

RESEARCH PAPER

The best way to the trap: An ecological study of coffee berry borer (*Hypothenemus hampei*) preference to several volatile compounds

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ABSTRACT

This study explored the effectiveness of ethanol and methanol as attractants for controlling the coffee berry borer (CBB), *Hypothenemus hampei* Ferr 1867, a significant pest in global coffee plantations. The research aimed to assess the efficiency of these substances as attractant traps for CBB in Kaliselogiri, Banyuwangi, Indonesia. Using a single-factor randomized block design, the experiment involved nine treatments, each replicated three times, with modified PTE traps of 2 L volume set up at 1.5 m height. Traps were checked weekly to monitor CBB attack intensity before and after applying attractants. Statistical analyses including the Mann-Whitney Non-parametric test and Duncan's test were used to examine variations across blocks and weeks. Abundance boxplot graphs offered descriptive insights. Over six weeks, 14,044 CBB's were captured. The peak was in the third week, with a decline in the fourth, followed by a resurgence until the end of the study. The Mann-Whitney U test showed a significant p-value ($p = 0.000$; confidence level = 5%), confirming the effectiveness of the attractants on CBB. Of the treatments, K6 (Ethanol: Methanol = 2:1) was most effective. Ethanol and methanol proved to be efficient lures in CBB management traps, with ethanol particularly effective in larger compositions, suggesting its wider application. These results support the use of attractant-based strategies for CBB control and highlight the importance of ethanol and methanol. Further research is needed to optimize attractant compositions and assess long-term impacts on CBB populations and agroecosystems. The ecological consequences, including potential effects on non-target species and overall sustainability, warrant further investigation. In summary, this study advances CBB management through attractant-based traps, emphasizing the importance of ethanol.

Key words: CBB, coffee, ethanol, methanol, and traps

INTRODUCTION

The coffee berry borer (CBB), scientifically identified as *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae), poses a significant threat to coffee crops (Abewoy, 2022; Johnson et al., 2020). In severe infestations, CBB can affect 30% to 60% of a crop, drastically reducing both the quality and quantity of coffee beans. Despite extensive use of various contact pesticides, their effectiveness is limited due to CBB's tendency to hide inside coffee berries (Fotso et al., 2021). Studies in coffee-growing

regions like Hawaii and Vietnam have explored the integration of biocidal methods with Integrated Pest Management (IPM) approaches, showing positive results in managing CBB (Bui et al., 2022; Lee et al., 2023). The use of attractants has emerged as a key strategy in controlling the spread of CBB, with notable effectiveness demonstrated in several studies (Bui et al., 2022; Tobing et al., 2022).

Attractant traps are appealing due to their efficiency, user-friendliness, and effectiveness in collecting various specimens (Carvalho et al., 2023). These traps often involve a mixture of ethanol and methanol in a polyethylene terephthalate (PET) container (Carvalho et al., 2023; Ruiz-Diaz & Rodrigues, 2021). Recommended sampling procedures, when combined with attractant traps, facilitate quick, precise, and accurate sample collection, maintaining an acceptable margin of error of about 25% (Ruiz-Diaz & Rodrigues, 2021; Tobing et al., 2022).

The variety of synthetic attractants available for CBB includes primary substances like ethanol and methanol (Fernandes et al., 2015). However,

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determining their effective concentration is crucial and varies depending on environmental conditions and geographical locations. There is a gap in our understanding of a unified sampling strategy using attractant traps, with only preliminary studies conducted within specific contexts. Therefore, this research aims to propose an effective trap model based on baited attractant traps, enhancing our understanding of CBB population dynamics and preferences for specific compounds through a refined ecological lens.

MATERIALS AND METHODS

Research Site. The research was conducted in a ten-year-old Robusta (*Coffea canephora*) plantation in Kaliselogiri, Banyuwangi, Indonesia.

Trap Preparation. In the trap preparation process, 2-L PET plastic bottles were modified and suspended at a height of 1.5 meters above the ground. Inside each bottle, a small, perforated vial containing the treatment solution was centrally placed. A detergent solution was added to the bottom of these bottles to submerge any Coffee Berry Borers (CBB) that entered the trap. The traps were situated approximately 40 cm away from the main stem of the coffee plants, as illustrated in Figure 1. A routine monitoring procedure was conducted weekly to record the number of CBB and other insects trapped.

Procedures. This study was conducted during the peak of the coffee harvest season, which occurs from October to November. This is when a significant number of coffee berries are ripe or overripe, just before the final harvesting stage. The research utilized a single-factor randomized block design (RBD) and incorporated nine different treatments. These treatments were:

Control = Water (10 mL);
 K1 = Ethanol (10 mL);
 K2 = Methanol (10 mL);
 K3 = Ethanol and methanol mixture in a 1:1 ratio (5 mL of each);
 K4 = Ethanol and methanol mixture in a 1:2 ratio (3.3 mL of ethanol and 6.7 mL of methanol);
 K5 = Ethanol and methanol mixture in a 1:3 ratio (2.5 mL of ethanol and 7.5 mL of methanol);
 K6 = Ethanol and methanol mixture in a 2:1 ratio (6.7 mL of ethanol and 3.3 mL of methanol);
 K7 = Ethanol and methanol mixture in a 2:3 ratio (4 mL of ethanol and 6 mL of methanol);
 K8 = Ethanol and methanol mixture in a 3:1 ratio (7.5 mL of ethanol and 2.5 mL of methanol);
 K9 = Ethanol and methanol mixture in a 3:2 ratio (6 mL of ethanol and 4 mL of methanol).

Each treatment was repeated three times as block (see Table 1). To avoid any interference between treatments, each group of treatments was kept at least 100 m apart. Additionally, each trap within a treatment was placed at least 25 m away from others to ensure they didn't affect each other. Alongside studying the target CBB, the research also examined non-target insect species to understand how the treatments might impact these other insects.

Observation Variables. The study also sought to understand the changes in the overall number of CBB and the severity of their attacks. This effort aimed to explore the common patterns of attacks and the possible competitive interactions among them. The relative abundance was calculated using the following formula (Suprapti et al., 2022):

$$RA = \frac{A_i}{\sum_n A}$$

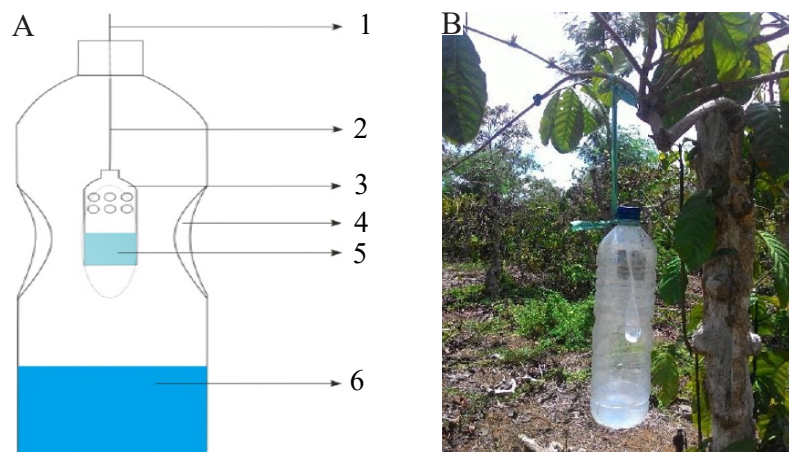


Figure 1. The CBB trap used in this research. A. A Scheme of trap; B. CBB traps in the field, 1. Mainline; 2. Treatment bottle line; 3. Treatment bottle; 4. Inlet; 5. Treatments, 6. Water and detergent.

- RA = Relative abundance;
- A_i = Abundance for each treatment;
- ΣA = Total abundance across all treatments.

The measurement of attack intensity was conducted using the following formula, as detailed by Suprapti et al. (2022):

$$AI = \frac{\sum(ns \times v)}{Z \times N} \times 100\%$$

- AI = Attack intensity;
- ns = Signifies the count of damaged fruits within each attack;
- v = Value assigned to each attack category;
- Z = Highest set scale value;
- N = Total number of leaves.

Data Analysis. The predefined scale values encompass distinct categories: Scale 0 = indicating the absence of observed leaf damage, Scale 1 = representing 1–25% damage to the observed leaves, Scale 2 = reflecting 26–50% damage to the observed leaves, Scale 3 = signifying 51–75% damage to the observed leaves, and Scale 4 = denoting 76–100% damage to the observed leaves. The results were then analyzed for normality using the Shapiro-Wilk test, and differences were assessed using the Kruskal-Wallis Non-Parametric Test, and Dunn Post Hoc Test, with SPSS 16.0 software. Changes in CBB populations were illustrated using Python programming language and the Seaborn library, and were analyzed in a descriptive manner.

RESULTS AND DISCUSSION

A total of 14,044 specimens were collected over the course of the six-week study period. The results

of the Kruskal-Wallis non-parametric test exhibited a p-value of 3.10×10^{-12} at a confidence level of 95%, indicating a significant disparity in trap abundance before and after treatment application across all experimental conditions (as presented in Table 2). Subsequent, statistical analysis using the Dunn test provided deeper insight into the effectiveness of the trap ratio, where all treatments had a significant impact. This thorough investigation also revealed notable differences in the effectiveness of each treatment, with the median values indicating that K6 had the highest median value (105.375), followed by K8 (75.87).

From a broader agricultural standpoint, the results of the treatments highlight a clear effectiveness in capturing CBB, with treatment K6 standing out as notably efficient, as shown in Figure 2. Notably, the performance of treatment K6 consistently improves over several weeks. This improvement is linked to the higher proportion of ethanol compared to methanol, a shared feature with treatments K8 and K9. In contrast, treatments with a higher proportion of methanol seem to show a narrower range of trapping effectiveness. Moreover, formulations that combine both ethanol and methanol outperform those with only one component. In summary, treatment K6 (Ethanol:Methanol = 2:1) emerges as the optimal approach, demonstrating superior trapping effectiveness for CBB specimen.

Abundance reached its highest point in the third week for all treatments, followed by a decline and then a rise again in the sixth week, marking the study’s end (as shown in Figure 3). These patterns are connected to the intricate behavior of CBB. They tend to increase and decrease in the same week across all treatments.

A meticulous comparison of abundance data further underscores the preeminence of treatment K6 (Ethanol:Methanol = 2:1, see Table 2 and Figure 3). However, it is of significance to note that the application of a singular ethanol concentration seems

Table 1. Experimental design

Block 1	Block 2	Block 3
K3	K2	K9
K4	K7	K1
K1	K6	K5
K2	K4	K7
K9	K1	K3
K5	K8	K6
K7	K3	K2
K6	K5	K8
K8	K9	K4

Table 2. Summary of statistical test results.

Treatments	Normalitas Shapiro-Wilk			Kruskal-Wallis test			Dunn Test			
	Statistics	p-Value	Interpretation	Statistics	p-Value	Interpretation	Dunn Results (Z)	Absolut Z (Bonferroni)	Interpretation	Median
Kontrol	1.000	1.000					-6.60			0.875
K1 = Etanol	0.928	0.182					-6.43			58.375
K2 = Metanol	0.798	0.001					-6.38			32.62
K3 = 1:1	0.849	0.008					-5.95			51.12
K4 = 1:2	0.871	0.0182	data are not normally distributed	73.51	3.10×10^{-12}	All treatment is significant (<0.05)	-5.62	3.26	All treatment is Significant (>3.26)	24.75
K5 = 1:3	0.869	0.017					-5.47			52.37
K6 = 2:1	0.728	0.0002					-5.21			105.37*
K7 = 2:3	0.781	0.0008					-4.59			27.5
K8 = 3:1	0.895	0.038					-4.05			75.87
K9 = 3:2	0.805	0.0018					-3.44			75

An asterisk (*) indicates the treatment with the best impact. (see methods for codes).

to have a moderating influence on CBB capture during the third and fourth weeks. This phenomenon can be attributed to the potent allure exercised by ethanol, one of the spectrum of Volatile Organic Compounds (VOCs) emitted by plants, which is believed to exert a compelling attraction on CBB. This inference aligns harmoniously with previous findings that highlight the potency of ethanol as an efficacious attractant against a range of pests, surpassing the performance of methanol.

Based on the analysis of preference data illustrated in the PCA graph (Figure 4), it becomes evident that the most significant upsurge in the CBB population occurred during weeks 3 and 6. Among the various individual and combined applications examined, it is apparent that treatment K6 stands out as the most effective attractant trap.

However, it's worth noting that treatments with higher concentrations of methanol (K4 and K5) showed comparatively diminished effectiveness when contrasted with the treatment featuring a higher concentration of ethanol in K6 (Figure 4). The study findings emphasize that traps employing higher methanol concentrations as attractants yielded reduced effectiveness. This suggests that while methanol is often utilized as a component of Volatile Organic Compounds (VOCs), achieving optimal results requires careful calibration, with minimal concentrations necessary for the best outcomes. The success of these compounds hinges on their ability to attract a greater number of CBB specimens in direct proportion to their concentration, ensuring a more substantial impact.

Each treatment's performance was estimated by comparing abundance and attack intensity. In general, the attack intensity was inversely proportional to the increase in the abundance of CBB caught (Figure 5). The single ethanol application improved in increasing abundance and reducing attack intensity starting in the fourth week (Figure 5K1). The performance of methanol application at a single concentration showed an increased activity up to the third week (Figure 5K2). Ethanol, at a higher concentration, showed a higher abundance performance, and the attack intensity decreased and remained stagnant (Figure 5K6, K8, K9). This evidence shows the effectiveness of ethanol in forming an attractant mixture with methanol.

The evidence demonstrates the efficiency of using ethanol to create an attractant mixture with methanol. This finding contrasts with the current standard practice of using methanol and ethanol in ratios like 3:1 or 1:1. Several studies suggest that different environmental conditions can affect the effectiveness of each attractant differently (Messing,

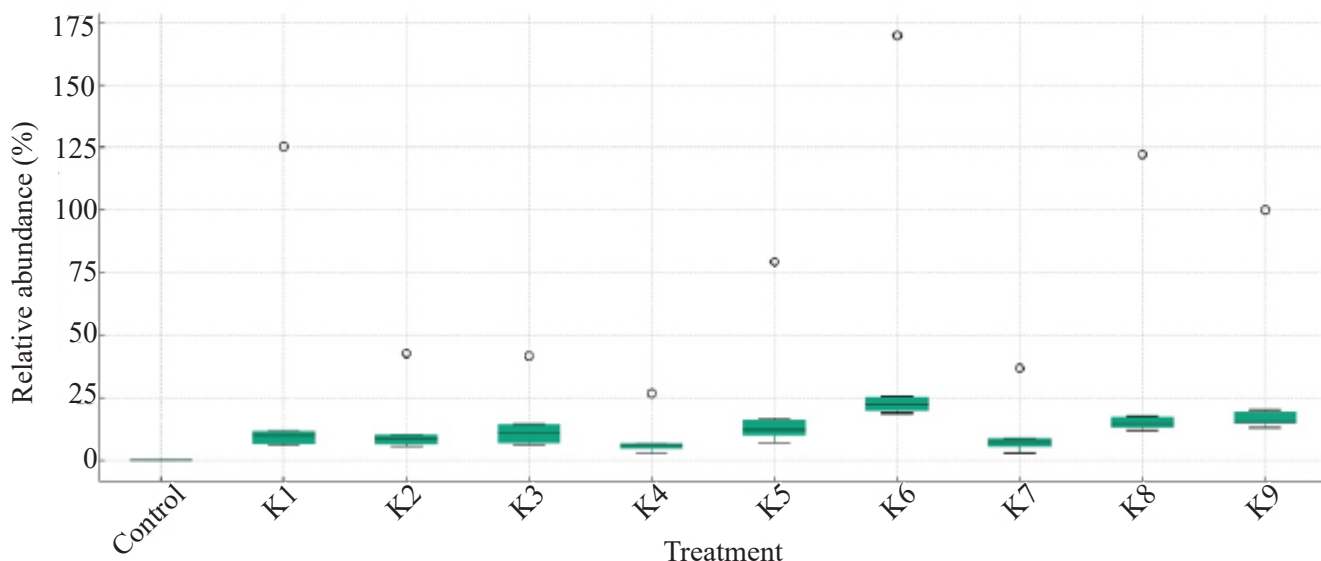


Figure 2. Boxplot graphs of the median and third quartiles on each treatment's performance in relative abundance for all weeks (see methods for codes).

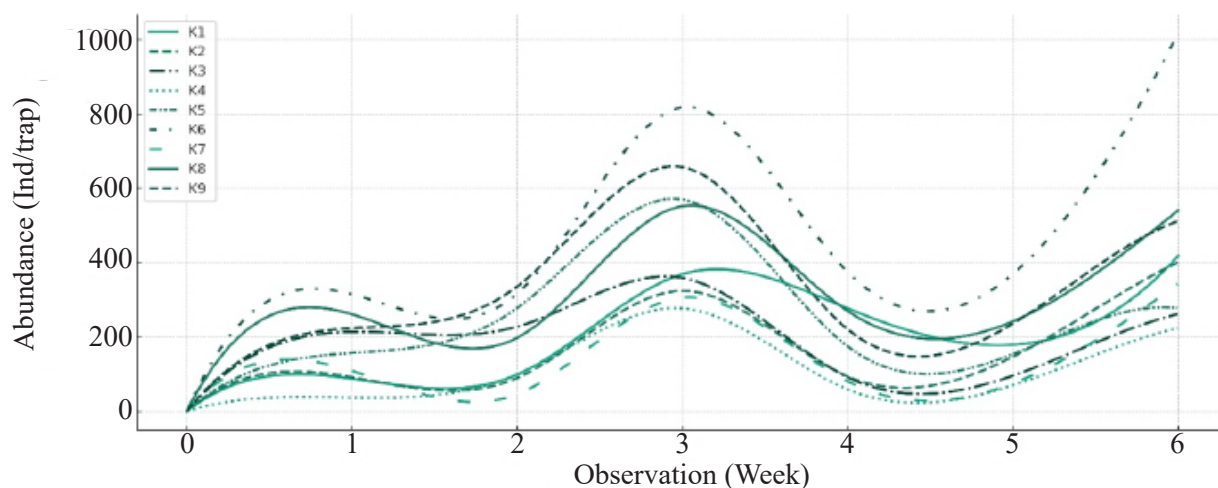


Figure 3. Comparison of performance between treatments at six weeks. Abundance tends to increase in the third week and peaks in the sixth week.

2012; Carvalho et al., 2023). Our research results aim to provide a clear picture and basis for the effectiveness of these combinations in specific environments.

The combination of ethanol and methanol in a 2:1 ratio has proven to be the most effective attractant not only for CBB but also for other types of insects. Among the notable captures, insects from the Coccinellidae (n= 3), Formicidae (n= 1), and Chrysomilidae (n= 1) families were observed (Table 3). It is important to note that while the Chrysomilidae family is considered a pest in plantation crops, its impact on reducing coffee yields appears to be relatively minor. These findings align with a similar study by Sitohang et al. (2022), which also highlighted that using ethanol alone as

an attractant has a stronger effect on attracting CBB. This can be explained by the fact that ripe coffee cherries naturally emit a significant amount of ethanol. As a result, attractants containing ethanol are more successful in eliciting a response from CBB due to the familiarity of the aroma with that of ripe coffee berries. Moreover, the utilization of a coffee fruit extract with a concentration of 150 mL, as opposed to a 10 mL Hypotan ethanol solution, also exhibited a considerable capacity to attract CBB (Rasiska et al., 2021). This attractiveness was further augmented when the coffee skin, housing chlorogenic acid, was combined with additional attractants, resulting in heightened efficacy. Interestingly, female CBB's also displayed a preference

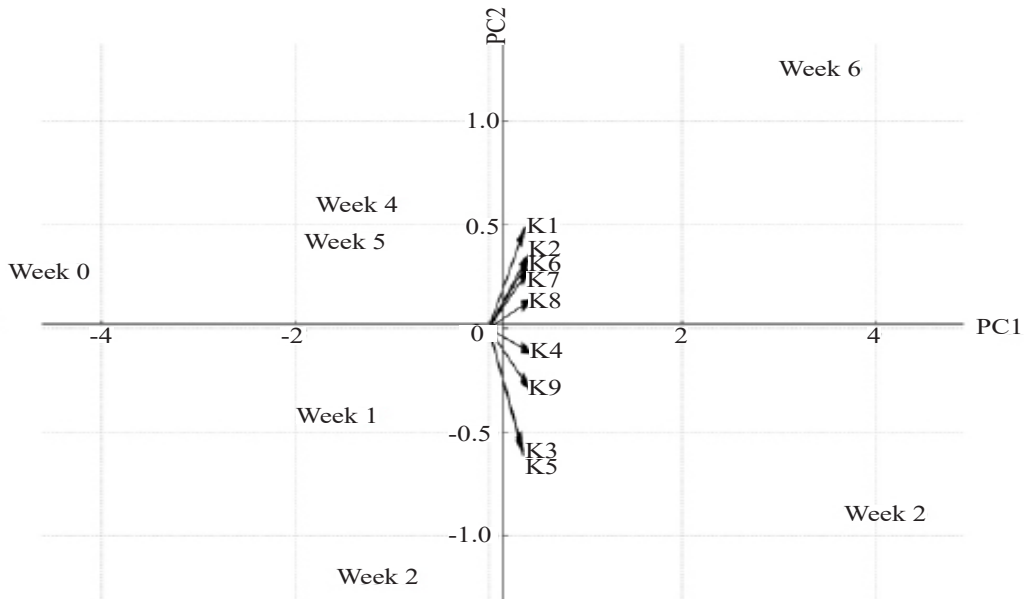


Figure 4. PCA graph of the CBB number by the treatments for six weeks.

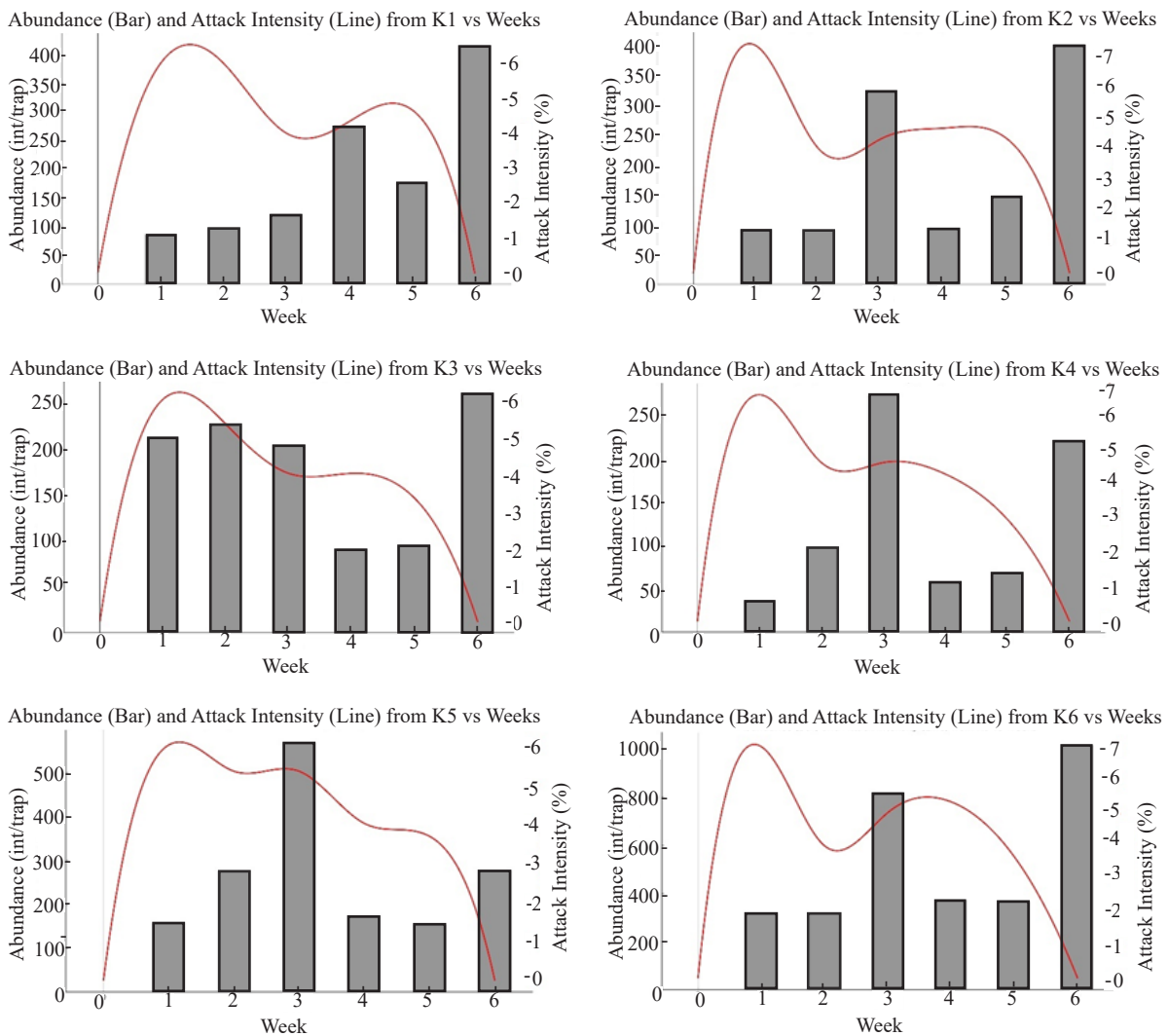


Figure 5. Dual axis graph on the performance of each treatment compared to attack intensity; here Gray bars shows attack intensity while smoothed line shows the CBB's abundance.

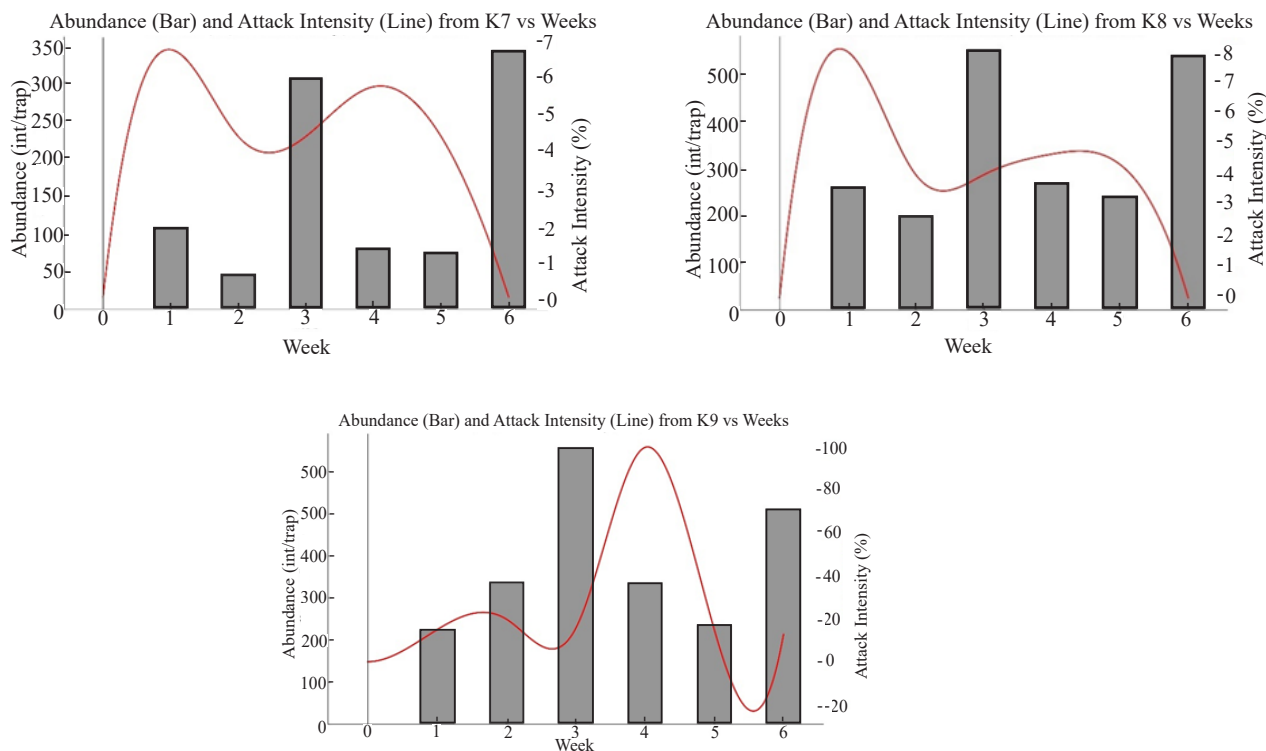


Figure 5. (Continued). Dual axis graph on the performance of each treatment compared to attack intensity; here Gray bars shows attack intensity while smoothed line shows the CBB's abundance.

Table 3. The number of other insects captured by the trap

Treatments	Blattodea: Blatellidae	Hymenoptera: Formicidae	Coleoptera: Chrysomilidae	Coleoptera: Coccinellidae
K0 = Control	0	0	0	0
K1 = Ethanol	0	1	1	2
K2 = Mehtanol	1	0	1	1
K3 = 1:1	0	0	0	1
K4 = 1:2	1	0	0	0
K5 = 1:3	1	0	0	2
K6 = 2:1	0	1	1	3
K7 = 2:3	2	0	1	0
K8 = 3:1	0	1	0	1
K9 = 3:2	0	0	1	1

for several other volatile compounds, including 2-heptanone, 2-heptanol, 3-ethyl-4-methylpentanol, phenyl ethyl alcohol, methyl salicylate, and α -copaene. These compounds naturally occur in coffee fruit skins, as previously identified by Rasiska et al. (2021).

The efficacy of the attractants begins with CBB's interest, drawn by the gradual release of volatile gases. Female CBB's actively seek the source of the scent, leading them to become ensnared within the attractant apparatus during this pursuit. The trapped insects

remain captivated by the scent, causing them to stay in proximity to the attractant. Consequently, CBB's become fatigued and eventually succumb to the soap solution, resulting in their demise (Girsang et al., 2020). It is noteworthy that a majority of the captured CBB's are females, a phenomenon attributed to the distinct behaviors of CBB's. While only female CBB's possess flight capabilities, the males inhabit the crevices of coffee berries. This gender-based difference in flight abilities is due to the more developed and efficient

wings of females, compared to the rudimentary and less functional wings of males (Siregar et al., 2023).

As previously mentioned, the synergistic blend of ethanol and methanol emerges as a recommended approach, as these chemical compounds effectively lure females for mating. In parallel, the mixture of ethanol and methanol also appeals to males for feeding, rendering this combination more efficacious due to its capacity to attract both genders.

Notably, the Chrysomelidae family captured in the traps did not exhibit significant variations in response to each attractant. However, it is important to recognize that Chrysomelidae, as herbivores, can inflict damage on coffee plants (Aristizábal et al., 2013). Nonetheless, their limited consumption scope—primarily confined to young leaves—prevents them from posing a significant threat that would cause substantial yield losses. In contrast, the Coccinellidae family embodies a crucial agricultural predator, feeding on mealybug eggs and nymphs present on coffee leaves (Rodrigues-Silva et al., 2017). The Formicidae family functions as a predator in coffee ecosystems, proficiently hunting CBBs within coffee fruits by accessing them through openings (Philpott et al., 2008). Furthermore, Coccinellidae's capability to consume aphids around coffee plants underscores their predatory prowess and adeptness at sourcing sustenance (Kibrom et al., 2012).

This research underscores the enhanced effectiveness of utilizing a blend of ethanol and methanol in comparison to individual applications. However, it is essential to note that the higher ethanol ratio also attracts insects other than CBB, albeit in reduced quantities.

CONCLUSION

In conclusion, the combination of ethanol and methanol has proven effective in attractant-based traps for Coffee Berry Borer (CBB) treatment. Specifically, the 2:1 ratio of ethanol to methanol stands out as the most favorable composition. Ethanol's heightened efficacy, even when used in larger proportions, highlights its superiority compared to methanol and other comparable formulations.

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AUTHORS' CONTRIBUTIONS

ASK designed the research and devised and analyzed the analysis, and is responsible for correspondence with the journal; NTH designed and conducted the research; ND conducted the research, ASPP conducted the research and data analysis, NLM is responsible for the manuscript and references, LS reviewed the manuscript

COMPETING INTEREST

The author declares that there is no competing interest in the research process and the creation of this manuscript.

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