficial enzymes, broadening the scope of this technology, and also leading to a large-scale need for environment friendly ways to eliminate pesticides.