

Behavioural Responses of Cowpea Bruchid to Three Volatile Organic Compounds from Cowpea

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ABSTRACT

Investigation was conducted to evaluate the behavioural responses of adult *Callosobruchus maculatus* (F.) to three volatile organic compounds (VOCs) (oleic acid, β -pinene, and p-xylene) at four mixed ratios (1:1:1, 1:2:1, 2:1:1, 1:1:2) and at different concentration (0.1, 0.2 and 0.3 $\mu\text{g g}^{-1}$). The attraction of both sexes to different concentration of oleic acid and p-xylene, oleic acid and β -pinene, and β -pinene and p-xylene were also investigated. Test odorant (VOC sample) and solvent (blank control) were separately applied to cotton wool and placed in each arm of the Y-tube olfactometer. Individual bruchid was introduced at the base of the olfactometer and given 15 minutes to choose an arm. Various results showed that the three compounds significantly ($p < 0.05$) attracted unsexed adult bruchids relative to control, and the percentage attraction ranged from 80.00 to 95.00%, irrespective of the VOCs. Similarly, VOC type, experimental concentration and sex significantly influenced the percentage attraction of adult bruchids relative to the control. However, only the two-way interaction of VOCs with concentration (VOC*C) significantly influenced the attraction of adult bruchids to VOCs. Relative to controls, β -pinene and p-xylene significantly attracted the highest (36-38) and lowest (32-35) number of bruchids. Female bruchids were significantly attracted to β -pinene at 0.3 and 0.1 $\mu\text{g g}^{-1}$ relative to p-xylene. All the four mixed ratios exhibited greater attraction compared to the individual compounds irrespective of the concentration. However, the highest (39) (97.5%) attracted bruchid was caused by 1:1:2 mixed ratio of oleic acid, β -pinene and p-xylene compounds at all the three concentrations relative to blank control. This study revealed that β -pinene and the mixed ratio of 1:1:2 caused the highest attraction of adult *C. maculatus*. Therefore, it could serve as excellent lures for baited traps for effective monitoring and management of cowpea bruchids infesting cowpea in the field and storage.

Keywords: *Callosobruchus maculatus*, oleic acid, Y-tube olfactometer, p-xylene, β -pinene

INTRODUCTION

The cowpea bruchid (*Callosobruchus maculatus*) is a major insect pest of stored cowpeas (*Vigna unguiculata*), causing significant losses and reducing the quality and marketability of this important legume (Fasuan et al., 2021; Hamzavi et al., 2022). Presently, the management strategies often depend on synthetic insecticides which have been widely used to mitigate the impact of *C. maculatus* infestations on cowpea seeds. However, these chemical control measures pose several challenges, such as environmental pollution, health hazards to humans and non-target organisms, and the development of insect resistance (Kalpna et al., 2022; Qasim et al., 2024). As a result, there is an increasing need for environmentally sustainable and biologically effective alternatives for pest management. One promising approach in sustainable pest control is the use of semio-chemicals naturally occurring volatile organic compounds (VOCs) that mediate insect behaviour.

Volatile organic compounds (VOCs) are emitted by plants, microorganisms, and even other insects, influencing insect behaviour in ways that can be beneficial or detrimental to

agricultural production (Brilli et al., 2019; Srikamwang et al., 2023; Makhoul et al., 2024). These compounds can function as attractants or repellents, providing opportunities for their application in integrated pest management (IPM) strategies. Understanding how insects respond to VOCs is essential for developing strategies to manipulate their behaviour for pest control and ecosystem management (Conboy et al., 2020; Razo-Belman and Ozuna, 2023; Qasim et al., 2024). Behavioural response of *C. maculatus* to VOCs is crucial for developing novel, eco-friendly pest control methods. The identification of effective attractants or repellents can lead to the formulation of biopesticides, pheromone traps, or push-pull strategies that reduce infestation levels while minimizing the effects of chemical pesticide commonly used. Oleic acid (fatty acid), β -pinene (monoterpene), and p-xylene (aromatic hydrocarbon) are naturally occurring volatile organic compounds found in plants, including cowpea (Al-Snafi, 2020; Temisan et al., 2025). Plant VOCs have been reported to influence insect behaviour, play a key role in insect communication, plant

defense mechanisms linked to insect attraction or deterrence (War et al., 2012; Yactayo-Chang et al., 2020; Divekar et al., 2022). In this study, we aim to provide insights into the behavioural response of cowpea bruchids to oleic acid, β -pinene, p-xylene and their mixed ratios in a Y-tube olfactometer. Finding in this study will contribute to the advancement of innovative, biologically based pest control strategies that are both efficient and sustainable.

MATERIALS AND METHODS

Study Design and Location

This study was designed and carried out at Entomology Laboratory Department of Biology, Federal University of Technology Akure to investigate the behavioural response of the cowpea bruchid (*Callosobruchus maculatus*) to ratios and concentrations of three selected volatile organic compounds (VOCs) namely: Oleic acid, β -pinene and p-xylene. A two-choice olfactometer bioassay was employed to assess the insect's preference or avoidance behaviour under controlled laboratory conditions.

Sourcing and Rearing of Insects

Adults *C. maculatus* was obtained from laboratory colony maintained at Entomology Laboratory Department of Biology, Federal University of Technology Akure. The colony was reared on cowpea seeds (*Vigna unguiculata*) in a temperature-controlled environment at $27 \pm 2^\circ\text{C}$, $65 \pm 5\%$ relative humidity (RH), and 12:12 h light-dark cycle following the method described by Ofuya, (1997) with little modification. Only unmated adult cowpea bruchids of 2 to 4 days old were used for behavioural assays. Prior to experimentation, insects were starved for 24 hours to ensure responsiveness to odour stimuli.

Preparation of Volatile Organic Compounds (VOCs) and Test Solutions

Method described by Barbosa-Cornelio et al. (2019) was adopted with slight modification. Oleic acid, β -pinene, and p-xylene were separately purchased from Bristol Scientific Company Limited, Liverpool Road, Apapa, 101254 Lagos, Nigeria with $\geq 98\%$ purity. The compounds were stored in amber glass vials at 4°C to prevent degradation. Each compound was prepared in ethanol at different concentrations 0.1, 0.2 and $0.3 \mu\text{g/g}$. Mixtures of the three VOCs were prepared in specific ratios (v/v) to evaluate synergistic effects. The first ratio mixture contain Oleic acid: β -pinene:p-xylene (1:1:1), the second ratio mixture contain Oleic acid: β -pinene:p-xylene (2:1:1), the third ration mixture contain Oleic acid: β -pinene:p-xylene (1:2:1), while the forth ration mixture contain Oleic acid: β -pinene: p-xylene (1:1:2).

Behavioural Bioassays and Experimental Set Up

A two-choice Y-tube olfactometer (glass, 10 mm internal diameter, 25 cm arms, 30 cm stem) was used to test insect response. Each VOC sample (test odorant) was applied to cotton wool, placed in one olfactometer arm, while the other arm contains solvent as blank control. The blank control was set-up to ensure any observed behavioural responses are due to odorant being tested and not from residual odours in the system. It is usually used to rule out false positives. A single cowpea bruchid was introduced at the base of the olfactometer and given 15 minutes to choose an arm. At first, individual VOCs was used at different concentrations (0.1, 0.2 and $0.3 \mu\text{g/g}$) after which the mixtures were used at different ratios. At each of the experimental concentration, oleic acid and p-xylene, oleic acid and β -pinene, and β -pinene and P-xylene were also tested using two-choice Y-tube olfactometer. Ethanol was used as blank control. A total of 40 male and 40 female respondents were used in the study. Each experiment was replicated thrice per concentration and ratio. This was carried out as described by Masini et al. (2024).

Data Collection and Response Measurement

Insect that moves toward the odour-treated arm and remains for at least 30 seconds were considered as attraction and response, while insect that moves toward the control arm were recorded separately. Insect that remains in the release chamber or moves randomly was recorded as noresponse. Percentage of responding insects = (Number of insects choosing treatd arm)/(Total number of Responding insect) x 100

Data Analysis

One-way ANOVA was used to determine the effect of concentration on behavioural response. New Duncan Range test was used to assess significant differences between VOCs treatments, significance was set at $p < 0.05$ using SPSS version 26.0. Two-sided binomial test ($\alpha=0.05$) was used to compare the proportion of insects choosing each odour source versus the control. General Linear Model (GLM-ANOVA) was used to determine main and interactive effects of the VOC type, sex and experimental concentration on the attraction of the bruchids

RESULTS

Response of unsexed adult *C. maculatus* to the three volatile organic compounds (VOCs) in a Y-tube olfactometer test

The percentage response of unsexed adult *C. maculatus* to different concentrations of oleic acid, β -pinene and P-xylene is shown in Table 1. Generally, all the three compounds significantly ($p < 0.05$) attracted adult bruchids relative to control and the percentage attraction ranges from 80.00 to 95.00%, irrespective of the VOCs. However, the attraction

of bruchids exposed separately to three experimental concentrations (0.1, 0.2 and 0.3 $\mu\text{g g}^{-1}$) of each of the VOCs were not significantly ($p > 0.05$) different from each other. Relative to the control, β -pinene and p-xylene exhibited the highest (95.00%) and lowest (76.25%) bruchid attraction at 0.1 $\mu\text{g g}^{-1}$.

Table 1: Percentage attraction of *C. maculatus* to three volatile compounds at different concentrations

Compounds	Conc. ($\mu\text{g g}^{-1}$)	Insect Attracted (%)
Oleic Acid	0.1	87.50 $\pm$ 3.72 ^{bcd}
	0.2	85.00 $\pm$ 4.02 ^{bcd}
	0.3	92.50 $\pm$ 2.96 ^d
β -Pinene	0.1	95.00 $\pm$ 2.45 ^d
	0.2	91.25 $\pm$ 3.18 ^{cd}
	0.3	93.75 $\pm$ 2.72 ^d
P-Xylene	0.1	76.25 $\pm$ 4.79 ^b
	0.2	86.25 $\pm$ 3.87 ^{bcd}
	0.3	80.00 $\pm$ 4.50 ^{bc}
Control	0	23.75 $\pm$ 1.38 ^a

Mean followed by the same letters are not significantly difference ($p > 0.05$) from on another using Duncan Multiple Range Test

Main and interactive effects of VOCs (oleic acid, β -pinene and p-xylene), sex (male and female) and concentration (0.1, 0.2 and 0.3 $\mu\text{g g}^{-1}$) on the attraction of adult *C. maculatus* in Y-tube olfactometer test

Figure 1 shows the response of male and female bruchids to each of the three volatile organic compounds. Generally, VOCs type significantly ($F_{2, 36} = 21.93$; $p < 0.001$) influenced the percentage attraction of adult *C. maculatus* relative to control. Similarly, the concentration (C) of the VOCs had significant ($F_{2, 36} = 4.64$; $p = 0.016$) effect on the percentage attraction of the bruchids to the VOCs. Significance ($F_{1, 36} = 7.76$; $p = 0.008$) difference also existed in the attraction of both sexes [Sex (S)] to the three VOCs. Also, only the two-way interaction of VOCs with concentration (VOC*C) significantly ($F_{4, 36} = 3.68$; $p = 0.013$) influenced the attraction of adult bruchids to VOCs relative to control. The three-way interaction of VOC with sex with concentration (VOC*S*C) had no significant ($F_{4, 36} = 0.42$; $p = 0.793$) effect on the attraction response of the bruchids to the VOCs relative to the blank control.

Generally, female bruchids were more attracted to oleic acid (Fig. 1A), β -pinene (Fig. 1B) and p-xylene (Fig. 1C) than male bruchids, especially at 0.2 and 0.3 $\mu\text{g g}^{-1}$. Irrespective of the experimental concentration and sex, significantly ($p < 0.05$) higher bruchids (32-38) were attracted to oleic acid relative to control (Fig. 1A). Also, significantly ($p < 0.0001$) higher (36-38) bruchids were attracted to β -pinene relative

to control, regardless of the experimental concentration (Fig. 1B). Lesser bruchids (32-35) were however significantly ($p < 0.05$) attracted to p-xylene relative to controls, irrespective of the concentration (Fig. 1C).

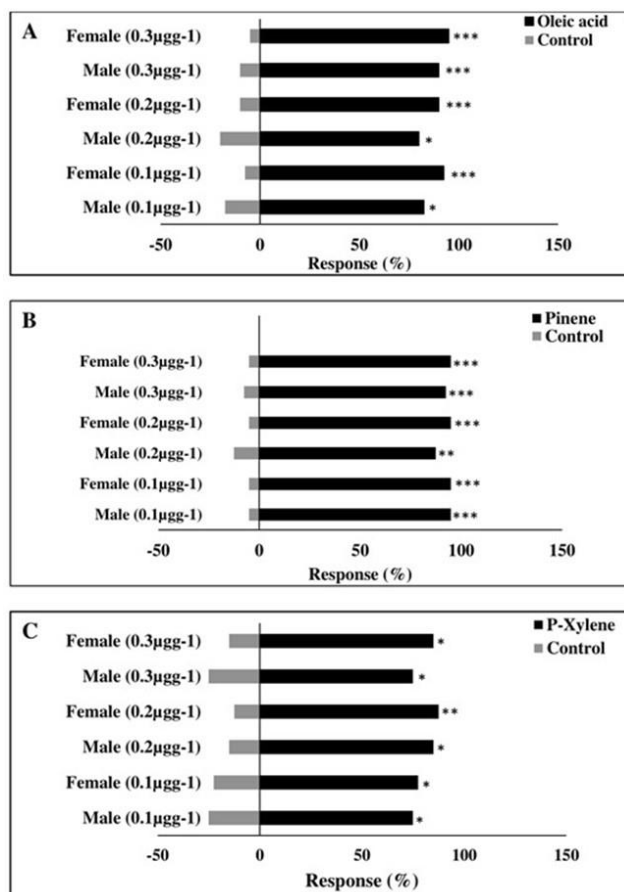


Figure 1: Response of male and female *C. maculatus* to oleic acid (A), β -pinene (B), and p-xylene (C) at 0.1, 0.2 and 0.3 $\mu\text{g g}^{-1}$ in a Y-tube olfactometer ($n = 20$). (*) $p < 0.05$, (**) $p < 0.001$, (***) $p < 0.0001$, (ns) $p > 0.05$ (two-sided binomial test $\alpha = 0.05$).

Attraction of male and female bruchids to different concentration of oleic acid and p-xylene, oleic acid and β -pinene, and β -pinene and p-xylene in a Y-tube olfactometer test

Figure 2 shows the percentage attraction of male and female bruchids to the three different concentrations (0.1, 0.2 and 0.3 $\mu\text{g g}^{-1}$) of oleic acid and p-xylene (Fig. 2A), oleic acid and β -pinene (Fig. 2B), and β -pinene and p-xylene (Fig. 2C). Higher male (30) ($p = 0.041$) and female (31) ($p = 0.027$) bruchids were significantly attracted to 0.2 and 0.1 $\mu\text{g g}^{-1}$ of oleic acid, respectively, relative to p-xylene (Fig. 2A). However, there was no significant difference in the number of bruchids attracted to oleic acid and β -pinene, irrespective of experimental concentration (Fig. 2B). Thirty (30) and thirty one (31) female bruchids were significantly attracted

to 0.3 μgg^{-1} ($p = 0.003$) and 0.1 μgg^{-1} ($p = 0.027$) of β -pinene relative to p-xylene (Fig. 2C).

Olfactory Response of male and female *C. maculatus* to four different mixed ratios (1:1:1, 1:2:1, 2:1:1, 1:1:2) of Oleic acid, β -pinene and P-xylene compounds at 0.1, 0.2 and 0.3 μgg^{-1}

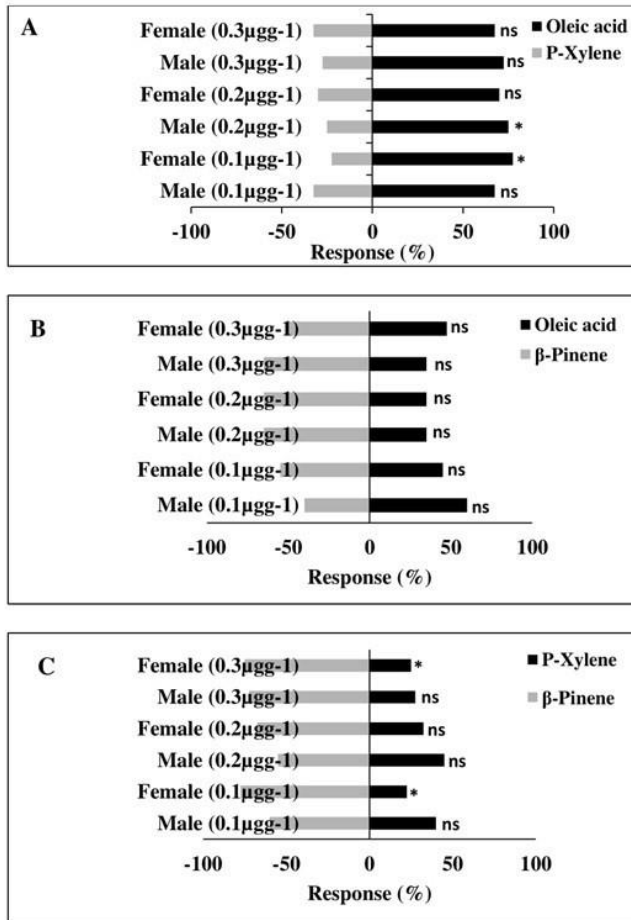


Figure 2: Response of male and female *C. maculatus* to different concentrations of oleic acid and p-xylene (A), oleic acid and β -pinene (B), and β -pinene and p-xylene (C) in a Y-tube olfactometer ($n = 20$). (*) $p < 0.05$, (**) $p < 0.001$, (***) $p < 0.0001$, (ns) $p > 0.05$ (two-sided binomial test $\alpha = 0.05$).

Figure 3 to 6 show the percentage attraction of male and female bruchids to the four different mixed ratios at three concentrations of 0.1, 0.2 and 0.3 μgg^{-1} . All the mixed ratios significantly ($p = 0.001$ or < 0.0001) attracted both male and female bruchids relative to blank controls, regardless of the experimental concentration (Figs. 3-6). At 0.1, 0.2 and 0.3 μgg^{-1} , more females (37-38) bruchids were significantly ($p < 0.0001$) attracted to 1:1:1 mixed ratio of oleic acid, β -pinene and p-xylene compounds relative to blank control (Fig. 3). However, 35 males were significantly ($p = 0.001$) attracted to 0.1 and 0.2 μgg^{-1} of 1:1:1 mixed ratio of oleic acid, β -pinene and p-xylene compounds relative to blank control (Fig. 3). More females (37) were significantly $p < 0.0001$ attracted to all the three experimental concentrations

of 1:2:1 mixed ratio relative to blank control (Fig. 4). On the contrary, 35, 36 and 36 male bruchids were significantly attracted to 0.1, 0.2 and 0.3 μgg^{-1} , respectively, relative to blank control (Fig. 4). The 0.1 μgg^{-1} mixed ratio of 2:1:1 significantly ($p = 0.001$) attracted 35 bruchids, irrespective of sex (Fig. 5).

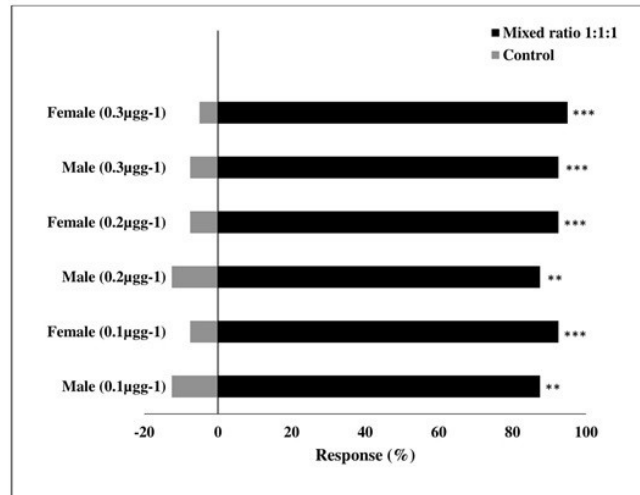


Figure 3: Response of male and female *C. maculatus* to 1:1:1 mixed ratio of oleic acid, β -pinene and p-xylene compounds at 0.1, 0.2 and 0.3 μgg^{-1} in a Y-tube olfactometer ($n = 20$). (*) $p < 0.05$, (**) $p < 0.001$, (***) $p < 0.0001$, (ns) $p > 0.05$ (two-sided binomial test $\alpha = 0.05$).

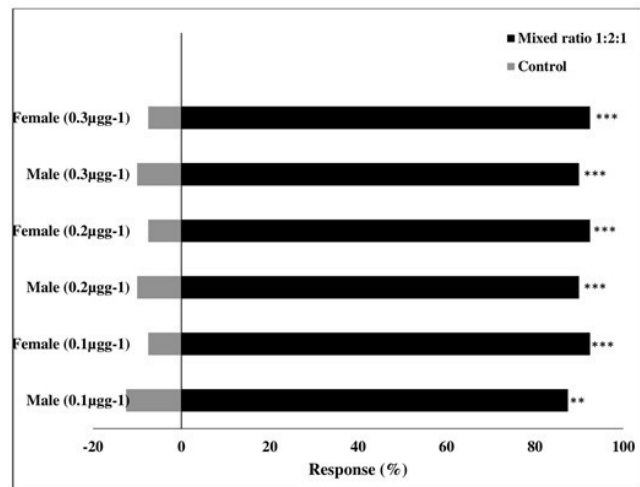


Figure 4: Response of male and female *C. maculatus* to 1:2:1 mixed ratio of oleic acid, β -pinene and p-xylene compounds at 0.1, 0.2 and 0.3 μgg^{-1} in a Y-tube olfactometer ($n = 20$). (*) $p < 0.05$, (**) $p < 0.001$, (***) $p < 0.0001$, (ns) $p > 0.05$ (two-sided binomial test $\alpha = 0.05$).

At concentrations of 0.2 and 0.3 μgg^{-1} of 2:1:1 mixed ratio, females (38 and 39) and males (37 and 38) bruchids were significantly ($p < 0.0001$) attracted, respectively, relative to blank controls (Fig. 5). Thirty nine (39) bruchids were significantly ($p < 0.0001$) attracted to 1:1:2 mixed ratio of oleic acid, β -pinene and p-xylene compounds at all the three

concentrations relative to blank control (Fig. 6). The only exception was observed at 0.1 $\mu\text{g g}^{-1}$ where 38 male bruchids were significantly ($p < 0.0001$) attracted relative to blank control (Fig. 6).

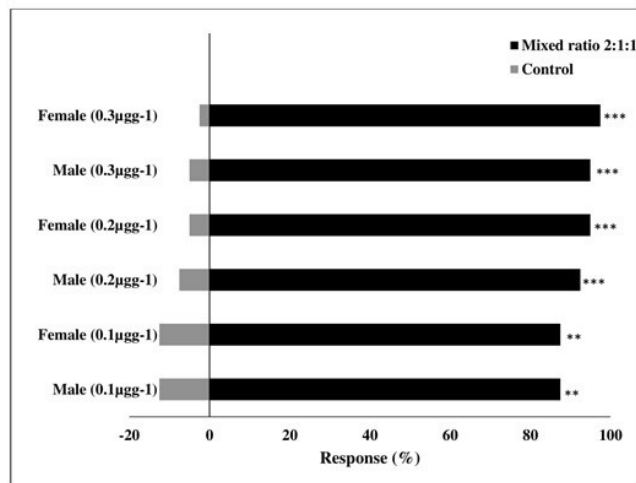


Figure 5: Response of male and female *C. maculatus* to 2:1:1 mixed ratio of oleic acid, β -pinene and p-xylene compounds at 0.1, 0.2 and 0.3 $\mu\text{g g}^{-1}$ in a Y-tube olfactometer ($n = 20$). (*) $p < 0.05$, (**) $p < 0.001$, (***) $p < 0.0001$, (ns) $p > 0.05$ (two-sided binomial test $\alpha = 0.05$).

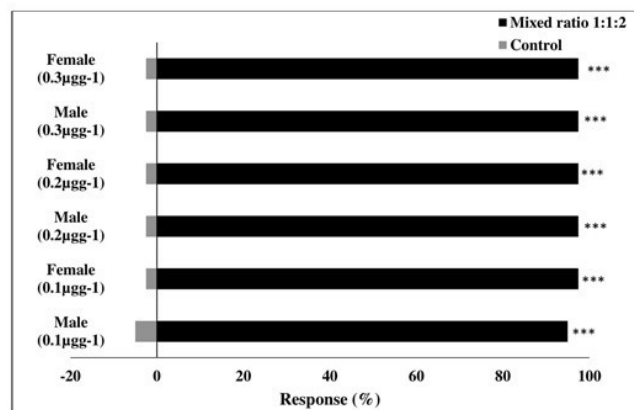


Figure 6: Response of male and female *C. maculatus* to 1:1:2 mixed ratio of oleic acid, β -pinene and p-xylene compounds at 0.1, 0.2 and 0.3 $\mu\text{g g}^{-1}$ in a Y-tube olfactometer ($n = 20$). (*) $p < 0.05$, (**) $p < 0.001$, (***) $p < 0.0001$, (ns) $p > 0.05$ (two-sided binomial test $\alpha = 0.05$).

DISCUSSION

The behavioural responses of cowpea bruchid to three volatile organic chemicals (oleic acid, β -pinene, and p-xylene) and their mixed ratios were examined. Regardless of experimental concentration, the three VOCs tested independently demonstrated significant attraction to sexed and unsexed adult *C. maculatus* compared to blank controls in Y-tube olfactometer. Given that the three compounds were previously identified in both local and improved

Nigerian cowpea varieties (Temisan et al., 2025), the attraction exhibited by *C. maculatus* towards these compounds corroborated the findings of Arnold et al. (2012) and Ndomo-Moualeu et al. (2015), which indicated that *C. maculatus* typically demonstrates a pronounced attraction to any cowpea-related stimulus. The significant attraction demonstrated by p-xylene in the present study aligns with the prior findings of Xiu et al. (2019), which confirm the attraction of p-xylene for *Adelphocoris lineolatus*. Although little information is available on the attraction of oleic acid and β -pinene for *C. maculatus*, this study shows that both compounds demonstrated higher attraction for adult bruchids relative to p-xylene. In fact, out of the three compounds, highest and lowest attraction for bruchids was generally shown by β -pinene and p-xylene, respectively. This shows that β -pinene exhibited considerable level of attraction for adult *C. maculatus* and it further corroborates the earlier findings by Ndomo-Moualeu et al. (2015).

The 1:1:1 mixed ratio of oleic acid, β -pinene, and p-xylene consistently achieves high attraction for both sexes across all the experimental concentrations relative to the blank control. This suggests that the mixed treatment has a strong and consistent effect. The mixed ratio 1:2:1 is equally effective as the previously tested 1:1:1 ratio, maintaining maximum responses in both males and females at all tested concentrations. This consistency suggests the mixed treatment is robust and effective across ratios. In previous studies, the role of β -pinene and other terpenes in attracting or insects has been well-documented. Ndomo-Moualeu et al. (2015), and Kelsey and Westlind (2020) reported that β -pinene was particularly attractive to certain species of weevils including *C. maculatus* when used in a blend with other compounds. This supports the results in the current study where β -pinene is mixed with other compounds (Oleic acid and p-xylene) resulting to increases in attraction. Additionally, the current findings of stronger responses from females align with similar observations in other research, where female weevils are often more responsive to certain blends of volatiles due to their reproductive behaviour (Kelsey and Westlind, 2020; Wang et al., 2020).

The mixed ratio 2:1:1 of oleic acid, Pinene, and P-xylene at all tested concentrations is highly effective in eliciting responses from both male and female *C. maculatus*. The responses consistently reach near maximum response whereas the control group shows minimal responses. This highlights the strong attractiveness or efficacy of the compound mixture under these conditions. Oleic acid, a fatty acid, has been shown to attract a range of insect species, including weevils. According to a study by Adhikary et al. (2016), fatty acids such as oleic acid can serve as potent attractants for *Callosobruchus* species, with some research showing that their presence in a mixture can increase attraction; likewise, this has been reported in Coleoptera *Philonthus decorus* (Falyet et al., 2024). The current study aligns with these findings.

The mixture of Oleic acid, β -pinene, and P-xylene in the tested ratio (1:1:2) is effective in attracting *C. maculatus*, with females showing stronger attraction. The concentration plays a critical role, with higher levels eliciting greater responses, supporting the potential use of these compounds in pest management strategies. P-xylene is an aromatic hydrocarbon that has been shown to influence insect behavior. Falyet al. (2024) found that xylene compounds can serve as either attractants or repellents, depending on their concentration and the insect species under study. In the study by Lvet al. (2024), p-xylene was found to act synergistically with other volatile compounds, enhancing attraction in some pest species. This corroborates the results from the current study, where p-xylene is included in the mixture and leads to high attraction, especially at higher concentrations. This confirms that the mixed ratio of Oleic acid, β -pinene, and P-xylene is effective in attracting *C. maculatus*, particularly females, and that its efficacy is concentration-dependent.

The study confirms that varying the ratio of oleic acid, β -pinene, and p-xylene impacts the attraction of *C. maculatus*, with the 1:1:2 ratio being the most effective. Higher concentrations amplified the attraction, and females exhibit stronger responses compared to males. These findings highlight the potential of these compound mixtures for pest management strategies, especially with optimized ratios and concentrations. The findings indicate that oleic acid is a more potent attractant for *C. maculatus* compared to p-xylene. This was not in conformity with the report of Lvet al. (2024); however, the findings were supported by Adhikary et al. (2016). Additionally, females exhibit a higher level of sensitivity to both compounds. These results suggest the potential for Oleic acid to be a key component in developing attractant-based pest management strategies for *C. maculatus*. The contrasting effects of Oleic acid and β -pinene highlight their potential roles in behavioral manipulation. Both β -pinene and Oleic acid are promising candidate for attractant-based pest management strategies. These findings emphasize the importance of concentration and sex-specific responses in designing effective pest control strategies. These findings agreed with Ndomo-Moualeu et al. (2015) and Wang et al. (2020). Female *C. maculatus* are more attracted to the compound mixture ratio of Oleic acid: β -pinene: p-xylene (1:1:2), this was also consistent with other studies. Wang et al. (2020) found that female *C. maculatus* exhibit stronger attraction to certain volatile blends due to their reproductive needs, which could explain the higher responses observed in females in this study.

CONCLUSION

The results demonstrated that the cowpea bruchid is highly attracted to specific bioactive compounds, with β -pinene being the most attractive, followed by oleic acid and p-xylene. This attraction is concentration-dependent, especially for oleic acid, where lower concentrations were

found to be more attractive to female beetles. Moreover, the synergistic effect observed when p-xylene was mixed with β -pinene and oleic acid further enhances the behavioural attraction of *C. maculatus*, indicating a complex interaction between these volatile compounds. These support the idea that volatile compounds, especially β -pinene and oleic acid, play a crucial role in mediating host selection and oviposition behaviour in *C. maculatus*. Furthermore, the study highlights the potential for using specific volatile compounds as tools for controlling pest behaviour in agricultural and storage environments.

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