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Particle Film Coverage Alters the Volatile Chemical Emission of Plants

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ABSTRACT

Pesticide application plays a critical role in ensuring agricultural productivity by minimizing crop losses due to infestations. However, there are long-term negative effects associated with this practice, prompting the need to develop greener and more sustainable alternatives. A promising alternative is particle film technology using kaolin clay. A suspension of kaolin clay in water is applied through a spray coating technique in which it forms a thin film on the surface of the substrate that serves as a protective barrier for plants and fruit crops. This protective barrier is known to deter pests through feeding and visual obstruction. In this study, we present preliminary evidence to suggest that particle film coverage not only acts as a physical barrier but also suppresses or masks volatile emissions from plants. Our study analyzed the effects of particle film coverage on the volatile chemical profile of a model plant, *Garcinia mangostana*. It was found that particle film coverage suppresses volatile chemical emissions as analyzed through solid-phase microextraction coupled with gas chromatography–mass spectrometry. The observed volatile suppression can be attributed to the fine and extensive coverage of the particle film on the plant surface.

Keywords: particle film, kaolin clay, volatile chemical emissions, solid phase microextraction

INTRODUCTION

Pesticide application is an effective strategy to increase agricultural productivity by limiting the damage caused by pests to crops (Wise & Whalon, 2009). However, pesticide use poses negative consequences to non-target species, especially to humans. Farmers are one of those who are directly affected by pesticide use. Since they prepare and apply these pesticides on their farms, their prolonged exposure to these on a pesticide application day can cause some immediate effects such as headaches, eye irritation, muscle weakness, and breathing difficulty (Athukorala et al., 2023). In the long term, those who are indirectly exposed to these pesticides such as children, pregnant women, and nearby communities can also acquire these serious effects on their health such as cancer, asthma, arthritis, Parkinson's disease, birth defects, and neurodevelopmental abnormalities, among others (Chittrakul et al., 2022; He et al., 2022; Sarwar, 2015; Tudi et al., 2022). These could have been avoided through proper training on best practices when using pesticides, but some users resort to excessive use just to catch up on the production (Lu, 2022; Sarkar et al., 2021). These consequences led to the development of alternative and greener pest management controls. A promising pest control measure that is effective and environmentally friendly is the particle film technology (Stanley, 1998). Particle film technology is a novel coating application wherein the particle film is created by making a water-based slurry with a chemically inert mineral with a particle size of <2 μm. This is then sprayed directly into the plant or the fruit crop. Once dried, a thin white powdery film layer that serves as a protective barrier on the surface will be formed. Currently, particle film technology uses kaolin clay as its main material (Unruh et al., 2000). Kaolin clay is a chemically inert aluminosilicate mineral and is considered an indirect food additive that is nontoxic and approved by the FDA (U.S. Food and Drug Administration, n.d.). Thus, using kaolin clay provides a green and safer alternative to pesticides and increases the yield and quality of crops. Kaolin has many uses in different consumer products such as a pigment and rheology modifier in paints and coatings, for oil absorption, and as a binder in cosmetic products (Bhavsar & Sardesai, 2022; Buyondo et al., 2022; de Carvalho-Guimarães et al., 2022; Iriany et al., 2020). As a particle film material, it has desirable characteristics such as cost-effectiveness, being readily available, and good wettability, and it exhibits non-Newtonian behaviors such as pseudoplasticity and thixotropy (Barbatoa et al., 2008). Ever since its introduction, kaolin clay particle film (KCPF) has been proven effective in the suppression of different pests such as codling moth (Lepidoptera: Tortricidae) in apples and pears (Unruh et al., 2000), fruit flies in blueberries (Diptera: Tephritidae; Liburd et al., 2003), Japanese beetle in woody and herbaceous ornamental plants (Coleoptera: Scarabaeidae; Baumler & Potter, 2007), and flower thrips in blueberries (Thysanoptera: Thripidae; Spiers et al., 2004).

The effectiveness of particle film technology as a pest management strategy emanates from the different forms of protection the particle film coverage confers to the plant. Aside from not interfering with any biological function of the crops, the particle film reduces the heat stress of plants leading to healthier leaves (Glenn & Puterka, 2010). The particle film coating also camouflages the leaves from the pests. Moreover, upon contact of the pest with the coated plant, kaolin particles would adhere to their bodies resulting in deterrence, reduced feeding, and even mortality due to its small particle size (Chiu, 1939; Glenn & Puterka, 2010). In addition, pest inhalation of the particles can result in suffocation (Briscoe, 1943), ingestion can affect their digestive system (Boyce, 1932), and physical contact may result in reactions to the body wall of the pest (Shafer & Lansing, 1913). Considering that plants release volatile chemicals that pests are attracted to (Agelopoulos et al., 1999), a particle film coating on the plant may possibly suppress, alter, or mask these emissions, thereby repelling or evading pests. However, this aspect of particle film coverage for pest control remains largely unexplored. Thus, this study aims to analyze the effects of particle film coverage on the volatile chemical profile of plants. The findings presented are expected to contribute to a deeper understanding of the effects of particle film coating on plants within the context of chemical communication.

MATERIALS AND METHODS

Collection of Samples

Fresh mature mangosteen leaves were collected in the plant nursery of the De La Salle University Laguna Campus at Biñan, Laguna, Philippines, in May 2018. The leaf samples were deposited at the De La Salle University Herbarium with voucher specimen number DLSUH 6213. The deposited samples were authenticated and identified to be *Garcinia mangostana* L. (Family Clusiaceae). The average length and width of the leaves obtained are about 25.91 cm and 3.64 cm, respectively. The collected leaves weighed about 1.50 g to 4.50 g.

Particle Film Preparation

The kaolin clay (NovaSource Surround® WP) used is a white powder with a mean particle size of <2 μm (Glenn, 2009). Two concentrations of kaolin clay slurry at 2.50% (Amalin et al., 2015) and 5.00% were made by mixing 25 g of kaolin clay in 1000 mL of tap water and 50 g of kaolin clay in 1000 mL, respectively. Coconut oil-based soap was dissolved in water and mixed with each slurry to act as a sticker spreader for the kaolin to stick onto the surfaces being sprayed. These mixtures were placed inside spray bottles and then shaken before use since kaolin tends to settle. Mangosteen leaves were sprayed once with kaolin clay slurries for both concentrations. The coated leaves were clipped and hung upside down on a clothesline to dry. Once dry, the leaves were placed in a resealable container prior to analysis.

Volatile Profile Analysis

Headspace analysis of the leaves were performed using Supelco® 100 μm Solid Phase Microextraction Fiber coated with polydimethylsiloxane (PDMS). The SPME fiber was conditioned according to the manufacturer's instructions prior to the analysis. Gas chromatography–mass spectrometry (GCMS) analysis was performed using Agilent Technologies 7890A GC System and Agilent Technologies 5977A MSD with HP-5 MS Ultra Capillary column (30 m × 250 μm × 0.25 μm). Volatile analysis of each sample was performed by placing a leaf sample inside a 500-mL Erlenmeyer flask covered with aluminum foil and parafilm that served as a headspace chamber. The chamber was heated at 30℃ to 40℃ for 25 minutes while the SPME fiber was exposed to collect the volatiles. This was done in 7 replicates—3 trials for the 2.5% treatment, 3 trials for the 5.0% treatment, and 1 trial for the untreated mangosteen leaf. Blank trials of the headspace chamber, the SPME, and the GCMS were also performed. After collection of the volatiles, the SPME fiber was directly injected in the GCMS for analysis. The temperature program used for the analysis is as follows: the injection temperature was set to 250℃ and operated in splitless mode. The oven was held at 50℃ for 5 minutes then programmed at 10℃/10 minutes until the final temperature of 200℃. Helium was used as the carrier gas with constant flow of 1 mL/min. Detection was performed in Electron Impact (EI) mode. Spectra acquisition was performed in scanning mode (mass range m/z 50–550). Chromatograms and spectra were recorded by means of the GC/MSD Chemstation Software and MassHunter Workstation with MSD Chemstation DA Software (Agilent Technologies). The identity of the compound was based on the match factor of the detected compound against the National Institute of Standards and Technology (NIST) Mass Spectral Library 2.0. Compound identification was limited to compounds that yielded a match factor of 80. Library match factors that are 80 and above are considered to be good matches (Stein, 1999, 2012).

Scanning Electron Microscope Analysis

The same procedure was followed in the preparation of the samples for scanning electron microscopic (SEM) analysis. The leaves coated with 2.5% concentration were examined using JEOL5300 from JEOL USA Scanning Electron Microscopes. Gold was used to sputter coat the samples.

RESULTS AND DISCUSSION

The use of particle film coating as an alternative to pesticide application directly contributes to the attainment of the Sustainable Development Goals. Particle film coating reduces the adverse environmental and health impacts of pesticide applications while increasing crop productivity, thus boosting food security. Thus, developing a deeper understanding of how particle film coats can deter pests is of paramount importance since this can pave the way for the formulation of more effective pest control strategies. This study sought to understand the effects, if any, of particle film coating on the volatile emission of plants since plants and insects use volatile organic compounds for communication.

Mangosteen leaves were used as the model plant for the study since the seedlings are easy to maintain within a controlled and confined environment. Figure 1 shows the actual dry mangosteen leaf treated with a 5.00% concentration of KCPF. Visually, it can be observed that the particle film did not significantly alter the color of the leaf, although the film introduced a small amount of opaqueness to it making the green color of the leaf lighter than it is supposed to be.

Figure 1. Mangosteen leaves with kaolin clay particle film at 5.00% concentration.

A volatile profile of the healthy mangosteen leaves was first analyzed that served as a control for the succeeding trials. Figure 2a shows the chromatogram of the healthy mangosteen leaves that are untreated, and Table 1 shows the corresponding identities of each peak, their retention times, and their percent relative abundance.

As observed in Figure 2a, the peaks are well-defined and with proper baseline throughout the analysis. The obtained profile for the untreated leaves is consistent with previous reports on the volatile chemical profile of *Garcinia mangostana* leaves (Tavera et al., 2018) wherein caryophyllene is the most abundant at 52.04%, followed by copaene at 13.03% and ß-germacrene at 10.05%. Compared with the chromatogram of the treated leaves, fewer chemicals were released and identified. Regardless of the concentration, KCPF was able to mask the release of volatiles in the leaf (Figs. 2b and c). From the chemicals released by the healthy untreated leaf, only the compound caryophyllene remained detectable in the treated leaves.

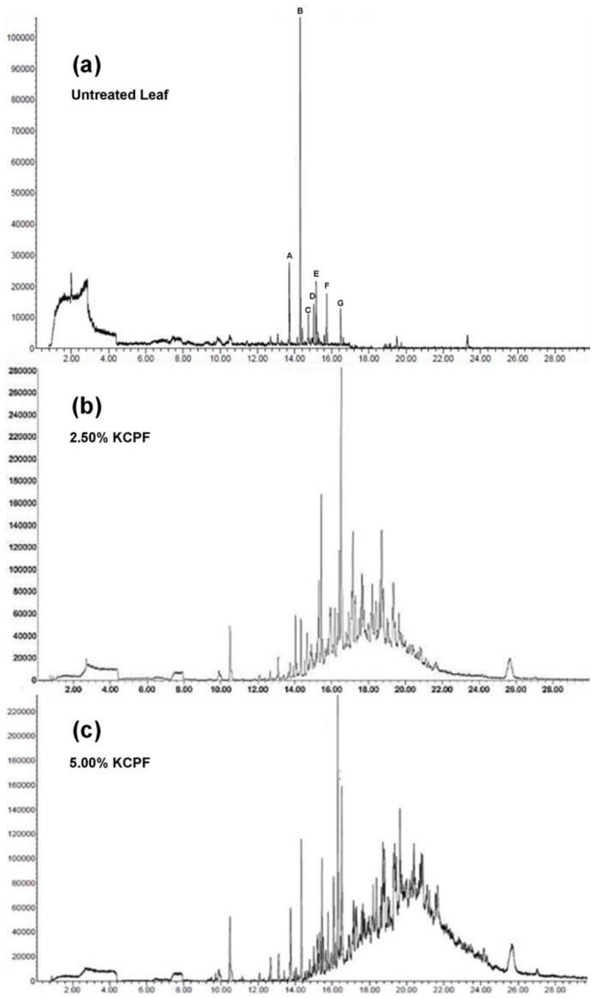


Figure 2. (a) Healthy untreated mangosteen chromatogram, (b) 2.50% KCPF treated mangosteen, and (c) 5.00% KCPF treated mangosteen.

In general, the application of the particle film has suppressed the overall chemical profile of *G. mangostana*. However, the amount of caryophyllene emitted by the leaf is much greater than the other volatiles that it could not be simply masked by the particle film. Meanwhile, it is worth noting that two new compounds were detected in Figures 2b and c: furan and sulfurous acid at retention times 16.275 min and 16.489 min, respectively. These two compounds possibly came from the kaolin clay slurry that dissolved when heated during the collection of volatiles in the headspace chamber (Song et al., 2012). Due to the application of the KCPF, there is an observable difference in the release of volatile chemicals and the alteration of the volatile chemical profile based on the comparison of the chromatograms.

The percent relative abundance of the treated leaves in Figures 2b and c could not be measured because the chromatogram does not have a proper baseline. Aside from comparing the volatile compounds, it is also worth noting that a baseline drift can be seen clearly and that the drift is not present in the chromatogram of the untreated healthy mangosteen leaf (Fig. 2a).

Table 1. Volatile Profile of Healthy Mangosteen (Fig. 2a)

Retention Time (min)	**Compound**	**% Relative Abundance**
13.718	Copaene[A]	13.03%
14.307	Caryophyllene[B]	52.04%
14.731	Humulene[C]	4.71%
15.023	Napthalene[D]	6.43%
15.171	ß-Germacrene[E]	10.05%
15.726	E-11(12-Cyclopropyl)dodecen-1-ol[F]	7.96%
16.482	2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester[G]	5.79%

The higher percentage of kaolin clay concentration has a bigger baseline drift compared to the lower concentration. These baseline drifts are a result of column bleeding that is caused by samples that have a low volatility (Paramasigamani & Aue, 1979). This is true as observed because the resulting chromatogram obtained when a blank sample was run also shows a baseline drift (Fig. 3). In this case, since a higher concentration of kaolin is more effective in masking the volatiles of the mangosteen, it is expected that the chromatogram of the 5.00% concentration would have a higher baseline drift.

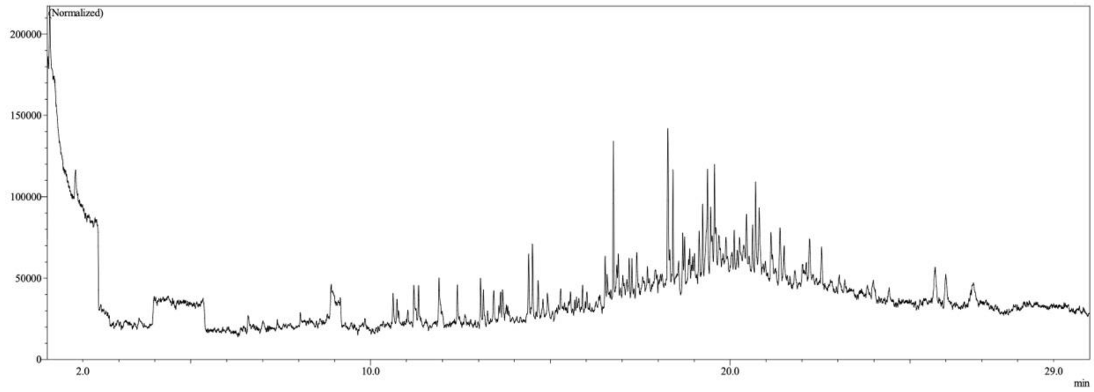
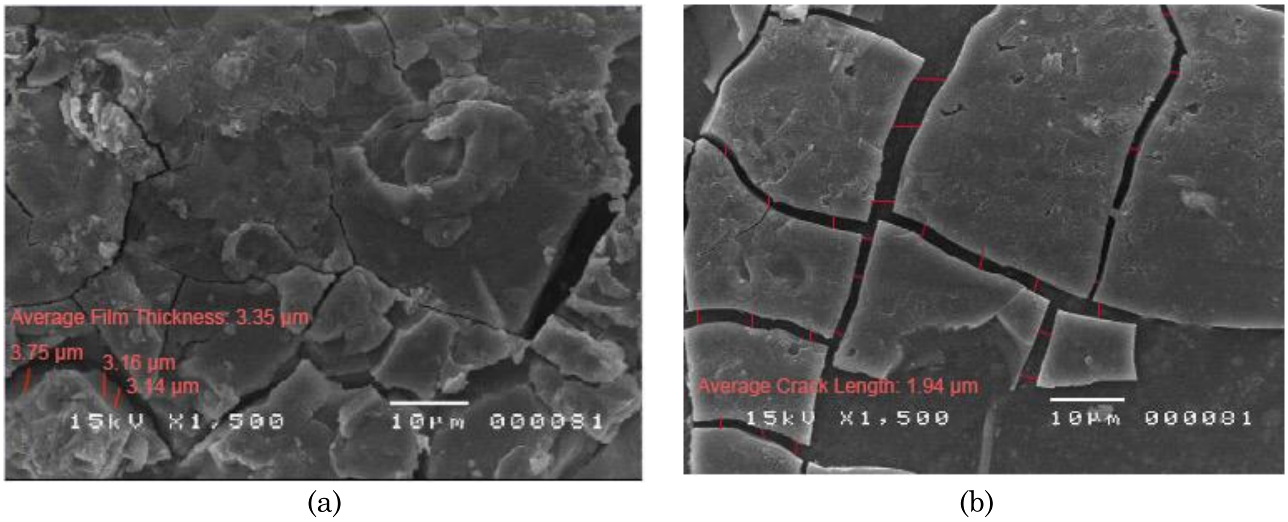


Figure 3. A blank chromatogram showing a baseline drift.

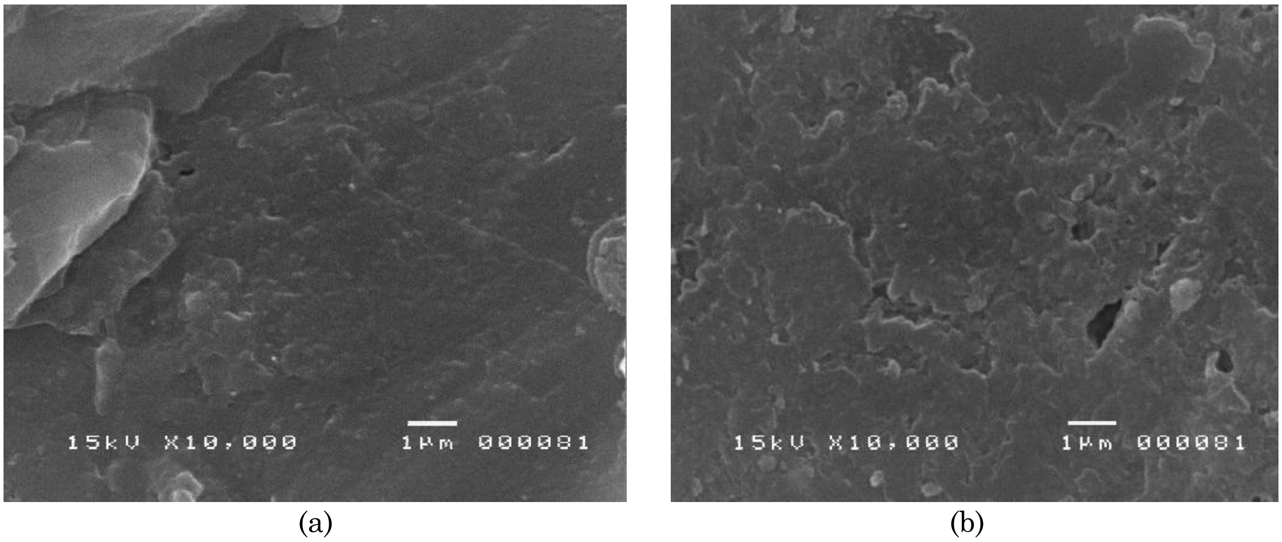
Investigation of the formation of the particle film through SEM at an accelerating voltage of 15 kV and at magnifications of both 1,500× (Figs. 4 a and b) and 10,000× (Figs. 5 a and b) showed a smooth, clear, and uniform formation of the particle film on the leaf. Although in Figure 4a there were occurrences of pores and clumping, these were very minimal overall. Using the same figure, the approximate average thickness of the film from three random areas inside a crack with a visible depth was measured using ImageJ (in Figure 4a). The average thickness of the film was determined to be 3.47 µm. In Figure 4b, a part of the applied area shows plenty of visible cracks. Cracks like these allowed the plant to release the volatiles that were detected in the GCMS. The average length of the cracks was obtained from 25 measurements using ImageJ and determined to be 1.94 µm. Meanwhile, Figures 5a and b present a higher magnification of the film surface in which it can be seen that the KCPF presented a relatively smooth surface in these areas. The shape and smaller size of the kaolin material could contribute to a more opaque and smooth formation of the film thus producing an effective coating (Jepson et al., 1997). The strong altering effect of the KCPF treatment on the volatile chemical profile of the mangosteen leaf and the reduction of identified chemicals may be attributed to the ideal particle film formed by the kaolin.

The results indicate that indeed particle film coverage alters the volatile chemical emission of plants. Considering that volatile chemical emission plays a large role in plant–insect communication, the effect of particle film coverage may go beyond physical and visual obstruction for pests. The presented results suggest that the suppression of the emission of the volatile chemical by the plant may alter the host-seeking behavior of the pest.



(a) (b)

Figure 4. SEM micrograph of US kaolin with 1,500× magnification.



(a) (b)

Figure 5. SEM micrograph of US kaolin coated on leaves at 10,000× magnification at different areas (a) and (b).

CONCLUSION

The pest control effectiveness of particle film coverage is known to work by physically coating the leaves and fruits of plants, thus obstructing pests to recognize and infest the covered plant. The particle film not only acts as a barrier to protect the plants but also suppresses the chemical emission profile of the applied substrate that pests are attracted to. This study provides promising preliminary data that demonstrated that particle film coverage significantly altered the volatile chemical profile of the plant, thereby suggesting a new perspective regarding the mechanism of action of particle film technology.

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