

ORIGINAL ARTICLE

Green Detox: A plant based Ecofriendly Bioenzyme Vegetable Wash for Effective Chlorpyrifos Removal in Brinjal

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ABSTRACT

Chlorpyrifos is an organophosphate pesticide commonly used by farmers to control insect and pests. It is highly toxic and has serious side effects on humans and environment. Pesticide contamination in vegetable poses significant health risks, with Chlorpyrifos being a commonly detected residue in brinjal. Conventional washing methods often fail to remove pesticide residues effectively. This study evaluates the efficacy of a medicated bioenzyme vegetable wash, in the dissipation and decontamination of Chlorpyrifos residue in brinjal. A bioenzyme vegetable wash is a natural, ecofriendly liquid cleanser made from plant-based ingredients and microbial fermentation. It is designed to remove pesticide residues, dirt and contaminants from fruits and vegetables. Brinjal samples were treated with Chlorpyrifos under different condition and were washed with bioenzyme vegetable wash. Residue analysis was performed using GC/MS to assess pesticide reduction. Results indicated that the bioenzyme wash significantly reduced chlorpyrifos levels compared to conventional washing methods. The enzymatic action, facilitated pesticide breakdown, making it a safe and eco-friendly alternative for residue removal and it is found to be more than 86% effective in the removal of Chlorpyrifos. The study highlights the potential of bioenzyme based washing solutions in ensuring food safety and reducing pesticide exposure in daily consumption.

Keywords: Pesticides residues, Chlorpyrifos, Bioenzyme, Vegetable wash

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INTRODUCTION

Pesticide contamination in vegetables is a global health concern, particularly in developing countries, where regulatory enforcement is limited. Chlorpyrifos, an organophosphate insecticide, is widely used in agriculture including the cultivation of brinjal (*Solanum melongena L.*), to control insect pests (1). Despite its effectiveness in pest control, chlorpyrifos residues persist on food crops, posing serious toxicological risks to human health and the environment (2). Chlorpyrifos exerts its primary toxic effects irreversibly inhibiting acetylcholinesterase (AChE), an enzyme essential for proper nerve function. This leads to an accumulation of acetylcholine at synapses, resulting in neuromuscular dysfunction, cognitive impairment and neurodevelopmental disorders, especially in children (3). Long term exposure to Chlorpyrifos associated with neurotoxicity: memory loss, cognitive decline, developmental disorders (4). Endocrine disruption: interferes with reproductive hormones, affecting fertility and fetal development (5). Carcinogenic Potential: Some studies suggest a link to hormone-related cancers, although further research is needed (6). Respiratory and immune effects: Increased risk of asthma, allergic reactions and immune suppression (7). Liver and kidney damage: bioaccumulation leads to hepatic and renal toxicity (8). There is currently an increasing concern and awareness on the bad effects of pesticides caused to consumers. Even with the adoption of integrated pest management, farmers still believe in the control of pests and diseases of plants by using pesticides because of its quick effects(9). The increasing use of pesticides in agriculture has led to significant concerns about residue accumulation in food products, particularly vegetables.

Despite the risks of pesticide residues, public awareness regarding pesticide residues remains limited. Surveys suggested that while consumers are concerned about food safety, they often lack specific knowledge about pesticide contaminants (10). Conventional methods for removing pesticide residues from fruits and vegetables, including washing, peeling and scrubbing. Research indicates that proper washing techniques can't significantly reduce pesticide residues to level below the maximum residue limit (MRL), thereby mitigating health risks (11). Traditional washing methods, such as rinsing with plain water, are often ineffective in completely removing pesticide residues from vegetables and fruits. Studies indicate that these methods may only reduce surface contaminants but fail to eliminate systemic pesticides that penetrate plant tissues (12). Leafy vegetable, in particular, retain higher pesticide residues due to their large surface area and waxy coatings, limiting the effectiveness of simple washing techniques (13). Research have shown that washing with tap water can remove up to 50%of some pesticide residues, but certain chemicals like chlorpyrifos and organophosphates persists even after thorough rinsing (14). Moreover, scrubbing and peeling, while more effective, result in the loss of the essential nutrients and may not be feasible for all produces types (15). Hence, alternative decontamination methods, such as bioenzyme washes, ozone treatment, and alkaline solutions, are being explored to enhance residue removal (16). The bio-enzyme wash derived from natural sources and containing enzymatic compounds, was tested for its ability to degrade and remove chlorpyrifos residues. Bio-enzyme based washes are a safe and effective option for cleaning all types of produce including porous fruits and vegetables. These technique uses natural enzymes to break down dirt, bacteria, and pesticides without leaving harmful residues. The bio-enzyme contains potential antioxidant and antimicrobial properties (17).

MATERIAL AND METHODS

Bioenzyme vegetable wash preparation method

A clean good quality plastic container was used for the fermentation process of bioenzyme production. The ingredients used are

Ingredients	Botanical name
Sireesha	<i>Albizia lebbbeck</i>
Nimba	<i>Azadirachta indica</i>
Lemon peel	<i>Citrus limon</i>
Tulsi	<i>Ocimum sanctum</i>
Jaggery	
Yeast	
Water	

The bio-enzyme vegetable wash was prepared by taking in the ratio of 1 part of jaggery, 3 parts of drugs (medicines in paste form), and 10 parts of water in total a ratio of 1:3:10. The quantity of ingredients taken are 100gms of jaggery, 300 gms of crushed ingredients (*sireesha, nimba, tulsi, lemon peel*) and 1 litre of water was taken. The ingredients are kept in the container and added with 1 teaspoon of yeast. Mix thoroughly. Then the lid is closed firmly and kept at room temperature in a shady place for 30 days. Bioenzyme Vegetable washes are produced through a microbial fermentation process, where beneficial micro-organisms (Mainly yeast and naturally occurring bacteria) break down organic materials to generate enzymes, organic acids, and bioactive compounds. This process enhances the wash's ability to degrade pesticide residues, dirt, and microbes from produce. Fermentation produces carbon dioxide gas. So the container lid was opened periodically, to release carbon dioxide gas. The Bioenzyme Vegetable wash was stored in cool, dry and dark place inside a food grade plastic container with a temperature 10°C to 30°C to maintain enzymatic activity.



Fig. 1 - Crushed drugs used in the preparation of bioenzyme vegetable wash

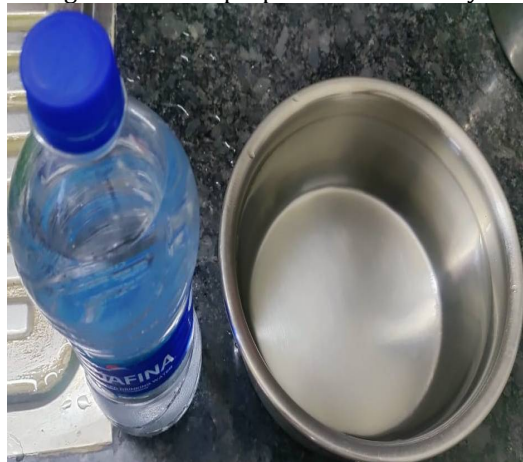


Fig. 2- Water used in the preparation of bioenzyme vegetable wash



Fig. 3- Mixed up ingredients for fermentation

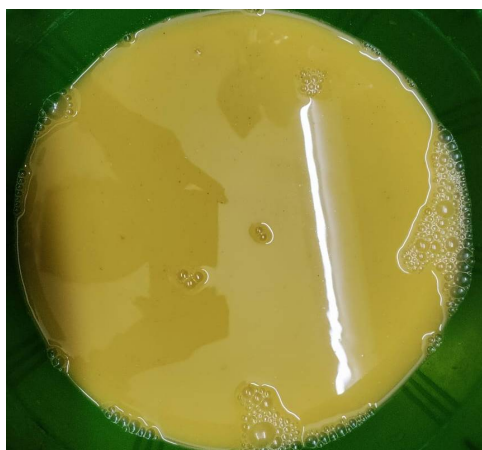


Fig.4 - Prepared bioenzyme vegetable wash solution

COLLECTION OF BRINJAL AND CHLORPYRIFOS PESTICIDE TREATMENT

Vegetable selected for the particular study is brinjal. Two types of samples were taken. First sample was cultivated in the fields and sprayed the pesticide Chlorpyrifos, in early morning from its flowering stage to the grown stage. The Chlorpyrifos (Banest brand is a systemic insecticide containing chlorpyrifos which is commonly used by farmers) was used in the study. The dose of pesticide and dilution was standardized based on common agricultural practices. Banest-Chlorpyrifos was diluted at 5ml per litre of water. Second sample of brinjal was purchased from the local market and was treated with Banest brand chlorpyrifos. The sample was dipped in the pesticide solution that is 25ml diluted in 5 litres of water for 10 minutes. The sample was removed and air-dried. After air drying subsampling or subgrouping was done for both the samples into 4 groups of 250gms each. One sample each from the subgroups was kept aside without decontamination for the comparison of pesticide residue.

The two samples were analyzed as per the criteria of QuEChERS (Quick, Easy, Cheap, Effective Rugged and safe). The samples were evaluated and it is concentrated with Rota vapor. The samples were centrifuged on centrifugation machine on 5 minutes. Then 10 ml of supernatant solution was collected in the test tube. Then the sample was ready to put in GC Mass Spectrometry machine.

The pesticide treated vegetables were washed with bio-enzyme vegetable wash solution. The quantity taken is 30 ml of bio-enzyme vegetable wash diluted with 1000ml of water. Soaked the vegetables and taken out air dried. Then the analysis was done.

Chlorpyrifos, the active ingredient in formulations such as Banest(20%EC) is an organophosphate insecticide characterized by phosphorothioate group linked to a 3,5,6-trichloro substituted pyridine ring. The concentration used 20% indicates that the active pesticide content, from which experimental dilutions can be easily calculated. Structurally chlorpyrifos exhibits lipophilic properties due to its diethyl ester side chains facilitating strong adherence to vegetable surface such as brinjal. This structural stability and surface affinity contributes to its persistence, thereby justifying its selection in residue analysis studies and evaluation of decontamination methods such as bio-enzyme washing.

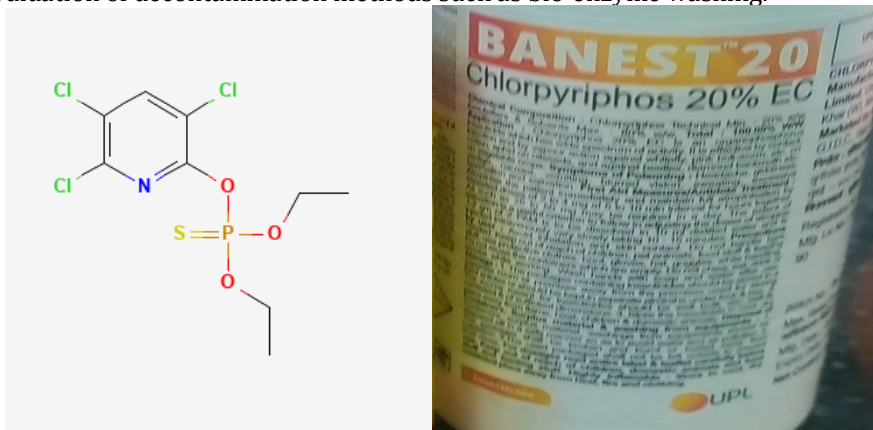


Fig.5 - The Chlorpyrifos pesticide used in the study for pesticide treatments and its chemical structure

RESULTS

Physiochemical Stability test & Microbiological test for herbal medicated bioenzyme vegetable wash

Table 1: Sample Details sent for Microbiology and stability test

Sample Reg. No (TRF)	461
Sample Name	Fertilizer
Sample Code	GTL-2401
Sample Description	Green colour liquid in a plastic bottle
Sample Quantity	1 x 100 ml
Customer Reference	Fermented Organic Product
Sampling Done By	Customer
Sample Condition	Ambient, Good

Stability Study-Results

The stability study of the bioenzyme-based vegetable wash was carried out to evaluate its physicochemical characteristics over a period of six months. The formulation was stored under ambient and accelerated conditions, and parameters such as colour, appearance, pH, density, moisture content, acid value, peroxide value, and electrical conductivity were assessed at regular intervals (0 day to 6th month). The results indicated no significant variation in colour and appearance, which remained as a green liquid throughout the study period. The pH values were consistently acidic, supporting the stability of the fermented formulation. Density remained constant, indicating no phase separation or degradation. Minor variations observed in moisture, acid value, and peroxide value were within acceptable limits, suggesting good physicochemical stability. Overall, the formulation demonstrated stability for up to six months under the tested conditions.

Table-2: Physiochemical Stability study for Bio enzyme vegetable wash

Sl.No	Test Parameter	Method	0 Day	1st Month	2nd Month	3rd Month	4th Month	5th-6th Month
1	Colour	GTL/CHE/SOP-36	Green liquid	Same	Same	Same	Same	Same
2	Appearance	GTL/CHE/SOP-36	Green liquid	Same	Same	Same	Same	Same
3	Overall Acceptance	GTL/CHE/SOP-36	Yes	Yes	Yes	Yes	Yes	Yes
4	Density	FCO	1.00	1.00	1.00	1.00	1.00	1.00
5	pH	FCO	3.17	3.17	3.18	3.17	3.18	3.17
6	EC	FCO	0.337	0.337	0.338	0.336	0.339	0.338
7	Moisture	IS 13579:1996	97.88	97.89	97.92	97.95	97.98	98.08
8	Acid value	IS 548 (Part 1 /Sec 2):2021	16.45	16.45	16.47	16.49	16.52	16.58
9	Peroxide value	IS 548 (Part 2):2021	11.25	11.26	11.28	11.29	11.31	11.38

Integrated Description of Stability and Physiochemical Evaluation Report: The prepared bioenzyme vegetable wash was subjected to a comprehensive physicochemical, chemical, and microbiological evaluation to assess its stability, quality, and safety over a defined storage period of six months.

Physicochemical parameters, including colour, appearance, pH, density, and electrical conductivity, were analyzed at regular intervals. The formulation consistently exhibited a green-colored liquid appearance without any visible changes, indicating physical stability. The pH remained stable in the acidic range (approximately 3.16–3.18), which is favorable for degradation of pesticide residues. Density values remained constant at 1.00, confirming the absence of phase separation or compositional changes, while electrical conductivity showed minimal variation, indicating ionic stability of the formulation.

Chemical stability was further evaluated through determination of moisture content, acid value, and peroxide value using standard methods. Moisture content remained consistently high, reflecting the aqueous nature of the formulation. Acid value and peroxide value showed only negligible variation throughout the study period, indicating the absence of significant chemical degradation or oxidative changes. These findings confirm that the formulation maintained its chemical integrity over time.

Microbiological Evaluation

Table-3: Microbiological testing for Bioenzyme vegetable wash

S.No	Test	Method	0 Day	1st Month	2nd Month	3rd Month	4th-6th Month
1	Aerobic Plate Count	ISO 4833	<10	<10	<10	<10	<10
2	Yeast and Mold	ISO 21527	<10	<10	<10	<10	<10
3	Coliforms	ISO 4831	Absent	Absent	Absent	Absent	Absent
4	<i>E. coli</i>	ISO 16649	Absent	Absent	Absent	Absent	Absent
5	<i>Salmonella</i>	ISO 6579	Absent	Absent	Absent	Absent	Absent
6	<i>Shigella</i>	ISO 21567	Absent	Absent	Absent	Absent	Absent
7	<i>Staphylococcus aureus</i>	ISO 6888	Absent	Absent	Absent	Absent	Absent

Microbiological Evaluation – Description and Significance: The bioenzyme vegetable wash formulation was subjected to comprehensive microbiological quality assessment to evaluate its safety and suitability for application on food materials. The analysis included determination of total aerobic plate count, yeast and mold count, and detection of specific pathogenic microorganisms, namely coliforms, *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, and *Staphylococcus aureus*, following standard ISO methods.

The total aerobic plate count was found to be less than 10 CFU/mL throughout the study period, indicating a very low microbial load and good overall microbiological quality of the formulation. Similarly, yeast and mold counts were maintained below detectable limits (<10 CFU/mL), confirming the absence of fungal contamination and indicating stability of the formulation against spoilage organisms.

Coliforms, which serve as indicators of fecal contamination and general hygiene, were found to be absent in all samples, demonstrating that the formulation was prepared and maintained under hygienic conditions. *Escherichia coli*, a specific indicator of fecal contamination and potential enteric infection, was also absent, confirming the safety of the formulation for use on edible materials.

Pathogenic bacteria such as *Salmonella spp.* and *Shigella spp.*, which are associated with gastrointestinal infections and foodborne diseases, were not detected in any of the samples, indicating that the formulation does not pose a risk of transmitting enteric pathogens. Additionally, *Staphylococcus aureus*, a common cause of food poisoning due to toxin production, was absent, further confirming the microbiological safety of the product.

The microbiological profile remained consistent throughout the study period, even under accelerated storage conditions (45°C and 75% relative humidity), indicating that the formulation possesses inherent antimicrobial properties and remains stable over time. These findings demonstrate that the bioenzyme vegetable wash is microbiologically safe, free from pathogenic contamination, and suitable for use in reducing potential microbial risks associated with food handling and consumption.

Table 4: Dissipation percentage –Chlorpyrifos (field sample before and after wash with bio-enzyme vegetable wash)

Days	Field samples-x dose	Field samples 2x dose	After wash X dose	After wash 2X dose	Field samples-x dose	Field samples-2x dose
0 Day	1.191	1.850	0.14	0.243	88.24517	86.86
1 day	0.852	1.189	0.095	0.122	88.84977	89.74
3 Day	0.587	0.915	0.057	0.094	90.28961	89.73
5 day	0.345	0.623	0.025	0.061	92.75362	90.21
7 Day	0.236	0.384	0.017	0.025	92.79661	93.49
10day	0.172	0.196	0.001	0.012	99.4186	93.88
12 ay	0.075	0.096	0	0.001	100	98.96
15 ay	0.013	0.011		0		100

Table-5: Dissipation percentage –Chlorpyrifos (market sample before and after wash with bioenzyme vegetable wash)

Days	market samples x dose	market samples 2x dose	After wash X dose	After wash 2Xdose	Dissipation %- x dose	Dissipation %- 2x dose
0 Day	2.794	3.124	0.415	0.512	85.14674	83.61076
1 day	1.418	2.709	0.189	0.442	86.67137	83.68402
3 Day	1.248	1.735	0.165	0.269	86.77885	84.49568
5 day	0.843	1.309	0.108	0.198	87.18861	84.87395
7 Day	0.524	0.938	0.065	0.134	87.59542	85.71429
10 day	0.201	0.345	0.012	0.048	94.02985	86.08696
12 Day	0.138	0.154	0.001	0.021	99.27536	92.07792
15 Day	0.099	0.065	0	0.0018	85.14674	98.46154

The efficacy of vegetable washing in reducing chlorpyrifos residues in brinjal was evaluated by measuring the concentration before and after washing them. The study was done to evaluate the effectiveness of removing Chlorpyrifos pesticide residue for 2 different concentrations such as single concentration and double concentration. Single concentration of pesticide residue of the field sample of brinjal was evaluated for different days varies from 0-15days. The single concentration of chlorpyrifos treated brinjal on first day was 1.191 and for double concentration it was 1.850 were reduced to 0.14 and 0.243 respectively. Likewise, all the market samples of brinjal were analyzed. For the sample dipped or the collected ones from market it was 2.794 and 3.124 and it was observed after wash were 0.415 and 0.512. The results after washing with the bio-enzyme vegetable wash demonstrated over 85% effectiveness in removing Chlorpyrifos from brinjal in field sample as well as dipped or market samples. This 85% reduction in Chlorpyrifos concentration highlights the importance of washing with bio-enzyme vegetable wash rather than using chemical containing vegetable washes.

DISCUSSION

Chlorpyrifos, a widely applied organophosphate insecticide, remains a major concern in food safety due to its persistence on agricultural produce and its well-documented toxicological profile. Dietary exposure through contaminated vegetables such as brinjal has been associated with adverse health outcomes, including neurotoxicity, endocrine disruption, and potential carcinogenicity (18). The persistence of chlorpyrifos residues is largely attributed to its lipophilic nature, which promotes strong adherence to the waxy cuticular layer of vegetables, thereby limiting removal through conventional washing methods.

In this context, bioenzyme-based vegetable washes represent a promising alternative to conventional chemical decontamination strategies. Unlike chlorine- or peroxide-based washes, which may generate harmful by-products such as chlorinated residues (20), bioenzyme formulations are derived from natural sources and are generally regarded as safe, leaving minimal or no toxic residues (19). Additionally, their biodegradable nature reduces environmental burden, making them suitable for sustainable food processing applications (23).

The enhanced efficacy of bioenzyme washes in removing chlorpyrifos can be attributed to their multifaceted mechanism of action. These formulations typically contain hydrolytic enzymes and naturally occurring surfactants that facilitate both the degradation and desorption of pesticide residues (21). Enzymes such as phosphotriesterases and carboxylesterases, produced during microbial fermentation (particularly by yeast), are known to catalyze the hydrolysis of organophosphate compounds into less toxic metabolites (25). This enzymatic transformation is complemented by microbial activity, which further enhances the breakdown of pesticide molecules into more water-soluble forms, thereby improving their removal efficiency (26).

In addition to enzymatic activity, the phytochemical constituents of the herbal ingredients play a critical role in the overall efficacy of the formulation. *Albizia lebbek* (Shirisha) contains saponins that function as natural biosurfactants, increasing the solubility and mobilization of hydrophobic pesticide residues (27). *Azadirachta indica* (Neem) contributes bioactive compounds such as limonoids and terpenoids, which have been reported to support pesticide degradation while simultaneously exerting antimicrobial effects (28). Citrus extracts, rich in citric acid, lower the pH of the washing medium, thereby facilitating acid-mediated hydrolysis and weakening the interaction between pesticide molecules and vegetable surfaces (29). Furthermore, fermentation-derived organic acids, including acetic acid, enhance residue desorption and contribute to the overall cleaning efficiency (30, 31).

The inclusion of *Ocimum sanctum* (Tulsi) adds an additional functional dimension to the formulation. Its main phytoconstituents, such as eugenol and rosmarinic acid, possess strong antioxidant and antimicrobial

properties, which may aid in reducing oxidative stress and microbial contamination associated with pesticide-laden produce (31). Moreover, the presence of probiotic microorganisms in bioenzyme formulations may inhibit the growth of pathogenic bacteria and fungi, thereby improving the microbiological safety of treated vegetables (22).

An important practical advantage of bioenzyme-based washes is their ability to preserve the sensory and nutritional quality of vegetables. Unlike oxidative chemical washes, which may degrade vitamins and antioxidants, bioenzyme formulations maintain the natural color, texture, and flavor of produce (24). This aspect is particularly relevant for consumer acceptance and marketability.

Overall, the findings support the hypothesis that bioenzyme-based herbal washes provide a synergistic and sustainable approach for pesticide residue mitigation. The combined action of enzymatic degradation, acid hydrolysis, biosurfactant-mediated desorption, and antimicrobial protection offers a comprehensive strategy that addresses both chemical and biological contaminants. However, further studies are warranted to characterize the degradation by-products, assess their toxicological safety, and validate the effectiveness of such formulations across a broader range of pesticides and agricultural commodities.

CONCLUSION AND FUTURE PERSPECTIVES

The formulated bioenzyme-based vegetable wash, comprising *Albizia lebbek*, *Azadirachta indica*, *Citrus limon*, *Ocimum sanctum* (Tulsi), jaggery, yeast, and water, demonstrated significant efficacy in the removal of chlorpyrifos residues from brinjal. The observed effectiveness can be attributed to a combination of mechanisms, including enzymatic degradation, biosurfactant-mediated solubilization, organic acid-driven desorption, and antioxidant synergy.

In contrast to conventional chemical washing agents, the bioenzyme formulation offers a natural, non-toxic, and environmentally sustainable alternative for pesticide residue removal without compromising the nutritional and sensory quality of vegetables. Its biodegradable nature further minimizes ecological impact, supporting its suitability as a green intervention for food safety applications.

Future research should focus on optimizing the formulation through the incorporation of diverse microbial strains and plant-derived extracts to enhance degradation efficiency. Comparative evaluations with standard chemical washing agents are necessary to establish relative performance benchmarks. Additionally, detailed investigations into enzymatic degradation pathways and the identification of transformation products are essential to ensure comprehensive safety assessment.

Further studies are also warranted to evaluate the long-term safety of residual bioenzyme components, as well as the stability and shelf-life of the formulation under varying storage conditions. From a practical perspective, scalability and process standardization for industrial applications remain critical areas for development.

In terms of economic feasibility, bioenzyme-based washes represent a cost-effective alternative to synthetic chemical detoxifiers, as they utilize readily available, fermented plant-based ingredients to achieve efficient pesticide removal. Their low production cost, combined with eco-friendly characteristics, makes them suitable for both household and commercial use. Continued research on formulation stability, microbial safety, and large-scale production technologies will further enhance their commercial viability.

Overall, the adoption of bioenzyme-based vegetable washes has the potential to significantly reduce dietary pesticide exposure, thereby contributing to improved public health outcomes and promoting safer consumption practices.

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