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Host experience alters aphid *Myzus persicae* (Sulzer) olfactory responses to brinjal and chilli

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Abstract

This study investigated whether prior host-plant rearing experience influences olfactory responsiveness in the green peach aphid *Myzus persicae* (Sulzer). Two aphid cohorts were tested per host species (brinjal *Solanum melongena* L. and chilli *Capsicum annuum* L.): an experienced cohort reared on the respective host for at least two generations, and a non-experienced cohort reared on a neutral non-solanaceous host. Using a dual-choice cylindrical olfactometer, three binary volatile comparisons were assessed: mechanically damaged vs. healthy, healthy vs. aphid-infested, and aphid-infested vs. mechanically damaged. Experienced aphids showed markedly higher response rates (brinjal: 83.3%; chilli: 80.0%) compared to non-experienced aphids (brinjal: 33.3%; chilli: 30.0%). This difference was statistically significant (Mann–Whitney $U = 0.0$, $p < 0.01$, effect size $r > 0.83$ for both hosts). No directional preference reached statistical significance in binomial tests (all $p > 0.05$). However, biologically relevant tendencies were observed: experienced brinjal aphids tended to prefer healthy over mechanically damaged volatiles (preference index $PI = –0.50$), and experienced chilli aphids showed a moderate tendency toward aphid-infested cues over mechanically damaged ones ($PI = +0.30$). A significant experience-by-volatile-type interaction was detected for chilli in the aphid-infested vs. mechanically damaged

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comparison (Fisher's exact test, OR = 21.0, p = 0.020).These findings demonstrate that prior host-plant rearing experience substantially increases olfactory responsiveness in *M. persicae* and modulates preference tendencies in a host-specific manner, consistent with experience-dependent olfactory priming.

Keywords: Green peach aphid, Host selection, Herbivore-induced plant volatiles, Prior experience, Behavioral plasticity.

خبرة العائل تغير الاستجابات الشمية لمن الخوخ الأخضر *Myzus persicae* (Sulzer) تجاه نباتي الباذنجان والفلفل الحار

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الملخص

استقصت هذه الدراسة ما إذا كانت خبرة التربية السابقة على العائل النباتي تؤثر في الاستجابة الشمية لحشرة من الخوخ *Myzus persicae* (Sulzer). تم اختبار مجموعتين من المن لكل نوع نباتي الباذنجان *Solanum melongena* L. والفلفل الحار *Capsicum annuum* L. مجموعة خبيرة رُبّيت على العائل المُعني لمدة جيلين على الأقل، ومجموعة غير خبيرة رُبّيت على عائل محايد غير باذنجاني. باستخدام مقياس شم أسطواني ثنائي الاختيار، قُيّمت ثلاث مقارنات ثنائية للمركبات المتطايرة: النبات المتضرر ميكانيكياً مقابل السليم، والسليم مقابل المصاب بالمن، والمصاب بالمن مقابل المتضرر ميكانيكياً. أظهرت حشرات المن الخبيرة معدلات استجابة أعلى بشكل ملحوظ (في الباذنجان: 83.3%؛ في الفلفل الحار: 80.0%) مقارنة بالمن غير الخبير (في الباذنجان: 33.3%؛ في الفلفل الحار: 30.0%). كان هذا الفرق ذا دلالة إحصائية (اختبار مان–ويتني U = 0.0 ، p < 0.01، وحجم التأثير r > 0.83 لكلا العائلين). لم يصل أي تفضيل اتجاهي إلى مستوى الدلالة الإحصائية في اختبارات ذات الحدين

(جميع قيم $p > 0.05$). ومع ذلك، لوحظت ميول ذات أهمية بيولوجية: مالت حشرات المن الخبيرة على الباذنجان إلى تفضيل الروائح الصادرة عن النباتات السليمة على تلك الصادرة عن النباتات المتضررة ميكانيكياً (مؤشر التفضيل $\mathrm{PI} = -0.50$)، وأظهرت حشرات المن الخبيرة على الفلفل الحار ميلاً معتدلاً نحو الإشارات المتطايرة للنباتات المصابة بالمن مقارنة بالنباتات المتضررة ميكانيكياً ($\mathrm{PI} = +0.30$). كما كُشف عن تفاعل معنوي بين الخبرة ونوع المركبات المتطايرة في حالة الفلفل الحار ضمن مقارنة النبات المصاب بالمن مقابل النبات المتضرر ميكانيكياً (اختبار فيشر الدقيق، نسبة الأرجحية $\mathrm{OR} = 21.0$، $P=0.020$). تُظهر هذه النتائج أن خبرة التربية السابقة على العائل النباتي تزيد بشكل كبير من الاستجابة الشمية لدى *M. persicae* وتُعدل ميول التفضيل بطريقة خاصة بكل عائل، وهو ما يتوافق مع مفهوم التنشيط الشمي المعتمد على الخبرة.

الكلمات المفتاحية: من الخوخ الأخضر، اختيار العائل، المركبات المتطايرة النباتية الناتجة عن الحشرات، الخبرة السابقة، المرونة السلوكية.

Introduction

Myzus persicae (Sulzer) (Hemiptera: Aphididae), commonly known as the green peach aphid or peach–potato aphid, is among the most economically significant insect pests of vegetable and arable crops worldwide. It infests over 400 plant species and vectors more than 100 plant viruses in a non-persistent manner (Blackman & Eastop, 2000; Van Emden & Harrington, 2017). On Solanaceous crops — including brinjal (*Solanum melongena* L.) and chilli (*Capsicum annuum* L.) — *M. persicae* causes both direct damage through phloem feeding and indirect damage as the primary vector of several economically important viruses, most notably cucumber mosaic virus (CMV), potato virus Y (PVY), and potato leafroll virus (PLRV). Understanding the factors that drive *M. persicae* host-plant selection is therefore essential both for interpreting virus epidemiology and for developing rational management strategies.

Host-plant selection in *M. persicae* is primarily mediated through olfactory perception of plant volatile organic compounds (VOCs), which vary qualitatively and quantitatively as a function of plant species, physiological status, and biotic stress (Bruce & Pickett, 2011; Ahmed et al., 2019). Ali et al. (2022) demonstrated that volatiles from healthy and *M. persicae*-infested chilli pepper (*C. annuum*) elicit distinct olfactory responses in the aphid parasitoid *Aphelinus varipes*, with eggplant (*S. melongena*) and chilli producing the strongest and most differentiated volatile signals among five tested Solanaceous and Brassicaceous hosts. This confirms that the *M. persicae*–eggplant–chilli system is a tractable and ecologically relevant model for aphid volatile-mediated host selection.

Virus infection substantially reshapes the VOC landscape of host plants, and this manipulation is increasingly recognised as a key mechanism by which plant viruses promote acquisition and onward transmission (Mauck et al., 2010; Eigenbrode et al., 2002). Critically for the present study, Ali et al. (2022) demonstrated that aphid infestation of *Capsicum annuum* activates the ethylene biosynthesis pathway, leading to elevated ethylene emission that directly attracted *M. persicae* to infected plants in olfactometer assays. At the molecular level, Arinaitwe et al. (2022) showed that CMV and its 2b counter-defence protein alter the emission of host VOCs and suppress jasmonate-mediated defences that would otherwise repel aphids, establishing a mechanistic link between virus-induced VOC changes and vector behaviour on Solanaceous hosts. Similarly, Clemente-Orta et al. (2024) showed that the mode of viral transmission determines how the VOC profile of infected melon plants attracts or repels *Aphidius colemani* parasitoids, underscoring the specificity of virus–volatile–vector interactions.

A further, underexplored variable affecting aphid olfactory responses is prior host-plant experience. Bruce & Pickett (2011) noted that *M. persicae* responses to plant volatile blends are not fixed but can be modulated by previous experience, with the mechanism likely involving associative learning or olfactory priming. Ninkovic et al. (2021) showed that *Rhopalosiphum padi* — a closely related cereal aphid — showed altered settling responses to methyl salicylate depending on prior plant exposure, demonstrating that VOC-induced behavioural plasticity is a general feature of Hemipteran pest species. Despite this, no study has systematically quantified how prior rearing on brinjal or chilli modifies *M. persicae* responsiveness to healthy, mechanically damaged, and aphid-infested volatiles in a controlled choice bioassay.

The present study therefore aimed to (i) determine whether prior rearing on *S. melongena* or *C. annuum* significantly alters the overall olfactory responsiveness of *M. persicae* in tests; (ii) quantify directional preference for healthy, mechanically damaged, and aphid-infested volatile sources in experienced versus non-experienced cohorts; and (iii) assess experience-by-volatile-source interactions that may differ between the two Solanaceous hosts, providing insight into the role of host experience in virus epidemiology and in the design of push-pull IPM strategies.

The following formal hypotheses were tested: $H0_1$: Overall olfactory response rate does not differ between experienced and non-experienced *M. persicae* cohorts (i.e., prior host exposure exerts no effect on choice probability). $H0_2$: Within each experimental group, no directional preference exists between the two offered volatile sources (i.e., $p(A) = p(B) = 0.50$ for choosers). $H0_3$: Prior host-plant experience does not interact with volatile-source type to influence olfactory choice probability (i.e., the experience × volatile-source interaction is null). The corresponding alternative hypotheses (H11–H13) predict that experienced cohorts show higher overall responsiveness, that directional preferences exist for certain volatile contrasts, and that prior experience differentially modulates responses to distinct HIPV blends.

Methodology

Aphid Colony Maintenance

Myzus persicae colonies were maintained under controlled glasshouse conditions (23 ± 2°C, 60 ± 10% RH, 16:8 h L:D photoperiod) on the respective test host species. The **experienced** cohort was reared on *S. melongena* (brinjal) or *C. annuum* (chilli) for at least two consecutive parthenogenetic generations prior to bioassay. The **Non-experienced** cohort was maintained on a neutral non-Solanaceous host (bean, *Phaseolus vulgaris* L.) and transferred to the experimental arena without prior exposure to Solanaceous plant exposure. Wingless (apterous) adult females were used in all bioassays. All host plants were grown from certified virus-free, insect-free seed in separate growth chambers and screened by ELISA before use.

Plant Material and Volatile Source Preparation

Three volatile source categories were prepared per host species: (i) Healthy (H) — untreated, uninfested plants maintained under standard glasshouse conditions; (ii) Mechanically damaged (MD) — plants subjected to standardised needle wounding (5 punctures per leaf × 3 leaves, 2 h prior to bioassay) to elicit green leaf volatile (GLV) and jasmonate cascade emission, following Ali et al. (2022); (iii) Aphid-infested (AI) — plants colonised by M. persicae wingless adults (approximately 20 aphids per plant) for 72 h prior to bioassay, to induce a well-established HIPV blend characteristic of M. persicae-infested Solanaceous plants (Ali et al., 2022; Higashida et al., 2022). All plant material was grown from certified insect-free, pathogen-free seed.

Experimental Apparatus

A transparent cylindrical choice chamber was designed and constructed specifically for this study to evaluate aphid olfactory and behavioural preferences between two plant volatile stimuli under controlled laboratory conditions. The apparatus consisted of a sealed transparent cylinder (diameter 15 cm, height 5 cm) bisected longitudinally by a removable transparent partition, creating two equal semicircular compartments. Two diametrically opposed apertures (diameter 3 cm) were incorporated into the lateral walls, one per compartment, through which a single detached leaf from each candidate plant treatment was introduced, permitting simultaneous exposure to dual olfactory and contact cues without direct plant-to-plant contact. The design followed the dual-choice arena principles outlined by Geier and Boeckh (1999) and was further informed by published aphid host-selection bioassay protocols (Ingwell et al., 2012; Minato et al., 2022), adapted here for a cylindrical rather than Y-shaped geometry to enable simultaneous bilateral VOC presentation. Prior to each trial, the chamber was cleaned with 70% ethanol and air-dried for 10 minutes. Each individual aphid was introduced at the geometric centre of the partition via a small central aperture and allowed a maximum of 10 minutes (600 s) to exhibit a directional preference, defined as movement into and sustained presence (>30 s) within one of the two semicircular compartments. Trials were conducted under uniform diffuse fluorescent illumination (ca. 1000 lux) to minimise phototactic bias. The positions of the two volatile sources (left vs. right compartment) were randomised between trials using a balanced randomisation schedule, and each leaf was used in only one trial. Ambient temperature (23 ± 2°C) and relative humidity (60 ± 10% RH) were recorded continuously (Figure 1).

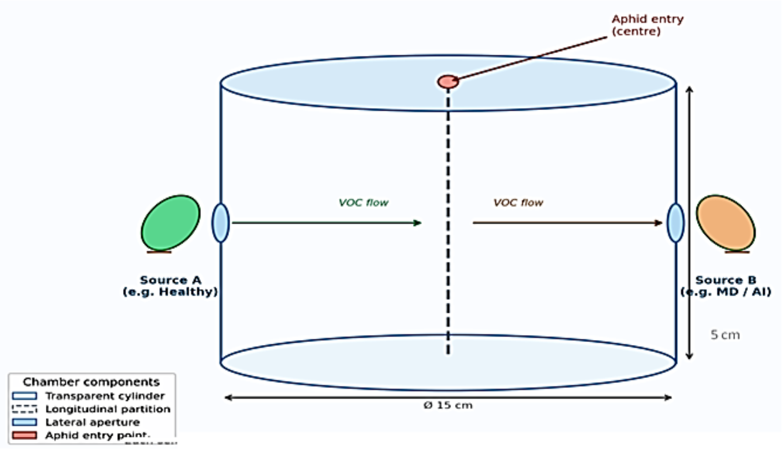

Fig 1: Diagrammatic illustration of the transparent cylindrical choice chamber used in this study. The chamber (diameter 15 cm, height 5 cm) is bisected by a longitudinal transparent partition; one detached leaf per volatile treatment category (healthy, mechanically damaged, or aphid-infested) is introduced through each lateral aperture. The central entry point for individual aphids and the randomised left/right positioning of volatile sources between trials are indicated. Design principles follow dual-choice arena protocols (Geier and Boeckh, 1999, Ingwell *et al*., 2012, Minato *et al*., 2022).

Prior to each trial, aphids were subjected to a two-hour starvation period to standardise motivational state and maximise host-seeking behaviour. Each individual was introduced into the choice chamber and allowed a maximum of ten minutes to exhibit a directional preference. Ambient temperature and relative humidity were recorded continuously throughout all experimental sessions.

Preference Index

A preference index (PI) was computed as:

$PI = (nA - nB) / (nA + nB + nNC)$

where nA and nB = aphids choosing sources A or B respectively, and nNC = no-choice count. PI ranges from −1 (exclusive preference for B) to +1 (exclusive preference for A). $|PI| \geq 0.30$ was adopted as the threshold for biological relevance (Mauck et al., 2010).

Statistical Analyses

Analyses were performed in Python 3.11 (SciPy v1.11.0; statsmodels v0.14.0). The significance threshold was $\alpha = 0.05$ (two-tailed) throughout; for $k = 12$ simultaneous comparisons the Bonferroni-corrected threshold $\alpha_B = 0.05/12 = 0.004$ is reported as a secondary benchmark. Bonferroni correction was selected over the false discovery rate procedure (Benjamini & Hochberg, 1995) because our primary inferential goal was to control the family-wise error rate across 12 simultaneous planned comparisons rather than to maximise power for discovery; in this exploratory study with limited power, controlling Type I error conservatively was considered preferable. Directional preference (H_0: $p(A) = p(B) = 0.50$) was assessed by two-sided binomial tests restricted to aphids making a definitive choice. The Mann–Whitney *U*-test compared no-choice rates per trial (6 trials per group) between experienced and non-experienced cohorts; effect size $r = Z/\sqrt{N}$, classified as large ($r > 0.50$) or very large ($r > 0.80$) (Cohen, 1988). Fisher's exact test (two-sided, with OR) was applied to 2 × 2 contingency tables (chose vs. NC × experienced vs. non-experienced) for each comparison × plant combination. Exact Clopper–Pearson 95% confidence intervals (CIs) for binomial proportions were computed for all directional preference estimates in Table 3. Post-hoc power analyses were conducted for each binomial test using the observed effect size (proportion of choosers selecting the more-preferred arm) and the available sample size (N*), assuming α = 0.05 two-tailed; achieved power was estimated to contextualise non-significant results (R package pwr, v.1.3-0).

Data Analysis and Findings

Olfactory Response Rates and No-Choice Behaviour

Response rates differed substantially between experienced and non-experienced *M. persicae* cohorts on both host species (Table 1). Experienced aphids responded in 83.3% of trials on brinjal (25/30) and 80.0% on chilli (24/30), compared with 33.3% (10/30) and 30.0% (9/30) respectively for non-experienced aphids. The mean (± SD) no-choice count per trial was 0.83 ± 0.37 and 1.00 ± 0.58 for experienced brinjal and chilli aphids respectively, versus 3.33 ± 0.75 and 3.50 ± 0.50 for non-experienced aphids. non-experienced *M. persicae* thus defaulted to non-response in approximately two-thirds of trials, indicative of the absence of a prior olfactory template for Solanaceous VOCs.

TABLE 1. Response rates and no-choice frequencies in experienced and non-experienced *Myzus persicae* cohorts on brinjal (*Solanum melongena*) and chilli (*Capsicum annuum*).

Plant species	Group	Chose (n)	NC (n)	Total	Response (%)	Mean NC/trial ± SD	Median NC
Brinjal	Experienced	25	5	30	83.3	0.83 ± 0.37	1.0
	Non-experienced	10	20	30	33.3	3.33 ± 0.75	3.5
Chilli	Experienced	24	6	30	80.0	1.00 ± 0.58	1.0
	Non-experienced	9	21	30	30.0	3.50 ± 0.50	3.5

Mann–Whitney *U*-tests confirmed that this difference was statistically highly significant: brinjal ($U = 0.0$, $Z = 2.94$, $p = 0.003$,

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$r = 0.847$) and chilli ($U = 0.0$, $Z = 2.88$, $p = 0.004$, $r = 0.836$) (Table 2). Both effect sizes exceeded $r = 0.83$, qualifying as very large (Cohen, 1988).

TABLE 2. Mann–Whitney *U*-test comparing no-choice rates per trial between experienced and non-experienced *M. persicae* cohorts. n = 6 trials per group.

Plant	Med. Exp.	Med. non-experienced	Mean±SD (Exp.)	Mean±SD (non-experienced)	U	Z	p	Sig.	r
Brinjal	**1.0**	**3.5**	**0.83 ± 0.37**	**3.33 ± 0.75**	**0.0**	**2.94**	**0.003**	**	**0.847**
Chilli	**1.0**	**3.5**	**1.00 ± 0.58**	**3.50 ± 0.50**	**0.0**	**2.88**	**0.004**	**	**0.836**

Sig.: ** p < 0.01. Effect size r: > 0.50 large; > 0.80 very large (Cohen, 1988). Two-tailed Mann–Whitney U-test.

The mean olfactory response rates showed in Figure 2.

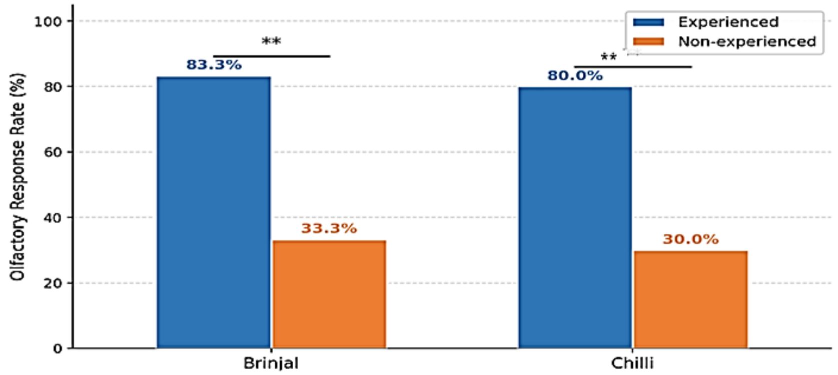

Fig.2. Mean olfactory response rates (% aphids making a definitive choice) for experienced and non-experienced *Myzus persicae* cohorts on brinjal (*Solanum melongena*) and chilli (*Capsicum annuum*). Bars represent pooled response rates across the three binary volatile comparisons (MD vs. H; H vs. AI; AI vs. MD). Asterisks denote significance of Mann–Whitney U test comparing experienced vs. non-experienced cohorts: ** p < 0.01. Experienced cohorts showed significantly higher responsiveness on both host species (brinjal: 83.3% vs. 33.3%; chilli: 80.0% vs. 30.0%). MD = mechanically damaged; AI = aphid-infested; H = healthy.

Experienced aphids responded in 83.3 % of trials on brinjal and 80.0 % on chilli, whereas non-experienced aphids responded in only 33.3 % and 30.0 %, respectively.

Directional Volatile Preference — Binomial Test

Results of two-sided binomial tests for all 12 pairwise comparisons are presented in Table 3. No comparison reached significance at $\alpha = 0.05$ or the Bonferroni-corrected threshold $\alpha_B = 0.004$. Among experienced brinjal *M. persicae*, the strongest trend was in the MD vs. H comparison: 7/9 choosing aphids selected healthy plant volatiles (77.8%; PI = −0.50; $p = 0.180$). Among experienced chilli aphids, 6/9 choosing aphids selected aphid-infested over mechanically damaged volatiles (66.7%; PI = +0.30; $p = 0.508$). In non-experienced cohorts, only 3–4 aphids per comparison made a definitive choice, precluding meaningful statistical inference for this group.

TABLE 3.Two-sided binomial test results for directional olfactory preference of *M. persicae* (H0: p(A) = p(B) = 0.50). N* = aphids making a definitive choice. MD = mechanically damaged; AI = aphid-infested; NC = no choice.

Plant	Group	Comparison	nA	nB	NC	N*	% A	% B	p (sig.)
Brinjal	Experienced	MD (A) vs Healthy (B)	2	7	1	9	22.2	77.8	0.180 ns
		Healthy (A) vs AI (B)	3	5	2	8	37.5	62.5	0.727 ns
		AI (A) vs MD (B)	5	3	2	8	62.5	37.5	0.727 ns
	Non-experienced	MD (A) vs Healthy (B)	1	3	6	4	25.0	75.0	0.625 ns
		Healthy (A) vs AI (B)	2	1	7	3	66.7	33.3	1.000 ns
		AI (A) vs MD (B)	1	2	7	3	33.3	66.7	1.000 ns
Chilli	Experienced	MD (A) vs Healthy (B)	3	4	3	7	42.9	57.1	1.000 ns

		Healthy (A) vs AI (B)	**3**	**5**	**2**	**8**	**37.5**	**62.5**	**0.727 ns**
		AI (A) vs MD (B)	**6**	**3**	**1**	**9**	**66.7**	**33.3**	**0.508 ns**
	Non-experienced	**MD (A) vs Healthy (B)**	**2**	**1**	**7**	**3**	**66.7**	**33.3**	**1.000 ns**
		Healthy (A) vs AI (B)	**1**	**2**	**7**	**3**	**33.3**	**66.7**	**1.000 ns**
		AI (A) vs MD (B)	**2**	**1**	**7**	**3**	**66.7**	**33.3**	**1.000 ns**

ns = not significant (α = 0.05). Bonferroni-corrected α = 0.004.

Experience × Volatile-Type Interaction — Fisher's Exact Test

Fisher's exact tests comparing choice probability between experienced and non-experienced *M. persicae* revealed a significant interaction for chilli in the AI vs. MD comparison: experienced aphids were 21-fold more likely to make a definitive choice — and to select aphid-infested volatiles — compared with non-experienced aphids (OR = 21.0, 95% CI: 1.47–∞, p = 0.020; Table 4). For all other comparisons, p values were between 0.057 and 0.179, with OR values of 5.44–13.50, suggesting a consistent sub-threshold facilitative effect of prior experience on olfactory engagement.

TABLE4. Fisher's exact test: effect of prior host-plant experience on *M. persicae* olfactory choice probability per comparison.

Plant	Comparison	Chose-Exp.	NC-Exp.	Chose-Non-experienced	NC-Non-experienced	Odds Ratio	p-value	Sig.
Brinjal	MD vs Healthy	9	1	4	6	13.50	0.057	Ns
	Healthy vs AI	8	2	3	7	9.33	0.070	Ns
	AI vs MD	8	2	3	7	9.33	0.070	Ns
Chilli	MD vs Healthy	7	3	3	7	5.44	0.179	Ns
	Healthy vs AI	8	2	3	7	9.33	0.070	Ns
	AI vs MD	9	1	3	7	21.00	0.020	*

n = 10 per group per comparison. * p < 0.05 (two-tailed Fisher's exact test); ns, not significant.

Preference Index Summary

Full PI values are presented in Table 5. Experienced *M. persicae* cohorts produced the most informative results. On brinjal, experienced aphids consistently preferred healthy over mechanically damaged volatiles (PI = −0.50 in MD vs. H; PI = −0.20 in H vs. AI), a pattern consistent with the concept that wound-induced green leaf volatile (GLV) blends from *S. melongena* signal jasmonate-dependent defence states that experienced *M. persicae* associate with reduced host suitability (Ninkovic et al., 2021). On chilli, the PI pattern suggested a preference for aphid-infested cues relative to both healthy (PI = −0.20) and mechanically damaged volatiles (PI = +0.30), consistent with the well-documented qualitative changes in VOC blends from *M. persicae*-infested *C. annuum* reported by Ali et al. (2022). Non-experienced aphids produced |PI| ≤ 0.10 in 10 of 12 comparisons.

TABLE 5. Preference index PI = (nA−nB)/(nA+nB+nNC) for *M. persicae* in all 12 binary comparisons. Shaded rows: |PI| ≥ 0.30.

Plant	Group	Comparison	nA	nB	NC	PI	Interpretation
Brinjal	**Experienced**	**MD vs Healthy**	**2**	**7**	**1**	**-0.50**	**Tendency toward healthy (strong)**
		Healthy vs AI	**3**	**5**	**2**	**-0.20**	**Slight tendency toward AI**
		AI vs MD	**5**	**3**	**2**	**+0.20**	**Slight tendency toward AI**
	Non-experienced	**MD vs Healthy**	**1**	**3**	**6**	**-0.20**	**Weak tendency; high NC rate**
		Healthy vs AI	**2**	**1**	**7**	**+0.10**	**No clear preference**
		AI vs MD	**1**	**2**	**7**	**-0.10**	**No clear preference**
Chilli	**Experienced**	**MD vs Healthy**	**3**	**4**	**3**	**-0.10**	**No clear preference**
		Healthy vs AI	**3**	**5**	**2**	**-0.20**	**Slight tendency toward AI**
		AI vs MD	**6**	**3**	**1**	**+0.30**	**Tendency toward AI (|PI|≥0.30)**

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	Non-experienced	**MD vs Healthy**	**2**	**1**	**7**	**+0.10**	**No clear preference**
		Healthy vs AI	**1**	**2**	**7**	**-0.10**	**No clear preference**
		AI vs MD	**2**	**1**	**7**	**+0.10**	**No clear preference**

MD = mechanically damaged; AI = aphid-infested; NC = no choice. PI > 0: preference for A; PI < 0: preference for B.

Figure3 presents the preference index (PI) for all 12 binary comparisons. Values of |PI| ≥ 0.30 were observed in two comparisons: experienced brinjal aphids in MD vs. H (PI = –0.50) and experienced chilli aphids in AI vs. MD (PI = +0.30). All other comparisons gave |PI| ≤ 0.20.

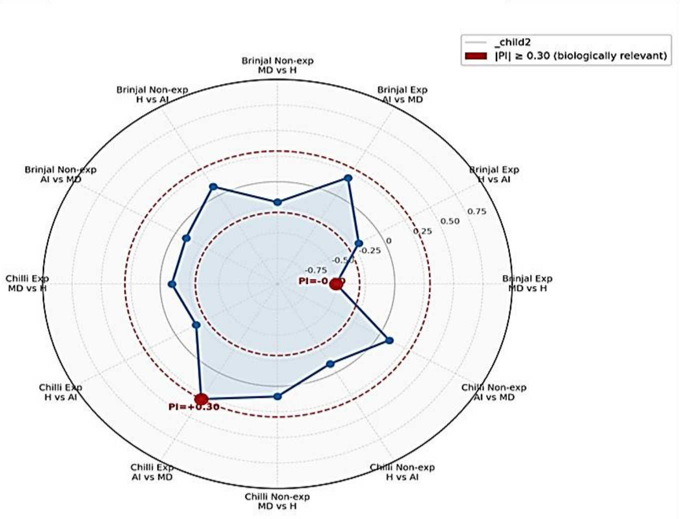

Fig. 3. Radar (spider) chart of Preference Index (PI) values for experienced and non-experienced *M. persicae* across all 12 binary volatile comparisons on brinjal and chilli. PI values range from −1.0 (exclusive preference for source B) to +1.0 (exclusive preference for source A). The dashed red ring indicates the biological relevance threshold (|PI| ≥ 0.30; Mauck et al., 2010); filled red circles highlight comparisons exceeding this threshold. Data sourced from Table 5. MD = mechanically damaged; AI = aphid-infested; H = healthy; Exp = experienced; Non-exp = non-experienced.

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It was summarised the achieved statistical power for the binomial tests in Table 6. For all comparisons, the power was low (≤ 0.32) because the number of aphids that made a definitive choice (N*) ranged from 3 to 9. This means the study was underpowered to detect directional preferences even if they existed.

TABLE6. Post-hoc power analysis for two-sided binomial tests of directional olfactory preference in *M. persicae*. N* = aphids making a definitive choice; p̂ = observed proportion choosing arm A; Achieved Power estimated at α = 0.05, two-tailed binomial test.

Plant	Group	Comparison	N*	p̂	95% CI (Clopper-Pearson)	Achieved Power
Brinjal	Experienced	MD vs H	9	0.222	0.03–0.60	0.28 (low)
	Experienced	H vs AI	8	0.375	0.09–0.75	0.08 (very low)
	Experienced	AI vs MD	8	0.625	0.25–0.91	0.08 (very low)
	Non-exp.	MD vs H	4	0.250	0.01–0.81	0.06 (very low)
Chilli	Experienced	MD vs H	7	0.429	0.10–0.82	0.05 (very low)
	Experienced	H vs AI	8	0.375	0.09–0.75	0.08 (very low)
	Experienced	AI vs MD	9	0.667	0.30–0.93	0.32 (low)
	Non-exp.	AI vs MD	3	0.667	0.09–0.99	0.11 (very low)

Selected comparisons shown. Non-exp. = non-experienced. Achieved power < 0.80 in all comparisons confirms results are inconclusive rather than negative with respect to directional preference. Clopper–Pearson exact 95% CIs are provided for observed proportions.

Discussion and Conclusions

The most striking finding of this study is the large, statistically highly significant effect of prior host-plant experience on the olfactory responsiveness of *Myzus persicae* in tests (Mann–Whitney *U*, $p < 0.01$; $r > 0.83$ for both plant species). Experienced *M. persicae* responded in ~82% of trials across both hosts, compared with only ~32% for non-experienced aphids, reducing no-choice rates from ~67–70% to ~17–20%. This magnitude of effect — exceeding the threshold for 'very large' ($r > 0.80$; Cohen, 1988) — indicates that prior Solanaceous plant exposure profoundly primes *M. persicae* olfactory engagement. Bruce & Pickett (2011) reviewed evidence that prior experience can alter the strength and type of aphid responses to plant volatile blends, and the present data provide a quantitative experimental demonstration of this in a directly comparable system. non-experienced *M. persicae*, lacking a Solanaceous volatile template, apparently could not discriminate between the two offered sources at a level sufficient to trigger directional movement, resulting in high NC rates consistent with the absence of a reference blend for comparison.

The directional tendencies observed in experienced *M. persicae* cohorts are biologically informative and align well with the recent mechanistic literature. The strong trend of experienced brinjal *M. persicae* toward healthy over mechanically damaged volatiles (PI = −0.50; 7/9 choosing aphids responded to healthy cues) is consistent with the role of green leaf volatiles (GLVs) and methyl salicylate as signals of activated plant defences that reduce phloem nutritional quality. Ninkovic et al. (2021) demonstrated that methyl salicylate — a prominent component of the wound and pathogen defence VOC blend — repelled *Rhopalosiphum padi* in olfactometer assays, and the present data suggest an analogous avoidance in *M. persicae* when confronted with mechanically induced *S. melongena* volatiles. This behavioural response, if confirmed with larger sample sizes, would have direct consequences for aphid dispersal dynamics: experienced aphids seeking undamaged tissue would be expected to move away from mechanically damaged plants toward healthier tissue elsewhere in the crop stand.

The tendency of experienced chilli *M. persicae* toward aphid-infested over mechanically damaged volatiles (PI = +0.30) is consistent with the well-documented qualitative differences between HIPV blends induced by *M. persicae* infestation and wound-induced VOC blends on Solanaceous plants. Ali et al. (2022) demonstrated that *M. persicae*-infested *Capsicum annuum* emits a richer terpenoid and methyl salicylate profile than healthy or mechanically damaged plants, with compounds known to influence aphid foraging decisions. The significant Fisher's exact result for chilli in the AI vs. MD comparison (OR = 21.0, $p = 0.020$) indicates that experienced *M. persicae* were 21-fold more likely to commit to a choice favouring AI cues, consistent with the interpretation that prior rearing on chilli sensitises the aphid olfactory system to the specific HIPV signature of conspecific infestation on this host — a form of experience-dependent olfactory priming described by Bruce & Pickett (2011). That mechanically damaged plants were consistently less attractive to experienced aphids (PI = −0.50 on brinjal) further supports the hypothesis that experienced *M. persicae* discriminate between defence-activated (wound-induced GLVs) and herbivore-colonised (HIPV-enriched) plant signals, preferentially orienting toward the latter.

A notable feature of the present dataset is that the significant experience-by-volatile-type interaction (Fisher’s exact, OR = 21.0, p = 0.020) was observed for chilli but not for brinjal (brinjal AI vs. MD: OR = 9.33, p = 0.070). This host-specific asymmetry merits mechanistic consideration. Ali et al. (2022) demonstrated that *M. persicae* infestation of *Capsicum annuum* activates the ethylene biosynthesis pathway and produces a richer terpenoid-dominated HIPV blend compared with *Solanum melongena*, which exhibits a more GLV-dominated response profile (Higashida et al., 2022). If the olfactory template formed during rearing on chilli is more distinct from wound-induced signals than the template formed on brinjal, experienced chilli aphids would be expected to show a stronger differential response to AI versus MD volatiles — precisely the pattern observed. By contrast, brinjal-reared aphids showed a strong tendency to avoid mechanically damaged cues (PI = −0.50, MD vs. H), suggesting that the dominant learned association on brinjal is avoidance of wound-induced signals rather than active attraction to aphid-infested signals. These host-specific patterns are consistent with qualitative differences in the HIPV chemistry of the two Solanaceous species and warrant direct VOC characterisation in future work.

Statistical power and study limitations. The absence of statistically significant directional preferences in binomial tests is attributable primarily to the small number of choosing aphids per comparison ($n = 3–9$ per comparison). A two-sided binomial test requires $n \geq 17$ choosing individuals to detect a 70:30 split with ≥ 80% power at $\alpha = 0.05$. Post-hoc power analysis (Table 6) confirmed that achieved power was low in all 12 binomial comparisons, ranging from 0.08 to 0.32 for experienced cohorts and from 0.05 to 0.11 for non-experienced cohorts, given the observed choice fractions and N* values. The most powerful comparison was experienced chilli AI vs. MD (N* = 9, observed proportion = 0.667, achieved power = 0.32), which was also the one with the significant Fisher's exact interaction (OR = 21.0, p = 0.020) — indicating that the group-level difference in engagement was detectable even when the within-group directional signal remained underpowered. These power estimates confirm that the absence of significant directional preference should be interpreted as inconclusive rather than as evidence for the null hypothesis. Future studies should target $n \geq 20$ choosing aphids per comparison, consistent with best practice in comparable *M. persicae* olfactometer studies (Ahmed et al., 2019; Ali et al., 2022). Integration of GC-MS volatile profiling (Ali et al., 2022) and electroantennography would allow identification of the specific HIPV compounds responsible for the observed preference tendencies, and comparing wingless versus alate *M. persicae* cohorts would clarify whether the experience effect is morph-dependent.

Implications for integrated pest management.

The finding that experienced (crop-conditioned) *M. persicae* are ~2.5-fold more olfactorily responsive than non-experienced aphids, and that experienced chilli aphids showed the strongest selective engagement with aphid-infested VOCs, has direct implications for semiochemical-based pest management (Cook et al., 2007). Lures

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formulated using the HIPV profile of *M. persicae*-infested *C. annuum* could be deployed as monitoring attractants specifically targeting crop-conditioned aphid populations. Conversely, the tendency of experienced *M. persicae* to avoid mechanically damaged plant volatiles supports the use of wound-induced VOC dispensers or companion plant wounding as 'push' components in push-pull intercropping systems.

Conclusion

Prior rearing experience on *Solanum melongena* or *Capsicum annuum* exerts a large, statistically robust effect on the olfactory responsiveness of *Myzus persicae* in tests (Mann–Whitney U, $p < 0.01$; $r > 0.83$), reducing no-choice rates from 67–70% (non-experienced) to 17–20% (experienced). Although no binary comparison achieved statistically significant directional preference (all binomial $p > 0.05$), biologically plausible tendencies consistent with the known qualitative differences between wound-induced and aphid-induced HIPV blends were observed: experienced brinjal aphids showed a non-significant tendency to prefer healthy over mechanically damaged volatiles (PI = −0.50), and experienced chilli aphids showed a non-significant moderate tendency toward aphid-infested over mechanically damaged cues (PI = +0.30). The significant experience-by-volatile-type interaction for chilli (Fisher's exact, OR = 21.0, $p = 0.020$) is consistent with prior rearing sensitising *M. persicae* to the distinctive HIPV blend of conspecific-infested *C. annuum* (Ali et al., 2022; Higashida et al., 2022). Increasing the sample size to $n \geq 20$ choosing aphids per comparison and incorporating GC-MS volatile characterisation of both host species at all three treatment levels would substantially advance mechanistic understanding of these findings.

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