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Adhesion, Microscopic, and Contact Test of Green Pesticides on Plants

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Abstract
The utilization of chemical pesticides will not be wholly absorbed by plants, resulting in the generation of residuals that are ultimately detrimental to the environment and public health. This research project was the impetus for the development of environmentally friendly pesticides. Its objective was to ascertain the adhesion of green pesticide formulations, their direct contact use, and the impact on plant microscopicity. This type of research is applied to the reformulation of green pesticides, with a particular focus on their adhesion and direct contact exposure to pests. This study employed a non-experimental design to elucidate the phenomenon of microscopic conditions in plants that had been sprayed with and without the use of green pesticides. The plant species utilized in this investigation were tomatoes, chilies, kale, and celery. The results of the green pesticide formulation, as determined by the stickiness of the pesticide in the good category, are reviewed based on the absence of pesticide droplets and the presence of a gloss on the morphology of the plant. Microscopic examinations revealed no discernible differences in the tomato, chili, and kale plants. However, the celery plants exhibited notable alterations, including the presence of chloroplast gaps and discoloration in specific regions, which were attributed to environmental factors (temperature) and the use of Span Tween. The tests demonstrated that the pesticide formulation is effective in repelling and killing the target pests, namely Plutella xylostella and Bemisia tabaci. It is imperative to develop formulations that will repel Aphis fabae and prevent damage to the leaf morphology of the plant.
Keywords: *green pesticide, food contamination, safety food*

Abstrak
Penggunaan pestisida kimia tidak sepenuhnya diserap oleh tanaman, sehingga akan ada residu yang terbuang dan berdampak pada lingkungan dan kesehatan masyarakat. Penelitian ini menginisiasi pembuatan pestisida hijau dengan tujuan untuk menentukan daya lekat formulasi pestisida hijau, penggunaan kontak langsung, dan kondisi mikroskopis tanaman. Jenis penelitian yang diterapkan adalah reformulasi pestisida hijau yang diuji untuk daya lekat dan paparan kontak terhadap hama. Desain investigasi non-eksperimental digunakan dalam penelitian ini untuk menjelaskan fenomena kondisi mikroskopis tanaman yang disemprot dan tidak menggunakan pestisida hijau. Tanaman yang digunakan sebagai sampel dalam penelitian ini adalah tomat, cabai, kangkung, dan seledri. Hasil formulasi pestisida hijau yang diperoleh berdasarkan daya lekat pestisida dalam kategori baik ditinjau dari tidak adanya tetesan pestisida dan terdapat kilap pada morfologi tanaman. Tes mikroskopis menemukan bahwa tidak ada perbedaan mikroskopis pada tanaman tomat, cabai, dan kangkung, namun pada seledri ditemukan celah kloroplas dan warna kuning pada bagian tertentu akibat faktor lingkungan (suhu) dan penggunaan span tween. Uji pada hama menunjukkan kemampuan yang baik untuk mengusir dan membunuh Plutella xylostella dan Bemisia tabaci. Diperlukan pengembangan formulasi untuk mengusir Aphis fabae agar tidak merusak morfologi daun tanaman.
Kata Kunci: *keamanan pangan, kontaminasi pangan, pestisida hijau*

1. Introduction

Pesticides, in general, play an important role in the sustainability and resilience of agriculture [1] [2] [3]. Their ability to control pests can contribute to increased crop productivity. This usefulness led to a rise in pesticide use during World War II (1939-1945). Moreover, from the 1940s onwards, the increased use of synthetic plant protection chemicals enabled further increases in food production [4]. Correspondingly, worldwide pesticide production increased by about 11% per year, from 0.2 million tons in the 1950s to more than 5 million tons in 2000 [5]. Three billion kilograms of pesticides are used worldwide annually

[6], while only 1% of the total pesticides are effectively used to control insect pests on target crops [4]. The group of substances called pesticides includes insecticides, fungicides, herbicides, rodenticides, molluscicides, and nematicides [4].

Pesticide terminology can be classified based on chemical class, functional group, mode of action, and toxicity [7]. Based on the toxicity of the substance, fungicides are used to kill fungi, insecticides are used to kill insects, and herbicides are used to kill weeds [8] [9]. Based on the chemical group, pesticides can be divided into organic and inorganic materials. Inorganic pesticides include copper sulfate, iron sulfate, copper, lime, and sulfur. Organic pesticide ingredients are more complex [10]. Organic pesticides can be classified according to their chemical structure, such as chlorohydrocarbon insecticides, organophosphorus insecticides, carbamate insecticides, synthetic pyrethroid insecticides, metabolite and hormone analog herbicides, synthetic urea herbicides, triazine herbicides, benzimidazole nematicides, metaldehyde molluscicides, metal phosphide rodenticides, and vitamin D-based rodenticides [11]. The use of chemical pesticides will not be fully absorbed by plants, so there will be residues that are wasted and affect the environment and public health [12].

The application or disposal of pesticides on target crops can potentially cause pesticides to enter the environment. Once in the environment, pesticides can undergo processes such as displacement and degradation [13] [14] [15]. Degradation of pesticides in the environment results in the formation of new chemicals [16]. Pesticides can move from the initial application site to other environmental media or non-target plants through adsorption, leaching, volatilization, spray drift, and runoff [17]. Different chemicals exhibit varied ecological behavior. Organochlorine compounds such as DDT, despite their low acute toxicity, tend to accumulate in tissues and cause long-term damage, leading to a ban on their use in most countries. However, their residues remain in the environment for long periods. Organophosphate pesticides, on the other hand, have low persistence but pose significant acute toxicity to mammals [18] [10].

The public and policymakers are very concerned about toxic pesticides in food due to their negative impacts on health and the environment. Food contamination results from pesticide spraying on non-target crops as well as pesticide behavior in the environment, such as volatilization from treated areas to air, soil, and non-target crops, and pesticide residues moving from soil and water to crops, vegetables, and fruits [19]. This environmental behavior of pesticides and their residues leads to food contamination and crop damage. In some areas, pesticide residues in crops and vegetables have exceeded the WHO maximum standards for food contamination [20] [21]. Research by Wanwimolruk et al. (2016) on pesticide contamination of fruits and vegetables and its health implications in Ghana showed that almost all fruits and vegetables studied contained pesticide residues above the maximum residue limit (MRL). Lozowicka et al. (2015) assessed pesticide residue levels in vegetables in the Almaty region of Kazakhstan and reported that more than half of the samples (59%) contained 29 pesticides, of which 10 were not registered in Kazakhstan, with levels between 0.01 and 0.88 mg kg-1, and 28% exceeded the maximum residue limit (MRL) [20]. Wanwimolruk et al. (2016) showed that detected pesticides exceeded MRLs at a rate of 48% (local markets) and 35% (supermarkets) for Chinese kale and 71% (local markets) and 55% (supermarkets) for pak choi [22].

Reducing pesticide use has become an urgent issue, driven by shared concerns across countries and reflected in evolving public policies that support food security and environmental sustainability. This research initiated the manufacture of green or environmentally friendly pesticides with the development of formulations made by Arora et al. in 2020 in India [23]. The content of the green pesticide to be made uses 13 ingredients that are adapted to local wisdom as well as the reformulation results of the research. Seven of them come from bio-botanicals, three animal products, one mineral salt, and two chemical products. It is hoped that with this reformulation, the green pesticide produced can work according to its function and become more effective in protecting plants from pests or plant-damaging microorganisms. The seven bio-botanical ingredients have efficacious substances including flavonoids, saponins, tannins, and curcumin which are owned by neem leaves, guava leaves, and white temu. These ingredients can have effects as insecticides, natural larvicides, antibacterials, antimicrobials, antifungals, and as nutrients for plants [24] [25] [26]. There is the efficacious substance allicin that comes from garlic. This allicin functions as a defense system against pests. Coconut oil is also used so that green pesticides stick to the plants, preventing pests from damaging the plants [27] [28]. This study aims to determine the adhesion of green pesticide formulations, direct contact use, and plant microscopicity.

2. Material and Methods

Type and design of research

This type of research is applied with green pesticide reformulation tested for adhesion and contact exposure to pests. The nature of investigation (non-experimental) design was used in this study to explain the phenomenon of microscopic conditions of plants sprayed and not using green pesticides. The plants used as samples in this study were tomatoes, chilies, kale, and celery. The data generated in this study are qualitative in the form of the results of the adhesive ability of green pesticides as seen from the sparkle indicator on plant morphology, pest mortality (*Plutella xylostella*, *Bemisia tabaci*, *Whiteflies*, and *Aphis fabae*), which are sprayed directly by green pesticides, and morphological conditions viewed from a microscope.

Tools and materials for making green pesticides

Table 1. Tool and materials Required in Making Green Pesticides

Number	Tools	Total
1.	Scale	1
2.	Water sprayer	4
3.	Shovel	4
4.	Blender	1
5.	Beaker glass 1 L	4
6.	1 L Measuring cup	2
7.	100 mL Measuring cup	2
8.	50 mL Measuring cup	1
9.	75 mL Evaporating dish	1
10.	125 mL Evaporating dish	4
11.	300 mL Evaporating dish	3
12.	Large stirring rod	1
13.	Plastic wrap	1
14.	Buckets of 5 L	2
15.	Glass funnel	2
16.	Measuring tape	2
17.	Gauze cloth	2
18.	Filter paper	2
19.	Wipe	4

Table 2. Ingredients Required in Making Green Pesticides

Number	Materials	Total
1.	Moringa leaves	100 grams
2.	Neem leaves	100 grams
3.	Guava leaves	100 grams
4.	*Curcuma zedoaria* (white turmeric)	50 grams
5.	Potassium aluminum sulphate dodecahydrate (alum)	25 grams
6.	Onion	50 grams
7.	Garlic	50 grams
8.	EM4	100 mL
9.	Coconut oil	50 mL
10.	Span 20	5 grams
11.	Tween 20	5 grams
12.	Water	3 L

Steps for making green pesticides

Prepare tools and weigh all ingredients

↓

Puree neem leaves, guava leaves, ginger, onions, garlic and cow dung using a blender with enough water.

↓

The pulverized material was mixed with alum and span 20 in a 1 L beaker glass, stirred well using a spoon/stirring rod

↓

Put coconut oil in another 1 L beaker glass, add tween 20

↓

The mixture was filtered using gauze and filter paper and the filtrate was taken

↓

The mixture was filtered using gauze and filter paper and the filtrate was taken

↓

The mixture was filtered using gauze and filter paper and the filtrate was taken

Fig. 1: Green Pesticide Manufacturing Procedure

3. Results and Discussion

Adhesion test results

The results of the observation of pesticide adhesion were tested on tomato, chili, water spinach, and celery plants. The test needs to be done on the ability of pesticides to stick to plants. The formulation used as adhesive material is Span Tween and coconut oil. The complete results are presented in **Table 1** below.

Table 3. Adhesion Test Results of Green Pesticides on Chili, Kale, Celery, and Tomato Plants

Before spraying	After spraying
Tomato	
 (a)	 (b)

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Chili

(a)

(b)

Yale

(a)

(b)

Celery

(a)

(b)

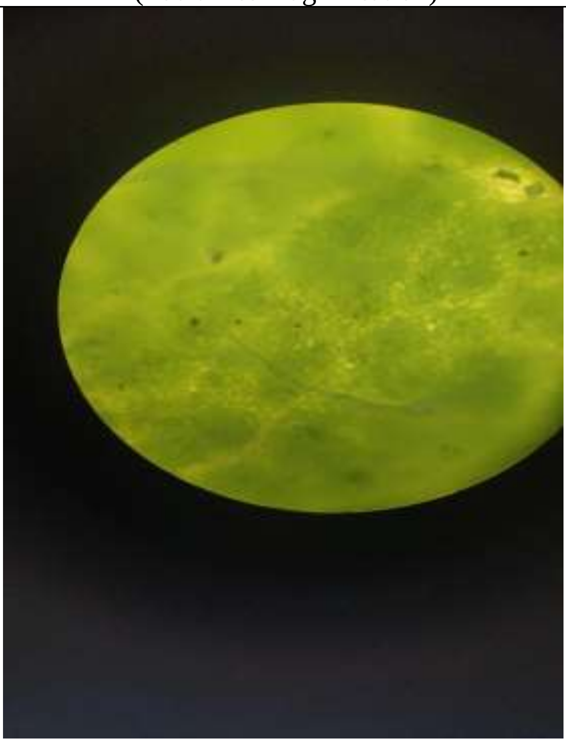
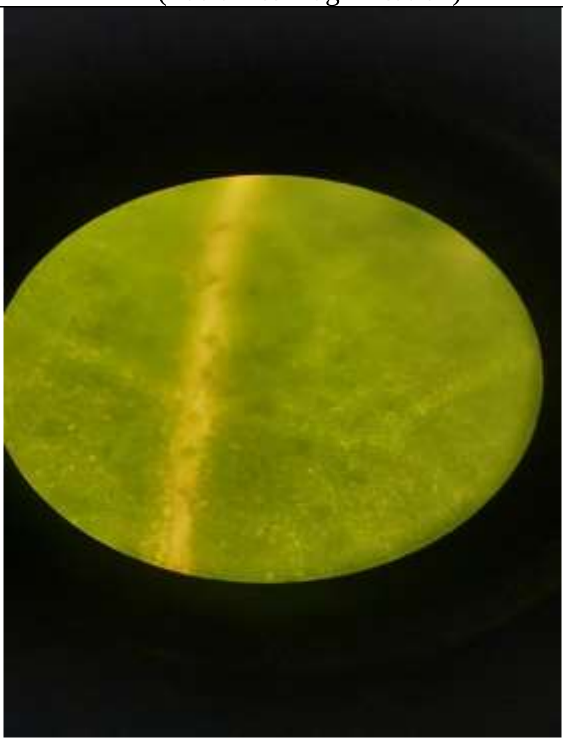
Figure tomato (b), chili (b), celery (b), and yale (b) leaf surface condition, the wet leaf indicates that the green pesticide adheres well to the leaf surface. There is no indication that the pesticide flows or disappears from the leaf. The spray appears evenly distributed over the entire surface of the marked leaves, indicating that the pesticide has good adhesion and can spread effectively. Leaf surfaces that still appear wet indicate that the pesticide may have components that prolong moisture or improve adhesion. The celery plant (b) demonstrates foliar discoloration after the application of green pesticides. Celery exhibits heightened sensitivity to phytotoxicity induced by specific chemicals. The active constituents of pesticides, as well as their combinations with surfactants and oils, can elicit phytotoxic responses, particularly in celery. Additionally, celery displays increased vulnerability to damage resulting from extreme variations in temperature or humidity. The application of pesticides under suboptimal conditions can exacerbate the risk of phytotoxicity. Moreover, the application of pesticides in direct solar exposure can elevate the potential for chemical burns on the foliage of celery.

The employment of a combination of Span 20, Tween 20, and coconut oil in green pesticide formulations confers superior adhesion, attributable to the physicochemical properties of these constituents [29] [30]. Span 20 (sorbitan monolaurate) and Tween 20 (polysorbate 20) are surfactants frequently utilized as emulsifying agents. These surfactants facilitate the homogenization of inherently immiscible substances, such as oil and water [31]. The synergistic interaction between Span 20 and Tween 20 results in the formation of a stable emulsion. Span 20 exhibits lipophilic characteristics, favoring dissolution in oil, whereas Tween 20 demonstrates hydrophilic properties, favoring dissolution in water [32]. This dual affinity is instrumental in preserving emulsion stability, thereby enhancing the persistence of the pesticide on foliar surfaces. These surfactants effectively reduce surface tension, promoting more uniform distribution and improved adhesion of the pesticide solution on leaf surfaces [33] [34]. Furthermore, they enhance the penetration of active pesticide ingredients into the leaf tissue.

Microscopic observation of plant morphology

Plants were sprayed using green pesticides and then plant morphology was tested to determine differences in the morphology of sprayed and unsprayed plants. Magnification was done with 160 times microscope magnification. **Table 4** below presents the results of the observation.

Table 4. Microscopic Test Results of Green Pesticides on Chili, Kale, Celery, and Tomato Plants

Surface of Tomato Leaf	
Before spraying (160 times magnification)	After spraying (160 times magnification)
 (a)	 (b)

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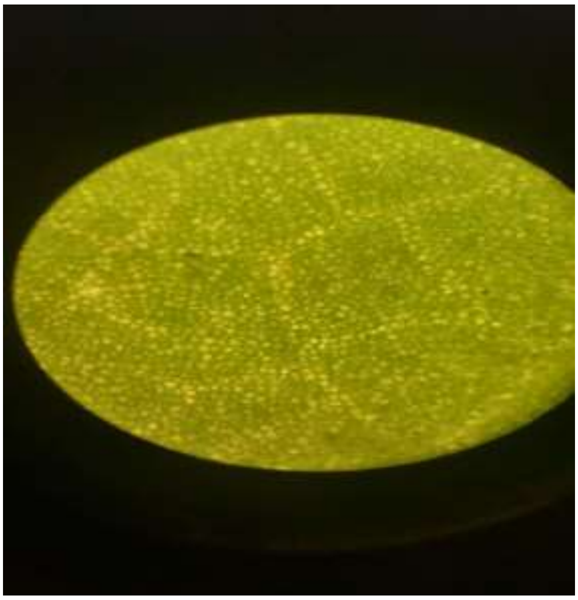
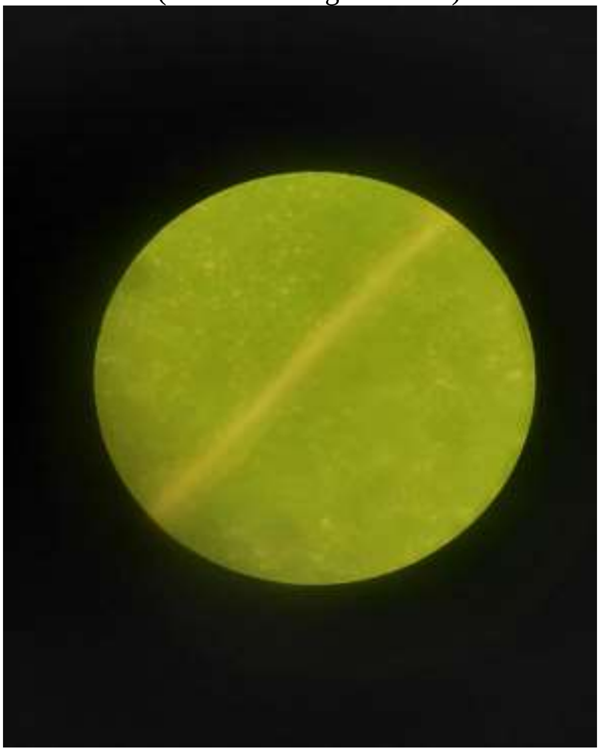
Description of no difference

Surface of Chili Leaf

Before spraying (160 times magnification)	After spraying (160 times magnification)
	
(a)	(b)

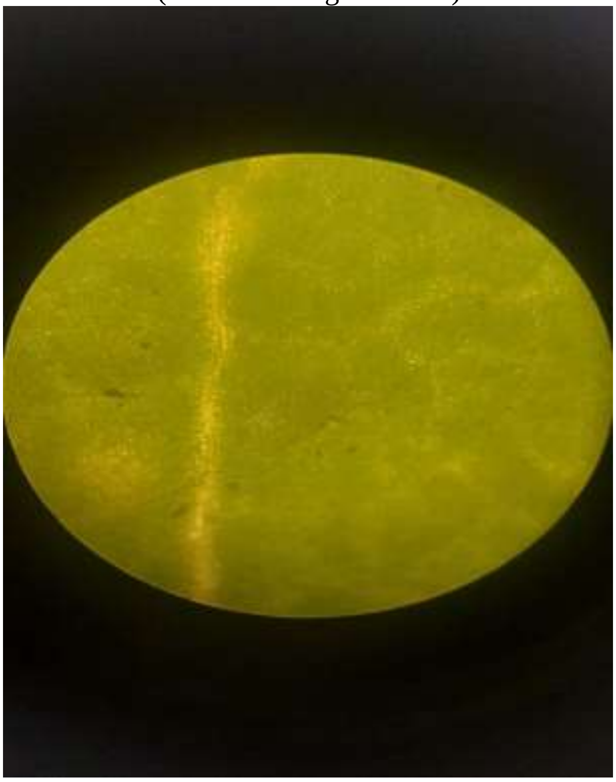
Description of no difference

Celery Leaf Surface

Before spraying (160 times magnification)	After spraying (160 times magnification)
	
(a)	(b)

Description of no difference

Surface of Kale Leaf

Before spraying (160 times magnification)	After spraying (160 times magnification)
	
(a)	(b)

The microscopic images of tomato (b), kale (b), and chili (b) leaves reveal no significant differences between those treated with green pesticides and those that were not. However, the image of celery (b) displays small dots on the leaves, identified as chloroplasts containing chlorophyll. The distribution of chloroplasts appears somewhat uneven, indicating areas of lower chloroplast concentration. Damage or alterations in chloroplast distribution may suggest a detrimental impact of the pesticide on photosynthesis. Darker or opaque regions could signify cellular damage or reduced chlorophyll content. A clear main vein structure, indicative of a vascular network, is observable. These veins appear brighter, possibly due to light reflection or distinct cellular structures. Some regions appear darker or opaque, potentially indicating cellular damage or decreased transparency due to the effects of green pesticides. The parenchymal structures may exhibit signs of stress or damage, and if parenchyma cells are compromised, it could lead to reduced photosynthetic efficiency and impaired nutrient transport within the leaf [35].

The observed conditions in celery may result from the application of green pesticides combined with Span 20, Tween 20, and coconut oil, which can induce phytotoxicity, leading to cellular damage and uneven chloroplast distribution. Surfactants such as Span 20 and Tween 20 reduce surface tension, enhancing pesticide adhesion and penetration into the leaves [36] [37]. While this facilitates more efficient pesticide application, excessive penetration can cause cellular damage. The deeper penetration of pesticides enabled by surfactants may adversely affect parenchyma cells and chloroplasts [38]. Furthermore, the uneven distribution of pesticides can result in certain areas of the leaf receiving higher concentrations, leading to localized damage.

Green pesticide of direct contact use

Green pesticide formulations were tested on several groups of plant pests. The mechanism of action was obtained to determine the effectiveness of the direct contact mode of action of green pesticides. Spraying applications were carried out on each of the three days and sprayed once. The observation results are presented in **Table 5** below.

Table 5. Observation Results of Pests on Plants after Spraying Green Pesticides

Before spraying	After spraying
	
(a)	(b)

Figure (a) initial condition of *Plutella xylostella* pests before spraying green pesticides. Spraying was done once in the morning, the caterpillars were weakened, and in the afternoon the new caterpillars died Figure (b).

Before spraying	Before spraying
	
(a)	(b)

Figure (a) is the condition of the plant that was not sprayed with green pesticide and there is *Bemisia tabaci*, while figure (b) after spraying green pesticide and the pest disappeared. Spraying was done once at 09.00 AM and at 13.00 PM *Bemisia tabaci* disappeared. The results of observations of Bemisia tabaci pests are presented below.

Before spraying	Before spraying
	

Figure (a) is a plant that was not sprayed and contained *Aphis fabae* pests. Figure (b) is the condition of the plant after spraying green pesticides. Spraying was done once at 09.00 AM and at 13.00 PM The picture shows that there are black spots even though the *Aphis fabae* pest has disappeared.

The results of observation and testing of green pesticides on plant pests are quite effective. The existence of *Moringa oleifera* as one of the green pesticide ingredients has an important role. The active ingredients found in these plants are bioactive compounds such as 13 flavonoids, 5 phenolic acids, 6 amino acids, 3 phenylpropanoids, 2 phytosterols, and 2 terpenoids (including 2 triterpenoid saponins), all of which possess antimicrobial and insecticidal properties [39]. The second ingredient formulation is *Azadirachta indica*, containing azadirachtin, one of the most effective natural insecticides known [40] [41]. Additionally, *Psidium guajava*, another component, contains flavonoids and tannins, both of which have significant antimicrobial and insecticidal properties [42] [43]. Furthermore, *Allium cepa* contributes sulfur compounds that enhance the antimicrobial efficacy of the pesticide mixture [44] [45]. Finally, *Allium sativum* contains allicin, a potent compound recognized for its strong antimicrobial and insecticidal effects [46] [47]. The synergistic action of these bioactive compounds from various plant sources makes the green pesticide formulation highly effective in managing and controlling plant pests, providing a sustainable and eco-friendly alternative to conventional chemical pesticides.

The black patches left by *Aphis fabae* are a mechanical effect that visibly marks the damage these pests cause to plants. *Aphis fabae* uses its piercing and sucking mouthparts to burrow into the plant's phloem [48]. By inserting their stylets into the plant tissue, they extract sap that is rich in sugars and other nutrients. This feeding process deprives the plant of essential nutrients, leading to reduced vigor and stunted growth. During feeding, aphids may inject saliva containing enzymes and other compounds that can be toxic to the plant. This can disrupt normal plant physiological processes and cause localized tissue damage [49]. Additionally, the saliva can interfere with the plant's ability to transport nutrients and water, exacerbating the overall decline in plant health [50]. The black patches are often accompanied by the presence of honeydew, a sticky substance excreted by the aphids, which can lead to the growth of sooty mold, further hindering photosynthesis and reducing the plant's ability to thrive [51]. Existing pesticide formulations can repel the pest but have not been able to restore leaves damaged by *Aphis fabae*. This indicates a need for integrated pest management strategies that not only target the aphids but also support plant recovery and resilience. Biological control agents, cultural practices, and resistant plant varieties are essential components of a comprehensive approach to managing *Aphis fabae* infestations and mitigating their detrimental effects on crops.

4. Conclusion

The results of the green pesticide formulation obtained based on the stickiness of the pesticide in the good category are reviewed by the absence of pesticide droplets and there is a gloss on the morphology of the plant. Microscopic tests found that there were no microscopic differences in tomato, chili, and kale plants, but in celery, there were chloroplast gaps and yellow in certain parts due to environmental factors (temperature) and the use of span tween. Tests on pests have a good ability to repel and kill Plutella xylostella and Bemisia tabaci. There is a need to develop formulations to repel Aphis fabae so that it will not damage the leaf morphology of the plant.

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