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Evaluation of Philippine-sourced clay particles as coating agents of cacao pods and carrier of entomopathogen against cacao pest, *Helopeltis bakeri* Poppius

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Evaluation of the efficacy of clay particles as a coating agent of cacao pods and carrier of entomopathogen, *Metarhizium anisopliae* Sorokin, was conducted for the control of cacao mirid bug (CMB), *Helopeltis bakeri* Poppius. Choice and no-choice tests were performed to evaluate Philippine-sourced clay particles as a coating agent of cacao pods to deter CMB feeding, in comparison with the commercially available particle film (US kaolin Surround®). To determine the most efficient local clay particles in protecting the pods from CMB feeding, six (6) treatments were evaluated namely, Philippine-sourced kaolin (PH kaolin), zeolite (PH zeolite), bentonite (PH bentonite), US kaolin, water (negative control), and a commercial synthetic insecticide thiamethoxam (Actara®) (positive control). All treatments were subjected to choice and no-choice tests. Among the Philippine clay particles tested in both tests, PH zeolite showed significant coating and deterred CMB from feeding. Since the US Kaolin and zeolite showed significant feeding deterrent effects on CMB, these treatments were tested as carriers of entomopathogenic fungi, *M. anisopliae*, including water (negative control) and thiamethoxam. Results showed that zeolite is a good carrier of the spores of *M. anisopliae* as its effects to deter CMB feeding started 24 hours after exposure. This was confirmed by positive *M. anisopliae* extraction from dead CMB through potato dextrose agar (PDA) plating.

KEYWORDS

cacao, kaolin, zeolite, bentonite, entomopathogen

1 Introduction

Theobroma cacao L., commonly known as cacao, is a shade-loving tree that is known to be the source of chocolate products. It is mainly grown for its seeds known as cocoa beans, which are used to make cocoa mass, cocoa powder, and chocolates. It has no product substitute, making it a good investment, especially for small-holder farmers. The primary producers of cacao in the world are the Ivory Coast and Ghana, and other equatorial countries in Southeast Asian like Indonesia, Malaysia, and the Philippines (Stecker, 2011). There is a growing demand for cocoa in the form of hard pressed beans for confection products (Morgan, 2014). According to the World Cocoa Foundation (Frimpong, 2013) disease and insect pest occurrences are important contributors to the decrease in the supply of cacao worldwide, where it is estimated that 30 to 40% of the loss in cacao bean production may be due to pest damage.

With the desire of the Philippines to increase local production to meet the growing market demands, it must face the threat of insect pests, specifically the cacao mirid bug (CMB) (*Helopeltis bakeri* Poppius) (Dormon et al., 2004). This is one of the four *Helopeltis* species endemic to the Philippines (Serrana et al., 2022). It feeds and oviposits on the cacao pods causing brown and black lesions, which serve as an avenue for microbial infection (Amalin et al., 2015). CMB-infested pods produce deformed and smaller beans as compared to healthy pods. Heavy infestation of CMB results in pod malformation and premature drop. In the province of Sorsogon alone, the number of damaged pods due to CMB feeding increased from 70% to 87% in just a period of three months (Tormes and Mostoles, 2021).

Management of cacao pests is usually through the application of chemical pesticides (Mboussi et al., 2018). However, that approach only offers short-term control and poses risks of harm to people and the environment. An alternative to chemical pesticides is the use of biological control agents, which use other living organisms to control the spread of insect pests and diseases. A potential biological control against CMB is the utilization of the microscopic entomopathogenic fungal species, *Metarhizium anisopliae* (Metschnikoff) Sorokin. It produces acidic substances that readily penetrates the proteinaceous cuticular barrier of insects (St Leger et al., 1999). Once inside, the fungi will reproduce and upon successful invasion of the host's tissue, will emerge from the dead host.

Another alternative to chemical pesticides is the utilization of particle clays as coating agents, also known as particle film technology, to deter insect feeding. This technology combines mineral technology, insect behavior, and light physics in controlling pests (Glenn and Puterka, 2010). When sprayed into the plants, the particle film forms a barrier that protects them from insect and microbial pests. Broadly, particle film is known to work as a repellent, irritant, and as an obstacle to feeding and oviposition. It also prevents fungal spores from propagating onto the plant. It was also noted that particle film blocks the spiracles of the insect, reduces mating success, impedes insect pest movement, alters the taste and smell of the host (Kljajić et al., 2011; Glenn and Puterka, 2010).

Particle film technology was originally based on kaolin clay, a secondary mineral that came from the weathering of primary minerals such as quartz and feldspar (Glenn and Puterka, 2010). It was patented under the commercial name Surround WP[®]. The United States Food and Drug Administration (FDA) granted kaolin the GRAS (Generally Recognized as Safe) status for use on fruits crops, such as apples, pears and citrus (Mazor and Erez, 2004; Daniel et al., 2005; Ramirez-Godoy et al., 2018). In the same manner, the US Environmental Protection Agency (EPA) found that kaolin has no adverse effects on non-target organisms such as spiders and beneficial insects. And since kaolin does not dissolve in water, it does not have adverse effects on aquatic organisms. Aside from the use of kaolin, other naturally occurring particle clays such as zeolite and bentonite can also be utilized in controlling pest attacks on plants. The insecticidal effect of zeolite was tested by De Smedt et al. (2016) against *Tuta absoluta* Meyrick, a major insect pest attacking tomatoes. They found out that zeolite affected the egg development process by weakening the first instar larvae and increasing mortality. Bentonite can also be used as a crop protectant though the study conducted by Abd El-Aziz (2013) concluded that kaolin is more effective against onion thrips as compared to bentonite.

Previous studies have reported the synergistic effects of particle film with entomopathogenic fungi. Storm et al. (2016) investigated the improvement of the efficacy of *Beauveria bassiana*, an entomopathogenic fungal species, against stored grain beetles (*Oryzaephilus surinamensis*) with synergistic co-formulant kaolin. In the same manner, Sabbour et al. (2012) tested the synergistic effects of *B. bassiana* and *M. anisopliae* with diatomaceous earth. In both studies, it was found that the efficacy of the biopesticide improved as compared to the control (water). To date, no studies have yet investigated the synergistic effect of kaolin or zeolite with *M. anisopliae*.

With this, the objective of the study is to evaluate the efficiency of Philippine-sourced particle clays (zeolite, bentonite, and kaolin) as a coating agent to deter CMB feeding on cacao pods. By determining which particle clay is most efficient, the study also assessed its synergistic effect with the spores of *M. anisopliae*.

2 Materials and methods

2.1 Coating agents, entomopathogenic fungi, and its preparation

One of the coating agents evaluated was the commercially available calcined kaolin particle film, Surround WP[®] (US Kaolin) (Engelhard Corporation, Iselin, New Jersey, USA). The study followed the standard formulation of 6 grams/100mL distilled water. Philippine-sourced kaolin (PH kaolin), zeolite (PH zeolite), and bentonite (PH bentonite) provided by the Department of Ceramic Engineering, Mariano Marcos State University (Ilocos Norte, Philippines) were also evaluated. The study followed the

standard formulation of Surround WP® (US Kaolin) by adding 6 g of the Philippine-sourced particle clay with 100ml distilled water with the addition of 0.6 g of Perla® soap as a sticker and spreader. The insecticide Actara® (Thiamethoxam) which served as the positive control was purchased from Syngenta® Philippines.

The entomopathogenic fungi, *M. anisopliae* was provided by the Crop Pest Management Division of the Bureau of Plant Industry, Manila, Philippines. In preparing the particle film with entomopathogen, 29 g of spore-coated corn kernels were added to the Philippine-sourced clay particle mixture agitated to dissolve all materials in a sprayer.

2.2 Helopeltis bakeri Poppius

In the study, third instars of (CMB) were used. This was acquired from the rearing facility of the Biological Control Research Unit (BCRU) at the De La Salle University – Laguna Campus in Biñan, Laguna, Philippines where they were fed with sweet potato shoots. The rearing facility maintained a temperature of 25 - 27 degrees Celsius and a level of humidity of 70 - 80%. Therefore, all conducted experiments in the study followed and maintained the same temperature and level of humidity.

2.3 Cacao pods

One-month-old cacao pods (approximately 3.5 to 5 cm in size) were used in this study. The pods were collected from the Quezon Agricultural Experiment Station (QAES), a pesticide-free plantation, in Brgy. Lagalag, Tiaong, Quezon Province, Philippines. Immediately after plucking the pods from the tree, wet cotton was placed on the stalk open tissue to prevent microbial infection and drying up. In the laboratory, the pods were cleaned thoroughly with running water, and the cotton was replaced on the open tissue of the stalk.

2.4 Experimental procedure

2.4.1 Biocoating experiments against CMB

Six prepared treatments were evaluated namely; (a) Surround WP® (US Kaolin), (b) Philippine-sourced Kaolin, (c) Zeolite, (d) Bentonite, (e) water as the negative control, and (f) insecticide thiamethoxam (Actara®) as the positive control. A hand spray containing the treatment was used to coat the cacao pods used in the study. Two kinds of experiments were conducted; a no-choice test and a choice test.

In the no-choice test, only the treated cacao pod was contained in the test chamber for each treatment. Consequently, in the choice test, two cacao pods were placed in a test chamber wherein one was treated while the other is untreated. During the experimentation, one third-instar cacao mirid bug was placed in each test chamber. Dead CMB were replaced with a new third instar CMB daily. The feeding punctures were counted daily over a 96-hour period, for the choice and the no-choice tests.

2.4.2 Particle film as a carrier of entomopathogen and extraction of *M. anisopliae* on CMB

After testing the coating efficiency of the clay particles as a deterrent for CMB feeding, the efficacy of the selected best coating agent/biopesticide was assessed as carriers of the spores of *M. anisopliae* (MA). The same procedure was done in the biocoating experiment with the incorporation of the *M. anisopliae* spores for each treatment; (a) US kaolin + MA, (b) PH zeolite + MA, (c) water + MA, (d) water and (e) thiamethoxam. To determine if the particle film had been effective in carrying the spores of the entomopathogen, each dead cacao mirid bug from the different treatments was collected to extract *M. anisopliae* using a potato dextrose agar (PDA) plate.

2.5 Statistical analyses

All data were subjected to one-factor repeated measures ANOVA and Mann Whitney U test using the IBM SPSS Statistics version 20.

3 Results

3.1 No-choice test

Statistical analysis demonstrated significant differences between the treatments 24 hours up to the 120 hours after infestation ($F = 13.55$, $df = 5$, $p < 0.000$) (Figure 1). *Post hoc* analysis using Tukey Honestly Significant Difference (HSD) showed that the effects of US kaolin was comparable to the effects of the insecticide thiamethoxam to deter CMB feeding starting from 24 hours to 120 hours. On the other hand, the effects of the Philippine-sourced Kaolin were statistically similar to that of the water, suggesting the inefficient coating of these particles. The coating efficiency of bentonite and zeolite are nearly comparable to US kaolin and insecticide thiamethoxam suggesting the potential as another coating agent to deter CMB feeding.

It can be noted that the number of feeding punctures increases over time across all treatments. However, the mean increase of the insecticide thiamethoxam-treated, US Kaolin, and zeolite-treated pods, was very minimal as compared to the other treatments. The mean number of feeding punctures of water-treated pods increased by almost a hundred daily which means that without any treatment present, the pods are very much susceptible to CMB feeding. Visual observations showed that water-treated pods were frequently visited by the mirid bug.

3.2 Choice test

The results demonstrated that the treated pods have lesser feeding punctures than non-treated pods up to a 96-hour observation period except for pods treated with water (Figure 2). Among all Philippine-sourced clay particles, zeolite was

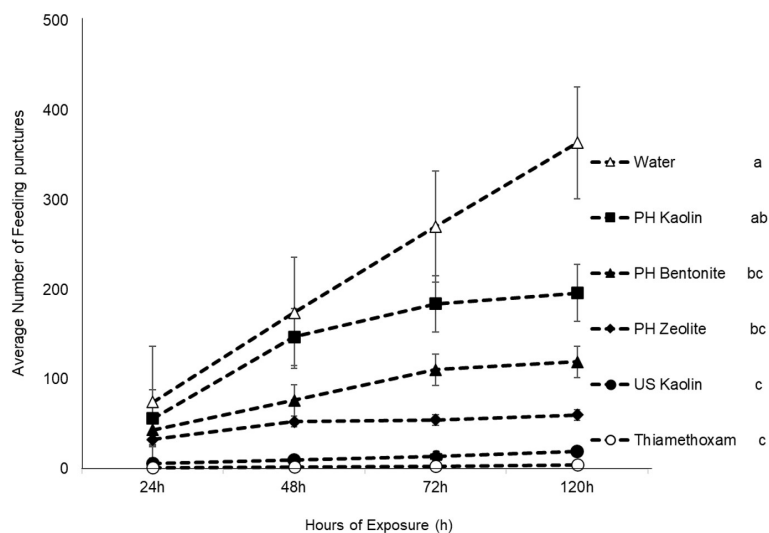


FIGURE 1
The average number of feeding punctures by cacao mirid bug in the no-choice test, treated with Surround WP® (US Kaolin), Philippine-sourced kaolin (PH-Kaolin), zeolite (PH-Zeolite), bentonite (PH-Bentonite), water, and Actara® (insecticide thiamethoxam) across 4 observation periods in hours (24h, 48h, 72h, and 120h) followed by different letters (a,b,c) using a *post-hoc* Tukey's test.

demonstrated as the best coating agent to deter the feeding of CMB with an average of 2.8 to 8.8 feeding punctures while the non-treated cacao pods had an average of 30.2 – 97.4 feeding punctures from a 24-hour to 96-hour observation time, respectively. Further analysis showed significant difference in the average number of feeding punctures between Philippine-sourced zeolite-treated and non-treated pods during the 48-hour ($U = 1, p < 0.05$), 72-hour ($U = 1, p < 0.05$), and 96-hour ($U = 2, p < 0.05$) observation periods. On the other hand, cacao pods treated with Philippine-sourced

kaolin also demonstrated to be a good coating agent in deterring CMB feeding. It was observed that there is an average of 22.2 to 57 feeding punctures in kaolin-coated cacao pods while an average of 45.6 – 188.6 feeding punctures were observed in non-treated pods from a 24-hour to 96-hour observation time, respectively. Further analysis showed significant differences between the number of feeding punctures treated with Philippine-sourced kaolin compared to the non-treated cacao pods in 24-hour ($U = 4, p < 0.05$), 48-hour ($U = 3.5, p < 0.05$), 72-hour ($U = 3, p < 0.05$) and 96-

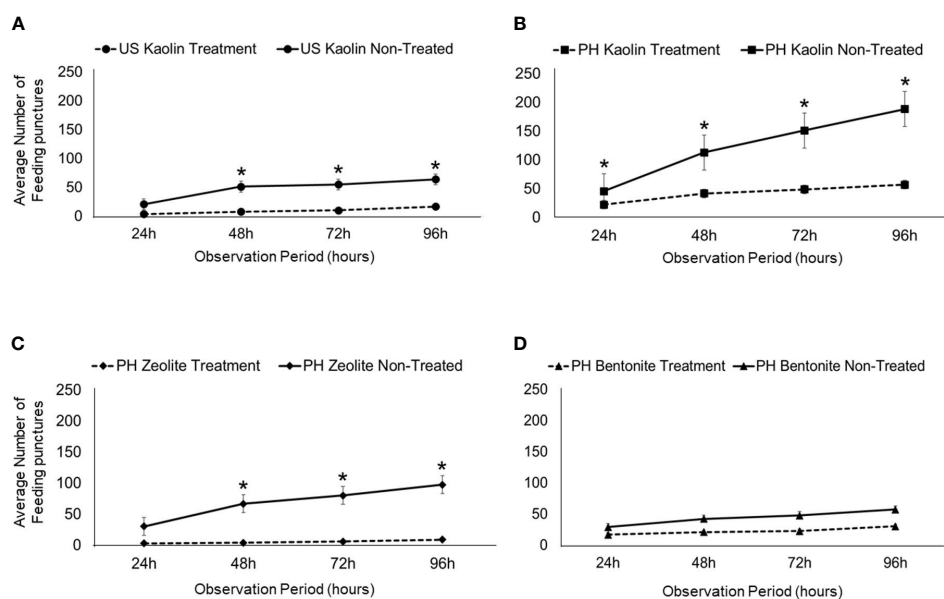


FIGURE 2
The average number of feeding punctures by cacao mirid bug using the choice test. Cacao pods were either non-treated or treated with (A) Surround WP® (US Kaolin), Philippine-sourced (B) kaolin, (C) zeolite, and (D) bentonite, with an asterisk (*) indicates a significant difference ($p < 0.05$) between treated and non-treated cacao pods.

hour ($U = 3$, $p < 0.05$) observation periods. The result emphasizes the potential early (24-hour) protective role as a coating agent against CMB feeding. These results were consistent with the results of the no-choice test.

3.3 Particle film as a Carrier of Entomopathogen, *Metarhizium anisopliae*

Based on the results of the choice and no-choice test, Philippine-sourced zeolite was deemed to be the best among all other Philippine-based clay particles. With this, the synergistic effect of this clay particle with *M. anisopliae* spores was assessed along with other treatments (Figure 3). Statistical analysis demonstrated there are significant differences between the treatments 24 hours up to 96 hours after infestation ($F = 8.15$, $df = 4$, $p < 0.000$). It is noteworthy that the treatment group PH zeolite + MA with an average number of feeding punctures of 19.8 – 39.8 is comparable with the US Kaolin + MA which had an average number of feeding punctures of 17 – 31.6. *Post-hoc* analysis indicated that the two treatments have the same effect against CMB feeding. This result clearly shows that the PH zeolite is as an effective carrier for *M. anisopliae* spores like US Kaolin.

To determine how long the pathogenicity of the *M. anisopliae* coated with PH zeolite on the cacao pods was, the study extended its observation up to seven days. Visual observations indicated a green sandy substance or green muscardine fungi found in the cacao pods which is the primary characteristic of *M. anisopliae*. Moreover, dead CMB collected from each treatment resulted in positive extraction of *M. anisopliae* spores using a potato dextrose agar (PDA) plate. Both results suggest that *M. anisopliae* developed on cacao pods, which in turn, also infected CMB as well. To further confirm the viability of *M. anisopliae* on the treated pods, the growth of *M.*

anisopliae was also observed on the PDA plates (Figure 4). This further strengthens that the presence of *M. anisopliae* even 7 days after the treatment, which infected and caused the death of the CMB in the test chambers.

4 Discussion

This study provides new knowledge as to the potential for particle films and entomopathogens in controlling CNB in cacao Integrated Pest Management. Results showed that both the Surround WP® and Philippine-sourced zeolite have similar levels of performance as the insecticide thiamethoxam (Actara®) in deterring CMB feeding. One of the most important modes of action of particle film is through masking the appearance of the target hosts. The coated pods were likely unrecognizable from the perspective of the insect pest, resulting in the deterrence of feeding, reduced oviposition, and reduced pest population in the plant (Sharma et al., 2015). The work of Glenn et al. (1999) showed that insect behavior was altered when exposed to plants coated with kaolin as compared to its behavior on natural plant surfaces. The physical barrier made by the particle film made it harder for the pest to locate its host.

There have been reports on the insecticidal properties of kaolin and zeolites. The use of kaolin as a biopesticide was documented by Daniel et al. (2005). A small field trial was conducted to determine the efficacy of kaolin as an insecticide against *Cacopsylla pyri* L. which attacks pear. Kaolin was introduced during the first flying period of *C. pyri* and its application reduced the egg laying and number of nymphs as compared to the untreated control. Kaolin-protected trees were as good as the standard insecticide, rotenone. Zeolite, on the other hand, was applied to wheat under laboratory conditions against *Sitophilus oryzae* and *Tribolium castaneum*

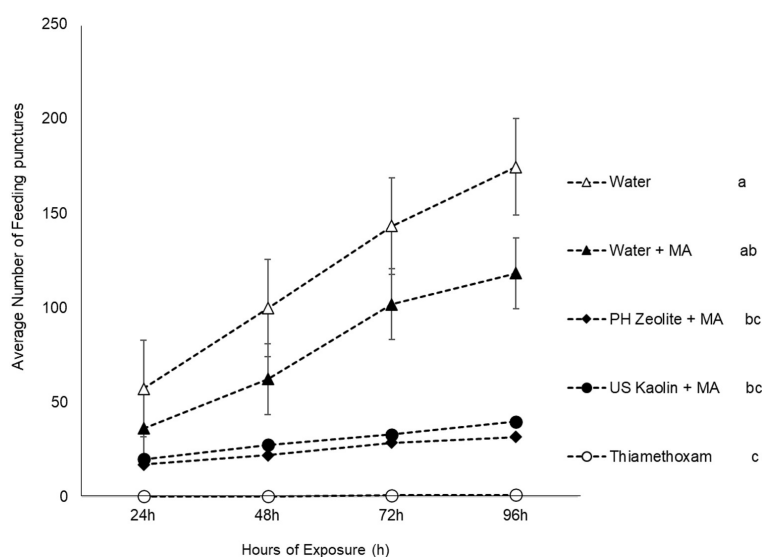


FIGURE 3

The average number of feeding punctures by cacao mirid bug treated with Surround WP® with *M. anisopliae* spores (US Kaolin + MA), Philippine-sourced zeolite with *M. anisopliae* spores (PH zeolite + MA), water with *M. anisopliae* spores (water + MA), water only and Actara® (insecticide thiamethoxam) across 4 observation periods in hours (24h, 48h, 72h, and 96h) followed by different letters (a,b,c) using a *post-hoc* Tukey's test.

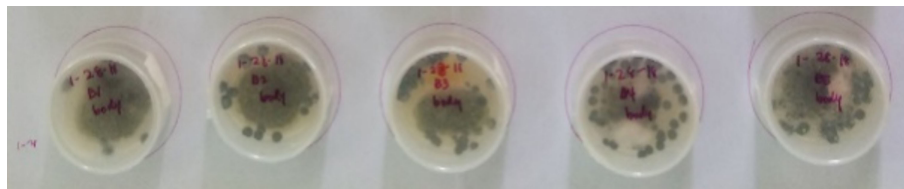


FIGURE 4

Selected Potato Dextrose Agar plates with the cultures of the green muscardine fungi from the insects which died on the 7th day of observation.

(Kljajić et al., 2011). Results showed that there was a 94–100% mortality on both insect pests for all zeolite dosages (0.25, 0.50, 0.75 g/kg). In addition, the progeny was reduced by more than 90% 21 days after exposure to zeolite. The non-choice study herein suggests that the Philippine-sourced kaolin does not have insecticidal properties, but based on the non-choice test causes the pods to be less desirable than those that are untreated.

Another result of this present study was that zeolite has a better synergistic effect with *M. anisopliae*. One reason might be the particle size. Surround WP[®] measures approximately 2 μm in size, whereas, zeolite is approximately 25 μm in size. Since the particle size of zeolite is bigger, spores have a bigger surface area for attachment, making it possible for better distribution on the treated pods. Malusá et al. (2012) noted that a good carrier of microbial inoculant may vary from 25 to 75 μm and be free of a lump. Furthermore, the same authors noted that a good carrier of beneficial bacteria and fungi must have a good moisture absorption capacity, good pH buffering capacity, and be porous. Coppin (2015) wrote that zeolite control moisture content, buffer pH of acid volcanic soils, and is porous.

There is no clear explanation yet as to the reason for the efficacy of the particle clays to carry the spores of entomopathogenic fungi. Samodra and Ibrahim (2006) observed that the cuticle of the insect was abraded due to the presence of kaolin. This gave fungal spores an avenue for easier penetration and greater conidial attachment. The increased water loss due to cuticle abrasion may result in favorable condition for spore germination and the increased stress of insects make them more susceptible to fungal infection.

Insecticides offer lethal-based control of harmful pests and diseases however, along with this comes the risks associated with these modes of action (Mahmood et al., 2016). Over time, many pest species have developed resistance to chemical pesticides which makes sustainable pest management more difficult. In this regard, biological pesticides or biological controls are becoming more attractive because they are relatively safe to humans, do not pose harm to the environment, and sometimes more economical, and pests are less likely to develop resistance and avoid pest resurgence.

5 Conclusion

This study has demonstrated that the effects of the commercially available kaolin (Surround WP[®]) and the Philippine-sourced zeolite are comparable with that of the insecticide thiamethoxam (Actara[®]) in deterring CMB feeding to cacao pods in both choice and no-choice

tests. Between these particle clays, zeolite is more efficient in carrying the spores of *M. anisopliae*, an entomopathogen known to infect different insect pest species because of its particle size. The potential of the combined formulation of zeolite and *M. anisopliae* for the control of CMB and other insect pests attacking the cacao pods should be further explored.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

SA, DA, and JW conceptualized the study. SA conducted all the experiments. SA and TC conducted the analysis under the supervision of JW. SA wrote and revised the manuscript under the supervision of DA, TC, and JW. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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