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Research Article

Utilising Plant Extracts as Lures to Capture Ambrosia Beetles (Coleoptera: Curculionidae) in Cocoa Plantation

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ABSTRACT
The ambrosia beetle (Coleoptera: Curculionidae) is a significant pest affecting cocoa plants in South Sulawesi. The high intensity of their attacks poses a serious threat to cocoa production, causing plants to wilt and die. This study developed traps baited with active ingredient compounds from various plant species, including coffee, carrot leaves, fermented cocoa wood, and eucalyptus oil, and compared their efficacy with ethanol. Beetles collected in these traps were identified based on morphological characteristics using a stereo microscope. The attraction test results indicated that all treatments successfully attracted ambrosia beetles, with ethanol capturing the highest number of individuals (1391). The results showed that ethanol and other extracts could capture ambrosia beetles of various kinds. The highest number of captures was found in ethanol and carrot leaf extract treatments. Additionally, eleven species were identified: *Coccotrypes* sp., *Diuncus quadrispinulosus*, *Eccoptopterus spinosus*, *Hypothenemus* sp. 1, *Hypothenemus* sp. 2, *Hypothenemus* sp. 3, *Xyleborus affinis*, *Xylosandru s mancus*, *Xylosandrus crassiusculus*, *Xylosandrus eupatorii*, and *Xylosandrus morigerus.* Traps baited with carrot leaf extract were most effective in capturing *Hypothenemus* sp. 3. These findings underscore the importance of developing various attractant traps utilising plant chemical compounds to detect and identify ambrosia beetle species and mitigate severe crop damage.

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INTRODUCTION

Cocoa is one of Indonesia's staple plantation commodities (Bhattacharjee & Akoroda 2018). Indonesia is the world's largest cocoa producer, ranking third in 2019 after Cote d'Ivoire and Ghana. Indonesia contributed 1.6 million tons or 44 % of the world's cocoa production. However, this position could not be maintained, and Indonesia currently ranks sixth in global cocoa production (Yemima & Novianti 2020; Ministry of Agriculture 2022). South Sulawesi is one of the provinces that produces the most cocoa, with the Luwu region being the largest cocoa fruit-producing center, accounting for up to 35 % of production (Ministry of Agriculture 2022). The development of cocoa production has declined over years due to pest and disease attacks, one of which is ambrosia beetle infestation.

The ambrosia beetles belong to the order Coleoptera, specifically within the family Curculionidae and its subfamilies, Scolytinae and Platypodinae (Kirkendall et al. 2015). These beetles cause significant plant damage, leading to wilting and death by boring into plant stems, entering the tissue, and causing structural damage to the woody stems. This activity results in cracks in the stem, causing the cocoa bark to dry out and peel. Ambrosia beetles feed on the juicy phloem tissue and dead plant parts, and some species can infect healthy trees (Triplehorn et al. 2005; Hulcr et al. 2007). In addition to direct damage, ambrosia beetles can transmit various pathogens, such as fungi and bacteria, which can accelerate plant damage and death. In East Luwu Regency, several fungi were isolated from cocoa plants attacked by ambrosia beetles, including Fusarium, Lasiodiplodia, Ceratocystis, and Diaporthe colonies (Asman et al. 2021).

More information is needed regarding ambrosia beetles infesting cocoa plants in South Sulawesi. Research results indicate a high number of ambrosia beetles in cocoa plants. To control this, farmers generally use chemical insecticide spraying technology to reduce the population of ambrosia beetles. However, this method is ineffective because the beetles attack and develop inside plant tissues (stems and twigs). Therefore, an alternative technology that can be offered is the use of compounds that facilitate communication between insects and plants (Norin 2001). One effective approach is to use lures to attract ambrosia beetles, which are attractants derived from plant extracts. Previous studies have tested attractants using coffee and carrot extracts against the Cocoa Pod Borer pest and found that ambrosia beetles were also caught (Witzgall et al. 2010; Rivay et al. 2023).

Ambrosia beetle attacks on stems occur because the bark layer of plant stems contains aromatic volatile compounds that insects use to find suitable host plants (Rohman 2020). Ethanol plays a crucial role in attracting ambrosia beetles as a kairomone, enabling them to detect suitable hosts, such as stressed or dying trees (Graham 1968; Ranger et al. 2015). Ethanol is not only present in healthy trees but also found in the xylem and phloem, with its concentration increasing when the tree is stressed (Kimmerer & Stringer 1988; Kelsey et al. 2014; Lehenberger et al. 2021). In addition to ethanol, semiochemicals, such as attractant kairomones derived from plant chemical compounds, are widely used to monitor insect pest populations and keep them below threshold levels (Komala et al. 2021).

The behaviour of ambrosia beetles favors the scent emitted by woody plants, making fermented extracts from the decaying wood of the host plant highly effective. Newly emerged female beetles are particularly attracted to volatile compounds from damaged trees, especially ethanol (Cavaletto et al. 2023). Eucalyptus oil, a key compound in attractant mixtures, can also test the attractiveness of beetles (Kuhns et al. 2014). Adult beetles can be more easily captured during their dispersal phase by luring them into traps that emit volatile compounds mimicking the plant odors to which they are naturally attracted (Mazon & Gaviria 2013). Based on the problem caused by ambrosia beetles attacking cocoa plantations, traps were set using bait-containing attractants to capture the presence of ambrosia beetles and reduce the attack.

MATERIALS AND METHODS

Study Site

This research was conducted in Tarengge Village, Wotu Subdistrict, East Luwu District, South Sulawesi Province, Indonesia is located between 2° 31' 58" – 2° 39' 57" South latitude and 120° 45' 20" - 120° 55' 38" east longitude (Figure 1) from March to August 2023. The study site is situated at an altitude of 15-68 m.a.s.l., with an average temperature, humidity, and rainfall of 26.97 °C, 82.39 %, and 15.57 mm, respectively. Observations were carried out in cocoa plantations featuring 10-year-old cocoa clone 45 trees infested with ambrosia beetles, as identified in a previous preliminary study.

Plant Extracts

The extraction of robusta coffee leaves and carrot leaves by performing a maceration process involves chopping the leaves until smooth then soaking them in a methanol solvent at a ratio of 1 kg of leaves to 3 liters of methanol. After three days, the extract is filtered to obtain a liquid extract, which was then evaporated using a rotary evaporator at 55 °C with a rotation speed of 100 rpm to separate the insoluble parts in methanol. The resulting extract was then placed in a water bath to obtain a 100 % plant extract. Furthermore, dilutions were made using the formula V1.M1 = V2.M2 to achieve a 10 % concentration by adding 10 g of extract into 90 mL of 70 % ethanol for carrot leaf extract. Dilution is done to reach a concentration of 15 % by adding 15 g of extract into 85 mL of 70 % ethanol for coffee leaf extract, so that the extract is ready to be used as an attractant.

Fermented cocoa wood is prepared from rotten cocoa wood, which is ground into a powder. Approximately 300 mg of this powder is weighed and placed into a container, then immersed in a water and molasses mixture at a 1:1 ratio. The container is tightly sealed to facilitate the fermentation process. After 14 days, the container is opened, the fermented liquid is filtered and used as an attractant. The eucalyptus oil utilised has been formulated and acquired from Cap Lang products of 100 % concentration, sourced from PT Eagle Indo Pharma, located in Tangerang City, Banten, Indonesia. The ethanol

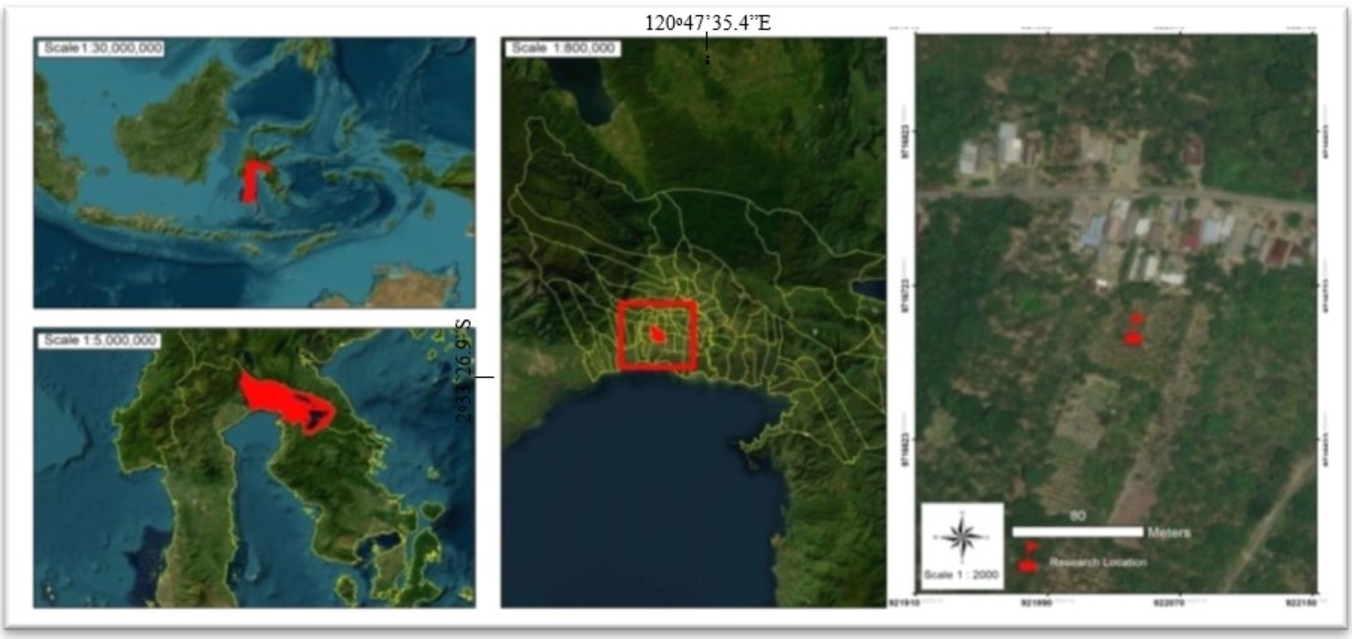

Figure 1. Study site in East Luwu District, South Sulawesi Province, Indonesia.

employed is sourced from One Med Alcohol 70 %, a PT Jaya Mas Medica Industri Product in East Java, Indonesia.

Attractant Trap

The traps utilised were created from plastic bottles measuring 32.2 cm in height and 8.2 cm in diameter, with one side of the bottle cut to form a 7×12 cm window. To attract insects, 0.5 g of cotton was hung inside the bottle, onto which 2 mL of attractant was sprayed. Additionally, an adhesive tube containing 5 mL of 70 % ethanol was placed at the bottom of the bottle to capture insects entering the trap. To protect against rainwater, a plastic plate was placed on top of the trap as a roof (Figure 2). The traps were then fastened to cocoa plants using plastic rope, positioned 50 cm above the ground. The treatments consisted of four types of plant extracts and ethanol. Each treatment involved two trees, with one trap set on each tree, and this was repeated three times, resulting in 30 traps set in each plot arranged in a randomized block design. The traps were positioned diagonally with 12 m spacing between them.

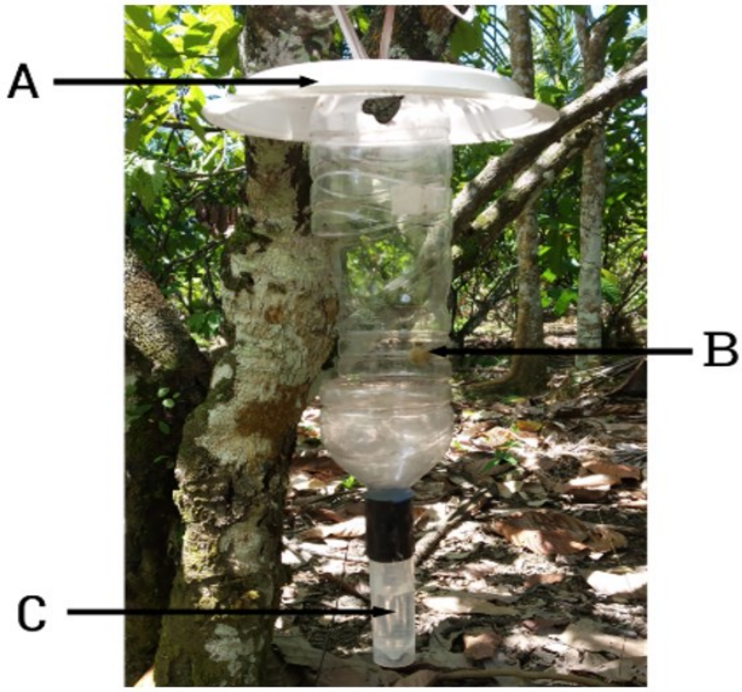

Figure 2. Bottle trap model. A) trap lid, B) cotton swab containing attractant, and C) tube containing soap/alcohol solution.

Insect Collection and Identification

The beetle population count was determined through the observation of ambrosia beetles trapped in the study. Observations were conducted 14 times, with 3-day intervals between each observation. Simultaneous observations were made on all sample trees. The collected samples were stored in specimen bottles, each labeled with the respective treatment and date. Ambrosia beetles were identified using an Olympus SZ microscope, following the guidance provided in the book "Bark and Ambrosia Beetles of South America (Coleoptera: Scolytidae)", which outlines morphological characteristics such as body size, pron otal shape, and elytral shape.

Data Analysis

The collected data underwent statistical analysis using analysis of variance (ANOVA) followed by a post hoc test, specifically the Fisher's Least Significant Difference (LSD) test at a significance level of 5 %.

RESULTS AND DISCUSSION

Populations of Ambrosia Beetles

The results showed the population of ambrosia beetles caught in the trap bottle. Analysis of the population of each species revealed that *Hypothenemus* sp. 3 was the most abundant species, with significantly different results compared to other species. Conversely, *Hypothenemus* sp. 2, X. *crassiusculus*, and X. *morigerus* showed no significant differences in their populations (Figure 3).

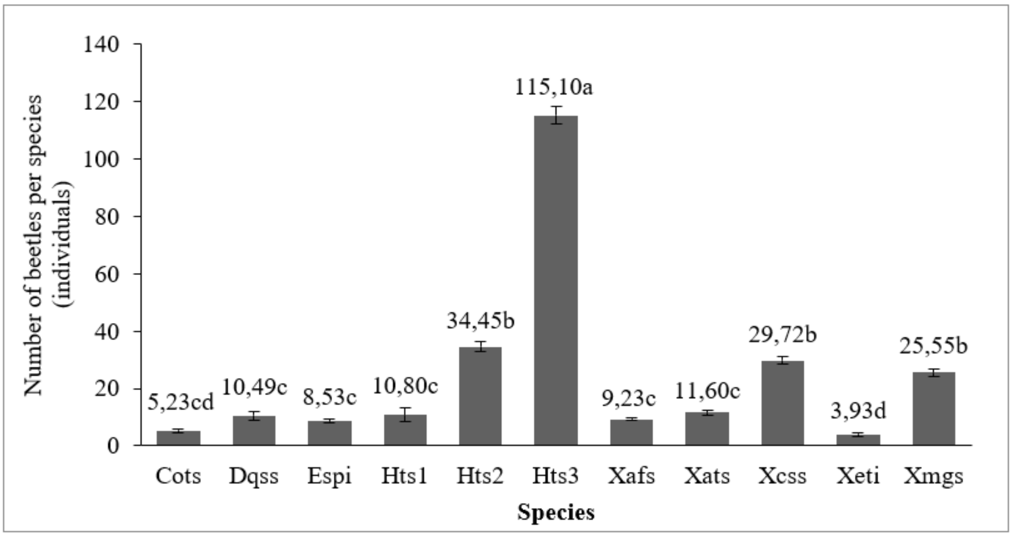

Figure 3. The number of individual ambrosia beetles captured, categorized by species, is as follows: Cots (*Coccotrypes* sp.), Dqss (*Diuncus quadrispinulosus*), Espi (*Eccoptopterus spinosus*), Hts1 (*Hypothenemus* sp. 1), Hts2 (*Hypothenemus* sp. 2), Hts3 (*Hypothenemus* sp. 3), Xafs (*Xyleborus affinis*), Xats (*Xylosandrus mancus*), Xcss (*Xylosandrus crassiusculus*), Xeti (*Xylosandrus eupatorii*), and Xmgs (*Xylosandrus morigerus*). The means ± standard deviation is presented and species denoted by the same lowercase letters at the top of the bars were not significantly different (LSD test at 0.05 significance level).

Hypothenemus emerged as the dominant species attracted to the baited traps, accounting for 82 % of the total identification results. This ambrosia beetle species is recognised for its ability to reproduce on a wide range of host plants. *Hypothenemus* is highly polyphagous, capable of colonising various plant families, including vines, fruits, live seeds, bark, and twigs, even those that are dead and nutrient-poor (Vega et al. 2015). Population diversity of these beetles is influenced by several factors, including high plant diversity, which in turn impacts the diversity of ambrosia beetle species (Haddad et al. 2001)

Based on the five treatments tested, ethanol captured the highest number of ambrosia beetles, with 1391 individuals, followed by carrot leaf extract with 1313 individuals, fermented cocoa wood with 1179 individuals, eucalyptus oil with 991 individuals, and coffee leaf extract with 964 individuals. Statistical analysis indicated a significant difference among the treatments involving ethanol, coffee leaf extract, and eucalyptus oil. However, no significant difference was observed between carrot leaf extract and fermented cocoa wood treatments, as depicted in Figure 4.

After conducting observations at the research site, 11 species of ambrosia beetles were identified, with varying occurrences in each treatment. All species belonged to the Scolytidae family, exhibiting distinct population distributions across the treatments. The species with the highest population was *Hypothenemus* sp. 3, particularly prevalent in the carrot leaf extract treatment (Table 1). The trend of beetle capture during 14 observations can be seen in Figure 5.

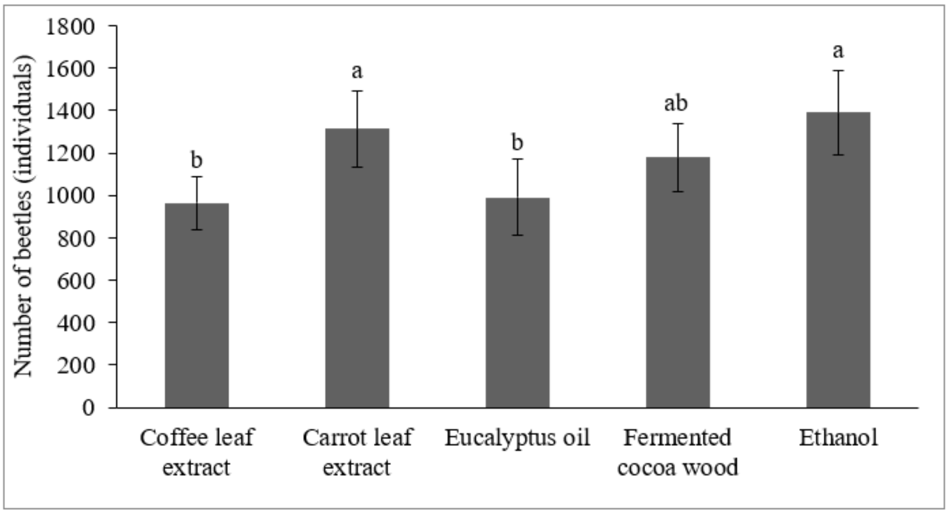

Figure 4. The number of beetles trapped from each treatment was recorded over 14 observations, with a 3-day interval between each observation. The means ± standard deviation was presented, and treatments sharing the same lowercase letters at the top of the bars were not significantly different (LSD test at 0.05 significance level).

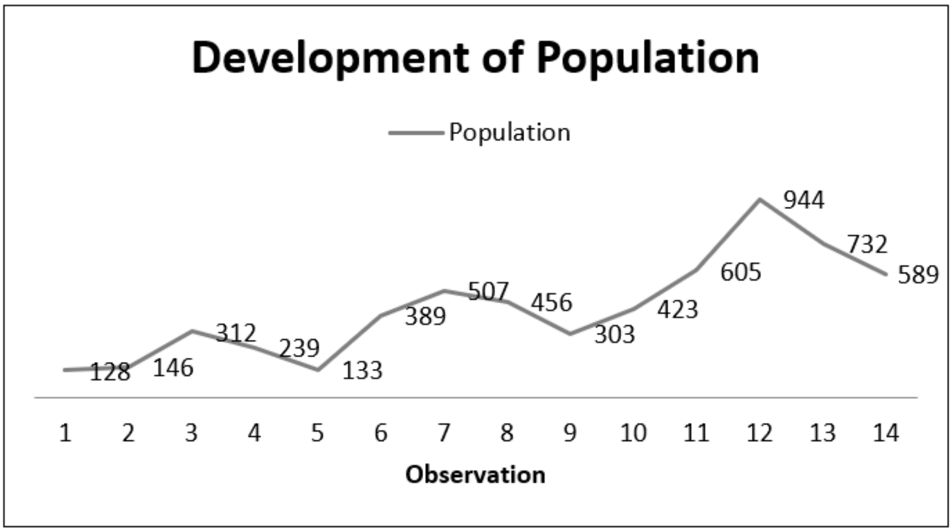

Figure 5. Population development of beetles caught during 14 observations.

The utilisation of traps baited with plant extract successfully captured ambrosia beetles, yielding significant results across all treatments with notably high catch rates. Increased plant species diversity is known to have a posi-

Table 1. Populations of several of ambrosia beetles trapped on each attractant trap.

Species	Population of each treatment				
	Coffee leaf extract	Carrot leaf extract	Eucalyptus oil	Fermented cocoa wood	Ethanol
Coccotrypes sp.	1	3	4	0	0
Diuncus quadrispinulosus	8	0	2	9	29
Eccoptopterus spinosus	1	1	11	5	6
Hypothenemus sp1	17	45	2	0	0
Hypothenemus sp2	89	43	36	74	107
Hypothenemus sp3	767	1150	801	943	1075
Xyleborus affinis	5	2	4	6	3
Xylosandrus mancus	5	13	6	3	8
Xylosandrus crassiusculus	51	30	73	70	94
Xylosandrus eupatorii	2	1	1	0	0
Xylosandrus morigerus	18	25	51	69	69

tive impact on insect abundance, particularly herbivorous species (Dinnage et al. 2012). Among the treatments, ethanol and carrot leaf extract yielded the highest number of captures from the post hoc test results, declared not significantly different with carrot leaf extract. This is attributed to the fact that ethanol, used as bait, mimics the ethanol emitted by stressed trees, serving as an olfactory signal for ambrosia beetles seeking susceptible host plants for colonization (Galko et al. 2014; Cavaletto et al. 2021). Furthermore, ambrosia beetles rely on ethanol for the development of fungal symbionts, essential for the production of their offspring (Ranger et al. 2018).

In addition to ethanol, plant-derived compounds with active ingredients can serve as effective attractants for detecting ambrosia beetles in cocoa plantations. These compounds have been evaluated for their attractiveness to insects and exhibit varying levels of efficacy as insect attractants (Maner et al. 2013; Kendra et al. 2014). Ethanol captured the most beetle populations, while carrot leaf extract captured the most *Hypothenemus* species. Chromatographic characterisation of these extracts has revealed that carrot extracts contain primary phenolic acids, with chlorogenic acid being quantitatively identified (Blando et al. 2021). Research on the preference of cocoa pod borers using carrot and coffee extracts as bait has shown that the traps can also capture other insects, particularly those from the Coleoptera order (Rivay et al. 2023).

Fermented extracts offer a cost-effective and environmentally friendly method for managing large pest populations. Fermented extracts have been discovered to also function as attractants, effectively luring insects. This is exemplified by cocoa wood extracts, which exhibit a high attraction potential for ambrosia beetles. The primary ingredient in this fermentation process is cocoa wood that has undergone decay due to ambrosia beetle infestation. The compounds believed to be present in the extract include ethanol, which is naturally found in the xylem and phloem of trees and increases in concentration during periods of tree stress (Lehenberger et al. 2021).

The results further demonstrate that eucalyptus oil can serve as effective trap baits for capturing ambrosia beetles and are comparably effective to coffee leaf extracts. Eucalyptol, the primary compound in eucalyptus oil has been identified as the key component responsible for beetle attraction. Eucalyptol is known for its lower volatility compared to other attractants, making it particularly suitable for capturing larger numbers of beetles. Previous research has shown that eucalyptol can attract beetles from the Coleoptera order, including *Xyloborus glabratus*, on avocado plants. Additionally, eucalyptus oil can aid in identifying specific beetle varieties that are attracted to it (Kuhns et al. 2014).

On the other hand, coffee leaf extract can also act as an effective attractant due to its chlorogenic acid content. This compound, classified as a secondary metabolite in the phenol class, has the ability to stimulate or attract adult insects to lay eggs (Siregar 2016) reported that chlorogenic acid attractants derived from coffee leaves and fruit peels effectively control *Hypothenemus hampei* Ferr.

From the results of the development of the ambrosia beetle population carried out during 14 observations with a span of 3 days, the results of the development of the trend show that at the beginning of the observation, which is still tiny, then at the seventh and 12th observations is the highest population peak with before that there is a decrease in population in the fifth and ninth observations, it shows a decrease in population which then increases due to the exposure period on the attractant plants used which can only maintain the aroma for 12-15 days so that when the population has decreased then replenish the attractant in the trap so that the population caught again increases as in the following observation.

Morphology Identification

Based on their morphological characteristics, 11 species of ambrosia beetles were identified specific morphological characteristics of each species were detailed in (Figure 6).

The results of identifying the morphological characteristics of the ambrosia beetles show that the dominant characteristics are body size, body color, pronotum, and the slope of the elytra, which are different for each ambrosia beetle obtained. A 1.2-2.5 mm length characterizes Genus *Coccotrypes.* The pronotum curves anteriorly from weak to strong, with a smooth to rough surface (Wood 2007). The pronotum looks flattened and somewhat flat when viewed from the lateral side. On the elytra, there are blackish spots. The elytra slope is convex and not equipped with additional tools (Figure 6.1a).

Diuncus quadrispinulosus found in this study has a yellowish brown body, medium body size, and length of 1.8 mm. The pronotum viewed from the lateral side is rounded and firm. The protibia are obliquely triangular (Figure 6.2a). From the dorsal side, the pronotum appears rounded. The scutellum is visible and flush with the elytra (Figure 6.2b). *Eccoptopterus spinosus* has a blackish brown body, a body measuring 2.8 mm, and a sturdy pronotum shape almost as large or more significant than the abdomen; at the pronotal base, there are dense setae. When viewed laterally, the slope of the elytra extends almost to the base of the elytra, conc ave with spines at the edges (Figure 6.3a).

Genus *Hypothenemus generally* has a length of 1.0–2.0 mm, and its color ranges from pale yellowish brown to black, with a broader pronotum and convex elytra slopes (Wood 2007). *Hypothenemus* beetles can differentiate based on vesture details, frontal sculpture, and surface texture (Vega et al. 2015). *Hypothenemus* sp. 1, when viewed from the lateral side, the elytra are convex, have emarginated compound eyes, and have a prominent lump on the pronotum (Figure 6.4a). The vestiure consists of erect, brightly colored setae that cover the entire body when viewed from the dorsal side. At the same time, *Hypothenemus* sp. 2 has morphological characteristics in the form of a body measuring 1.9 mm with a dark brown color. When viewed from the lateral side, the elytra are convex, have emarginated compound eyes, and have a conspicuous lump on the pronotum (Figure 6.5a). The vestiture consists of erect, brightly colored setae that cover the entire body when viewed from the dorsal side (Figure 6.5b) (Wood 2007).

Hypothenemus sp. 3 on the lateral side (Figure 6.6a), it is clear that the whole body is covered with setae, the tip of the pronotum is hairy and pointed downwards, and the slope of the elytra is convex, and di is covered with setae and spots (Hulcr et al. 2015). This indicates that the dominant characteristics of ambrosia beetles were small bark beetles with a black coloration that did not exceed 1.2 mm in size. When observed laterally (Figure 6.6a), the body is densely covered with setae, the tip of the pronotum is hairy and points downwards, and the elytral slope is convex, also covered with setae and spots. Unlike other ambrosia beetle species, the wings of *Hypothenemus* sp. do not extend to cover the tip of the abdomen (Hulcr et al. 2015; Dzurenko & Hulcr 2022).

Xyleborus affinis is characterised by a slender body of dark red to brown (Figure 6.7). The oblique elytra are curved at the end of the eel, there are fine hairs on the pronotum, and the slanting eel on all parts of the body has short setae, the head is directed downwards and curved, and the mouth is pointed (Figure 6.7a). The *Xylosandrus mancus* determined in this research has a yellowish-brown body. The elytra looks like it has been cut off, and the slope of the elytra suddenly separates from the disc (Figure 6.8a). The pronotum is the same length and width, and when viewed from the dorsal side, the pronotum appears rounded, and the base is shiny (Figure 6.8b) (Wood 2007).

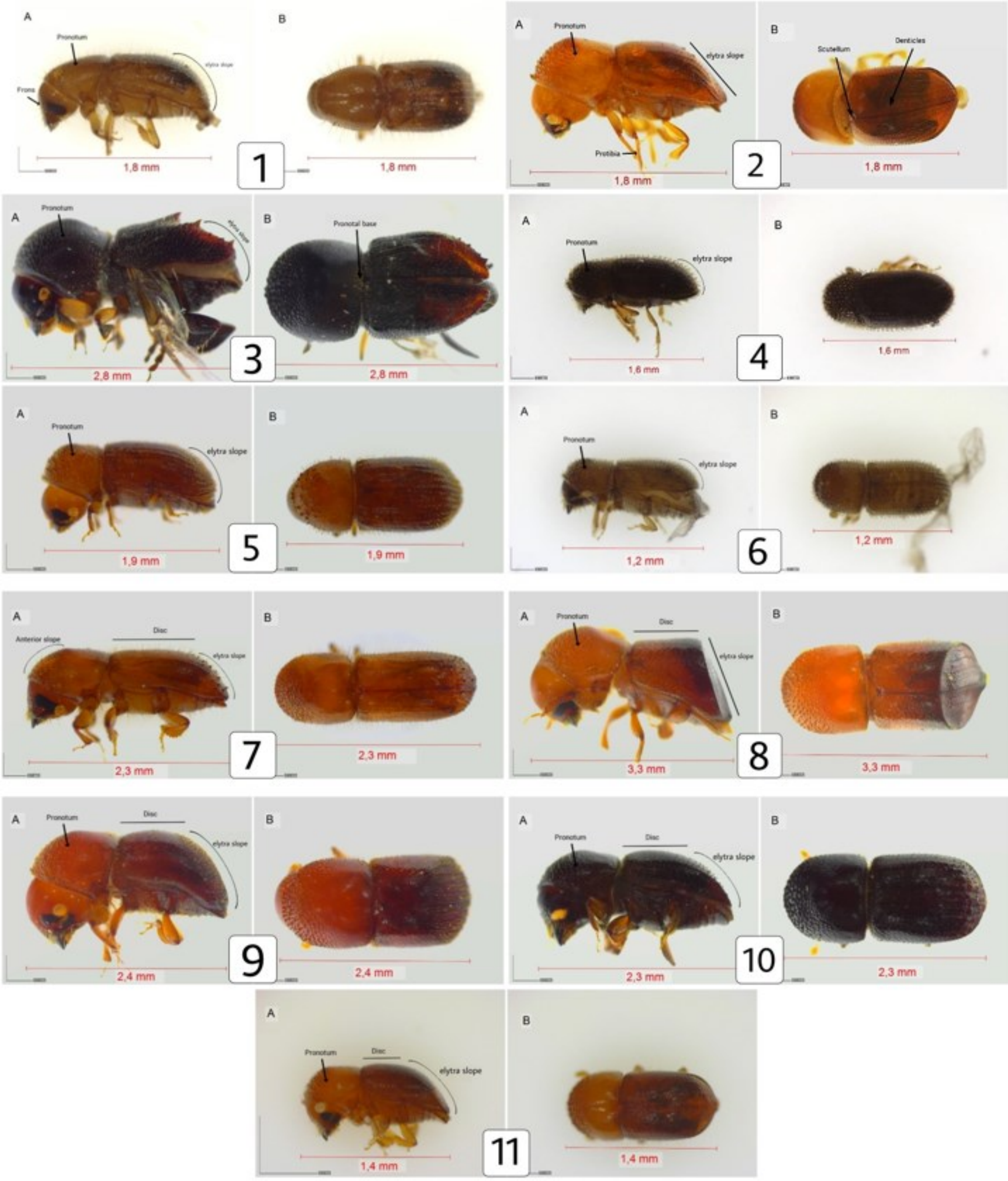

Figure 6. Ambrosia beetles trapped in attractant trap. (1) *Coccotrypes* sp., (2) *Diuncus quadrispinulosus*, (3) *Eccoptopterus spinosus*, (4) *Hypothenemus* sp. 1, (5) *Hypothenemus* sp. 2, (6) *Hypothenemus* sp. 3, (7) *Xyleborus affinis*, (8) *Xylosandrus mancus*, (9) *Xylosandrus crassiusculus*, (10) *Xylosandrus eupatorii*, (11) *Xylosandrus morigerus*. (A) Lateral view, (B) Dorsal view.

Xylosandrus crassiusculus, characterised based on the pronotum, is round when viewed from the lateral side. The granulate ambrosia beetles have a "granulated" region located on the front portion of the downward-facing head and setae on the back end of the elytra (Figure 6.9a) (Poudel et al. 2023). The slope of the elytra is very convex and has setae all over the body surface evenly (Wood 2007). In the *Xylosandrus eupatorii*, when viewed from the dorsal side, the pronotum appears rounded, has the same length and width, and the basal part is smooth and shiny (Figure 6.10b). The slope of the elytra is convex and appears rounded, with the elytra disc gradually curving towards the slope (Smith et al. 2020). The *Xylosandrus morigerus* is characterized by a length of 1.4-1.7 mm, yellowish to reddish brown color, convex, steep elytra slopes with a rounded base (Wood 2007).

Hypothenemus sp. 3 is the most dominant species, reaching 82 % of the total species obtained. Due to the large population of ambrosia beetles found on cocoa plants, the attacks are also getting worse. This can be the basis for controlling this population and minimizing the damage and loss of yield.

CONCLUSION

The study indicated the efficacy of utilising plant extracts as lures to capture ambrosia beetles in cocoa plantation. Ethanol, carrot leaf extract, and fermented cocoa wood emerged as promoting attractants, offering effective means of monitoring beetle population. Ethanol captured the most beetle populations, while carrot leaf extract captured the most *Hypothenemus* species. Chromatographic characterisation of these extracts has revealed that carrot extracts contain primary phenolic acids, with chlorogenic acid being quantitatively identified. These findings underscore the importance of developing alternative pest management strategies to safeguard cocoa production.

AUTHOR CONTRIBUTION

The authors SS and AR contributed to the conceptualisation, implementation, and preparation of the scientific article. AA contributed to the preparation of the scientific article, MBM contributed to the identification and preparation of the scientific article, ES and AA contributed to the research design, S and N contributed to the implementation of the research, NAF contributed to the preparation of the article, and AS contributed to the implementation of the research and identification. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declared that the present study was conducted in the absence of any conflict of interest or competing interests.

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