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Research Article

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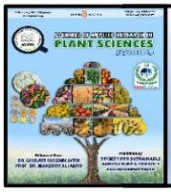

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Evaluation of Cucurbit Hosts and Low-Cost Protein Sources as Candidate Bait Components for Melon Fly, *Zeugodacus cucurbitae (Diptera: Tephritidae)*

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ABSTRACT

The melon fly *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae) is a major pest of cucurbit crops, with reported losses of 30-100% depending on cucurbit species and season. Bait-based monitoring and control tools need knowledge of the cues that guide host and food selection, but the responses of males and females to local cucurbit hosts and protein sources have not been compared under standardised conditions. The aim of this study was therefore to compare the attraction of adult *Z. cucurbitae* to five cucurbit vegetables (ash gourd, ridge gourd, cucumber, bitter gourd and muskmelon) and four protein sources (a commercial protein, fish protein hydrolysate, defatted soybean hydrolysate and a vegetable protein) under laboratory cage no-choice and choice assays (30 cages per treatment) and to measure oviposition punctures on the vegetables (10 cages per treatment). In the no-choice test, cucumber received the most visits by both sexes, followed by ash gourd and ridge gourd, while bitter gourd and muskmelon received the fewest visits ($P < 0.001$). In the choice tests between ash gourd and cucumber, ash gourd was preferred over cucumber, while in the three-way choice tests, cucumber was the most visited. Cucumber had the highest number of oviposition punctures whereas ash gourd was preferred over cucumber in the pairwise visitation test, indicating that host rank depends on the assay. In the no-choice tests, the commercial protein was visited the most by both sexes, followed by fish protein hydrolysate and defatted soybean hydrolysate, while the vegetable protein bait had the fewest visits; in the choice tests, the commercial bait was again preferred. The results identify candidate host and protein components for further field evaluation in protein bait sprays and attract-and-kill traps.

KEYWORDS: Ash gourd, cucumber, oviposition punctures, fish protein hydrolysate, bait spray, attract-and-kill, smallholder vegetable production

INTRODUCTION

Cucurbit crops are economically important vegetables in South Asia and play an important role in human nutrition and farm income. In Pakistan, cucumber, bitter gourd and *Luffa* (ridge and sponge gourd) were reported on 3,200, 5,600 and 5,800 ha, respectively, with production of 124,000, 113,000 and 97,000 t, respectively, in 2023–24, while melons were reported on 53,000 ha with production of 1.62 million t (MNFSR, 2025). The melon fruit fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae), is one of the most serious pests of these crops. It is widely distributed in tropical and subtropical areas, and attacks over 80 plant species, especially cucurbit species in Pakistan (EPPO, 2026). The larvae feed within the fruit and losses of 30–100% have been reported, varying widely with the cucurbit species, location and season (Dhillon *et al.,* 2005).

This species was originally described as *Dacus cucurbitae* Coquillett, and until recently it was believed to be the same as *Bactrocera cucurbitae* (Coquillett) in the subgenus *Zeugodacus* of *Bactrocera*. However, molecular phylogenetic analyses of the tribe Dacini revealed that the *Zeugodacus* group was not monophyletic, as then defined, since it was found to form a lineage separate from the other *Bactrocera* species (Virgilio *et al.,* 2015). On this basis, *Zeugodacus* was elevated to genus and the melon fly is now known as *Z. cucurbitae* (Coquillett) (Doorenweerd *et al.,* 2018). The two names are synonyms. Most of the older literature, including some references cited here, uses *B. cucurbitae*, but *Z. cucurbitae* is used throughout this paper.

Insecticide cover sprays are still the mainstay of managing *Z. cucurbitae*, and repeated application of these products is a cause for concern about resistance, residues, and impacts on non-target organisms (Rashmi *et al.,* 2025). Measures to control adults (field sanitation, protein baits and cue-lure traps) are important in area-wide management (Dhillon *et al.,* 2005), because the larvae are protected within the fruit. A protein bait spray has proven to be an important method of controlling this species in Hawaii since the introduction of a bait containing spinosad (GF-120) in 2001 (Shikano *et al.,* 2022). Heavy use of a single active ingredient resulted in resistance: spinosad resistance increased 34-fold over nine years on a single Oahu farm (Hsu *et al.,* 2021). A rotation of GF-120 with baits containing malathion, abamectin or zeta-cypermethrin significantly reduced melon fly

populations and increased zucchini yield on a commercial farm in Maui (Shikano *et al.,* 2022). These tools are most effective when their design is guided by quantitative knowledge of the cues that lead adults to hosts, oviposition sites and food. Of special practical interest are two sets of cues: host-fruit cues, which identify the crops visited and those used for oviposition, and protein-food cues, which can be used to formulate baits for the adults of both sexes.

Host-fruit odours are critical to host location. Cucumber odour is highly attractive to female *Z. cucurbitae* (Miller *et al.,* 2004; Piñero *et al.,* 2006), a blend of cucumber volatiles has been developed into a female-biased lure (Siderhurst and Jang, 2010), and a synthetic blend of cucumber and tomato volatiles attracted both sexes (Njuguna *et al.,* 2018). Cucumber has been found to be the most visited and most oviposited host in choice and no-choice experiments (Farooq *et al.,* 2020; Pradhan *et al.,* 2023); however, the rankings are not consistent across the studies. Bitter gourd was the most preferred host in a machine learning analysis (Nazir *et al.,* 2024), watermelon was the most infested among three vegetables under natural and semi-natural conditions in Tanzania (Mwatawala *et al.,* 2015), and gravid females preferred ridge gourd volatiles over the volatiles of cucumber, bitter gourd and snake gourd in olfactometer assays (Shivaramu *et al.,* 2022). These rankings are based on several assays (visitation, olfactometer choice, oviposition and field infestation) and a host that ranks first in one assay may not rank first in another. Therefore, host preference should be determined by both attraction and oviposition, and oviposition preference among hosts can be determined by counting the number of punctures on fruit (Su *et al.,* 2025). However, the relative preference of *Z. cucurbitae* among the five cucurbit species most popular in Pakistan (ash gourd, ridge gourd, cucumber, bitter gourd and muskmelon) has not been well studied under standardised no-choice, choice and oviposition conditions with the sexes analysed separately.

Protein cues form the second group. Tephritid flies require protein for sexual and egg development, and females in particular forage actively for proteinaceous food (Biasazin *et al.*, 2018). The volatiles that signal protein sources are largely by-products of microbial activity and protein breakdown, and include nitrogen-containing compounds that are detected by the antennae (Biasazin *et al.*, 2018); ammonia and its derivatives serve as cues for locating protein-rich food (Piñero *et al.*, 2017). Bait type affects the feeding response of the melon fly (Barry *et al.*, 2006), and in bitter gourd fields a commercial protein hydrolysate captured more flies than soybean, yeast or casein baits (Sruthi *et al.*, 2023). In small-scale production systems, the high price of commercial fruit fly products limits control options, which has stimulated interest in low-cost, locally available materials (Figueroa Candia *et al.*, 2019). For example, beer waste enriched with ammonium acetate performed as well as the standard bait GF-120 in tests with the melon fly and the oriental fruit fly (Piñero *et al.*, 2017). Male lures such as cue-lure are the main monitoring tool for males of this species, but they do not attract females, which are the sex that damages fruit by oviposition (Dhillon *et al.*, 2005); thus, female-targeted protein baits are still needed. Protein source refers to protein-based materials provided as attractants to flies, and 'protein bait' refers to a protein source that contains an insecticide to be used in sprays or traps. No toxicant-containing baits were tested in the present experiments, only protein sources as attractants.

Host and protein cues are not independent of each other. The response of female melon flies to host-fruit odour and to protein odour depends on their physiological state: protein-fed females in cages preferred cucumber odour to protein odour (Miller *et al.*, 2004), and in the field, egg-free females responded similarly to both odours, whereas more wild females responded to cucumber odour than to protein odour overall (Vargas *et al.*, 2018). In Pakistan, Iqbal *et al.* (2020) compared protein and ammonia sources, including a cucumber-based mixture, for the attraction of *Z. cucurbitae* under laboratory and field conditions. Combining host and protein cues in one lure therefore requires information on both groups of cues, obtained in the same system and for both sexes.

However, it is unclear if protein sources that are locally available in Pakistan, such as fish protein hydrolysate and defatted soybean hydrolysate, are as attractive to *Z. cucurbitae* as a commercial protein. Thus, the objectives of the current study were to determine (1) the attraction and oviposition preference of *Z. cucurbitae* among ash gourd, ridge gourd, cucumber, bitter gourd and muskmelon, and (2) the attraction of male and female *Z. cucurbitae* to a commercial protein, fish protein hydrolysate, defatted soybean hydrolysate and a vegetable protein, under no-choice and choice conditions. The five vegetables were chosen because they are widely grown, marketed and consumed in Pakistan (MNFSR, 2025), are recorded host plants of *Z. cucurbitae* (Dhillon *et al.,* 2005), and were ranked differently in previous host preference studies (Farooq *et al.,* 2020; Shivaramu *et al.,* 2022; Nazir *et al.,* 2024); hence, a standardised comparison among them is informative. The protein sources were chosen to compare the commercial protein with inexpensive materials that could be produced locally. Because cucumber is already known to attract *Z. cucurbitae*, the contribution of this study is not this finding itself but the standardised comparison of several locally important hosts and protein sources under the same laboratory conditions, with male and female responses considered separately. We hypothesised that cucumber would be the most attractive host and that the commercial protein would be the most attractive protein source. This information can be used to select host and protein components for protein bait sprays and attract-and-kill stations in cucurbit fields.

MATERIALS AND METHODS

Study site and insect culture: The experiments were carried out at the Insect Rearing Laboratory, Department of Entomology, MNS University of Agriculture, Multan, Pakistan, from April 2026 to August 2026. *Zeugodacus cucurbitae* was cultured in the laboratory from pupae obtained from naturally infested cucurbit fruits collected from farmers' fields and the vegetable market in Multan. The infested vegetables were kept under ambient rearing conditions (26–30 °C and 60–70% RH) for pupation. Pupae were extracted from the soil and kept in Petri dishes in ventilated plastic cages (45 × 45 × 45 cm, length × width × height), and emerged adults (6–7 days after pupation) were given a standard artificial diet consisting of honey, Cerelac, yeast and water (1:1:1:2) and water through a moistened cotton wick. The colony was established from field-collected pupae (F0). The F1 generation was reared to remove field and maternal effects. All bioassays were performed exclusively on F2 generation flies (18–22 days old). Later generations were used only to maintain the colony and were not used in bioassays. During the bioassays, the temperature and relative humidity were 26–28 °C and 60–70% RH, respectively. Each cage contained equal numbers of males and females. Fresh flies were used in every replicate, and observations began at 09:00 h. All bioassays used a completely randomised design (CRD).

Experiment 1: Attractiveness of cucurbit vegetables:

No-choice test: The five vegetables commonly cultivated in the region, namely ash gourd, ridge gourd, cucumber, bitter gourd and muskmelon, were bought fresh from the local market, rinsed with reverse osmosis (RO) water and air-dried, then grated into fine pulp using a manual grater. Pulp was prepared immediately before each replicate and fresh pulp was used for each replicate and cage. Each vegetable pulp (10 g) was individually placed in a Petri dish (10 cm diameter) in separate cages (45 × 45 × 45 cm, length × width × height). Ten pairs (1 male : 1 female) of 18–22-day-old mated *Z. cucurbitae* were starved for 12 h and introduced into each cage. Fresh flies were used in every replicate and were not reused. The number of visits was recorded by instantaneous count, i.e. the number of flies present on each pulp at that instant was recorded every hour for 8 h. For analysis, cumulative 8-h visits per cage, i.e., the sum of eight hourly instantaneous counts, was used. Thirty replicate cages were used for each vegetable. Similar no-choice and choice cage tests have been used in host preference studies of this species (Farooq *et al.,* 2020; Pradhan et *al.,* 2023).

Choice test: The three vegetables that were found to be more attractive in the no-choice test, namely cucumber, ash gourd and ridge gourd, were then used in the choice test. These three hosts were tested in all pairwise combinations (cucumber × ridge gourd, cucumber × ash gourd and ridge gourd × ash gourd) and in a three-way combination (cucumber × ridge gourd × ash gourd). The adults (18–22 days old) were starved for 12 h and released into each cage, and the visits to each vegetable pulp were noted every hour for 8 h. Thirty replicate cages were used for each combination. Dish positions were randomised between replicates.

Oviposition-puncture bioassay: A modified protocol (Jaleel *et al.,* 2019) was used for the assessment of oviposition preference. Prior to being added to cages with 20 pairs of adult *Z. cucurbitae*, vegetables were washed, dried and weighed. Each vegetable was cut into slices and the surface area that was exposed was measured. A single slice was added to each cage. The surface area of the fruit was estimated by multiplying the length × diameter (cm^2) of the exposed surface of the fruit slice, and this value was used as a proxy for the exposed surface area to normalise puncture counts. The same approximation was applied to all vegetables. To keep the flies active and reduce nutritional stress during the oviposition period, a 10% sugar solution was provided on separate cotton wicks. Each test vegetable was exposed to the flies for 24 h. The vegetables were then taken out and the oviposition punctures on each were counted under a microscope. The number of punctures was normalised per 100 cm^2 of fruit surface area (Total number of punctures/Fruit surface area in cm^2 × 100). The data obtained were normalised and then statistically analysed. This bioassay was conducted 10 times with each vegetable. Ten repeats were carried out instead of the 30 repeats carried out for the visitation experiments because the assessment of oviposition was more time-consuming and required fresh slices and longer handling time per replicate.

Experiment 2: Attraction to protein sources:

No-choice test: The four protein sources used were a commercially available protein bait (CA; BioGuard, Evyol Group), fish protein hydrolysate (FPH), defatted soybean hydrolysate (DS) and a vegetable protein (VP). The same base recipe was used in the laboratory to prepare FPH, DS and VP, with only the raw material changed. The quantities of fish, defatted soybean and mixed vegetables used for FPH, DS and VP, respectively, were 300 g each. The raw material, 20 g of yeast, 500 mL of water, 200 mL of molasses, 180 g of citric acid and 12 g of potassium sorbate were added to each preparation, and each preparation was brought to a volume of 1 L. The mixtures were fermented for 7 days at room temperature. Twenty millilitres of each protein source was placed on cotton in a separate Petri dish (10 cm diameter), which was then placed in a 45 × 45 × 45 cm cage (length × width × height). Cotton equal in quantity and sufficient to completely absorb the 20 mL solution was used. The cotton did not dry during the 8-h assay. Ten pairs (1 male : 1 female) of adult *Z. cucurbitae* (18–22 days old) were starved for 12 h and placed in each cage and the number of times *Z. cucurbitae* visited the protein source was counted

hourly for 8 h. Thirty replicate cages were used for each protein source.

Choice test: The three protein sources that were most attractive in the no-choice test (CA, FPH and DS) were then tested in all two-way combinations (CA × FPH, CA × DS and DS × FPH) and in a three-way combination (CA × FPH × DS), with the same caging, starvation, fly age and observation time of 8 h as in the no-choice test. Vegetable protein was excluded from the choice tests because it was the least attractive source in the no-choice test, and DS was retained as the third source because it ranked third in that test. Thirty replicate cages were used for each combination (Jaleel *et al.,* 2019).

Statistical analysis: One-way ANOVA was performed on data from the no-choice tests, the three-way choice tests and the oviposition-puncture bioassay, and the means were separated by the least significant difference procedure. The experimental unit was the cage; cumulative 8-h visits per cage were analysed (hourly counts were not treated as independent observations), and males and females were analysed separately. A paired t-test was used to analyse pairwise (two-way) choice tests. The data were analysed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA) at $\alpha = 0.05$. In this paper, 'visitation' means the number of flies counted on a test material, 'attraction' the number of times flies landed on a material in the cage assays, and 'preference' means the relative ranking of the materials in the cage assays based on the number of times flies landed on them; 'oviposition' is assessed separately in the cage assays by counting the number of punctures.

RESULTS

Experiment 1: Vegetable host attraction:

No-choice test: In the no-choice test, the numbers of visits by females differed significantly among the five vegetables ($F = 75.78$; $df = 4, 145$; $P < 0.001$, Mean ± SE = 46.80 ± 0.47): cucumber received the most visits, ash gourd and ridge gourd were intermediate and did not differ from each other, and visits then fell further on bitter gourd and were lowest on muskmelon. In the no-choice test, the numbers of visits by males also differed significantly among the vegetables ($F = 33.89$; $df = 4, 145$; $P < 0.001$, Mean ± SE = 26.02 ± 0.48): males followed the same order, with cucumber highest, ash gourd and ridge gourd intermediate, then bitter gourd, and muskmelon lowest. Muskmelon received the fewest visits from both sexes (Figure 1).

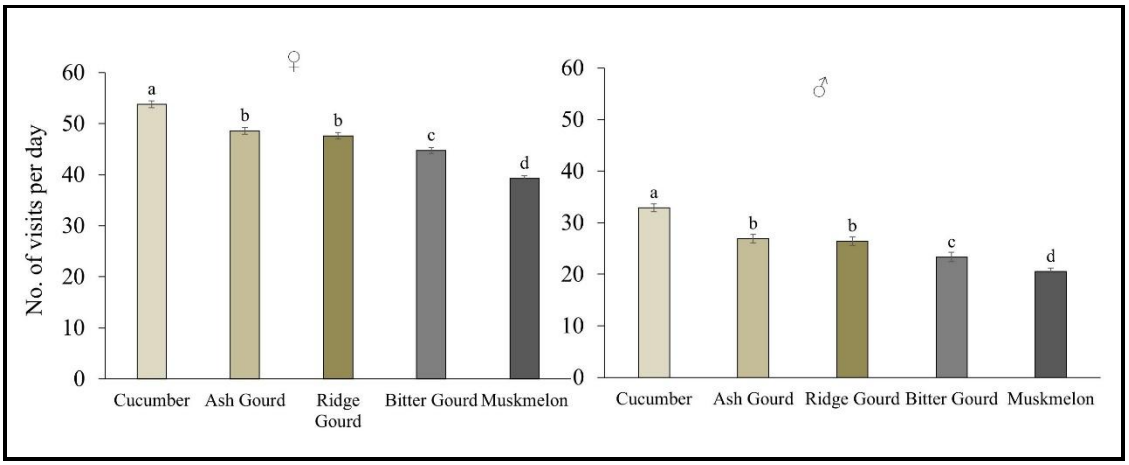

Figure 1. Mean (± SE) number of visits per day by female (a) and male (b) *Z. cucurbitae* to different vegetable hosts in the no-choice test. Bars sharing the same letter are not significantly different (Tukey HSD, $P < 0.05$). Error bars indicate SE.

Choice test: In the pairwise choice tests, ash gourd received significantly more visits than cucumber (females: $t = –39.38$, $df = 29$, $P < 0.001$, Mean ± SE = 42.57 ± 0.99; males: $t = –19.33$, $df = 29$, $P < 0.001$, Mean ± SE = 43.72 ± 2.24) and than ridge gourd (females: $t = –92.52$, $df = 29$, $P < 0.001$, Mean ± SE = 48.80 ± 0.49; males: $t = –62.19$, $df = 29$, $P < 0.001$, Mean ± SE = 30.18 ± 0.43). Also, in the pairwise choice tests, cucumber received significantly more visits than ridge gourd (males: $t = –34.10$, $df = 29$, $P < 0.001$, Mean ± SE = 50.10 ± 0.69; females: $t = –63.93$, $df = 29$, $P < 0.001$, Mean ± SE = 30.58 ± 0.77). In the three-way choice test, the ranking changed: cucumber received the most visits, followed by ash gourd and ridge gourd, for both females ($F = 37.85$; $df = 2, 87$; $P < 0.001$, Mean ± SE = 50.55 ± 0.49) and males ($F = 25.78$; $df = 2, 87$; $P < 0.001$, Mean ± SE = 29.87 ± 0.50) (Figures 2–3).

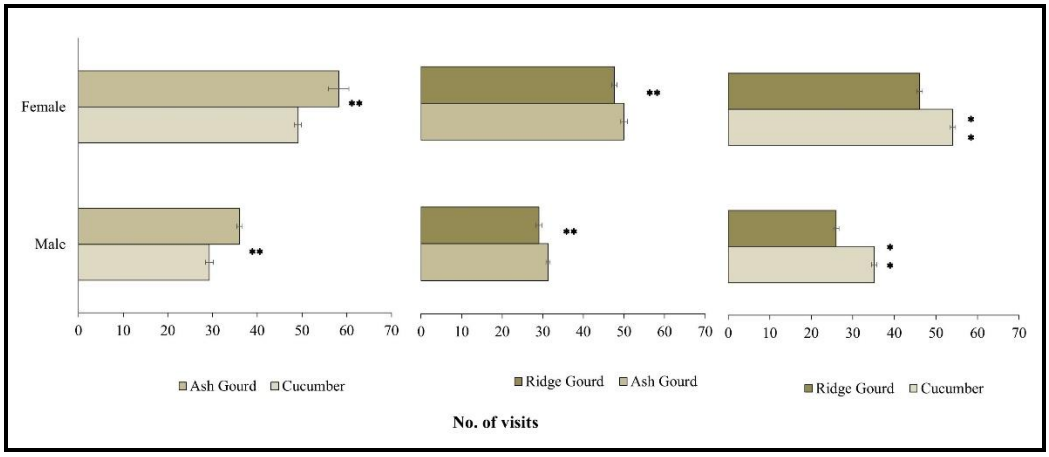

Figure 2. Mean (± SE) number of visits per day by female and male *Z. cucurbitae* in pairwise choice tests: (a) Ash gourd vs. cucumber, (b) ash gourd vs. ridge gourd, and (c) cucumber vs. ridge gourd. ** indicates a significant difference between the pair (paired t-test, $P < 0.001$). Error bars indicate SE.

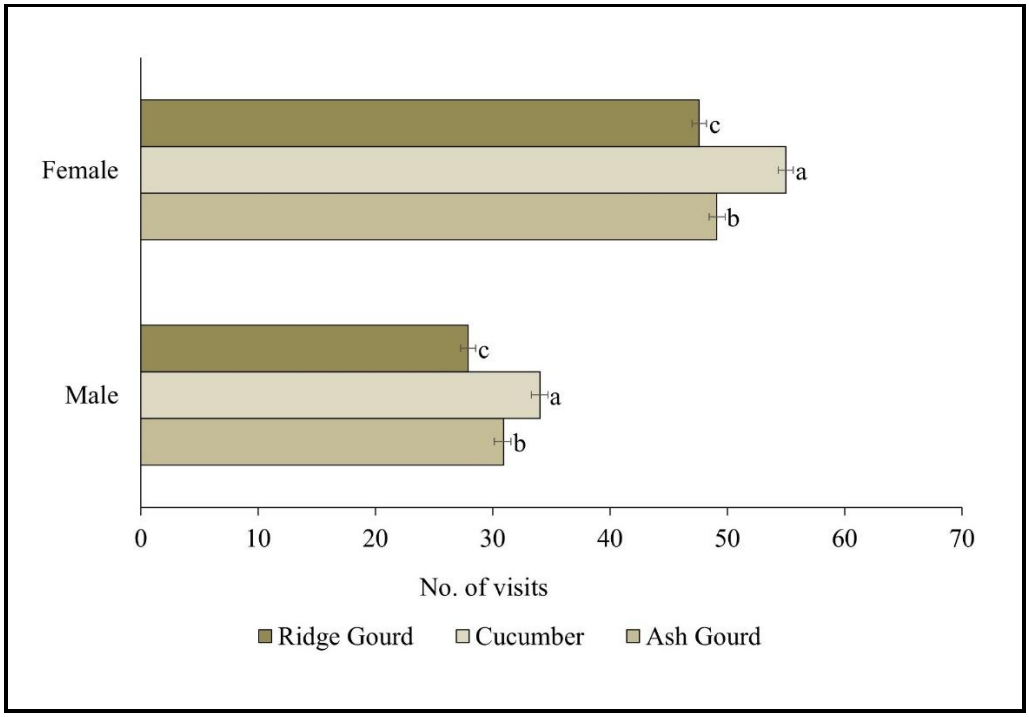

Figure 3. Mean (± SE) number of visits per day by female and male *Z. cucurbitae* in the three-way choice test among cucumber, ash gourd and ridge gourd. Bars sharing the same letter are not significantly different (Tukey HSD, $P < 0.05$). Error bars indicate SE.

Oviposition-puncture bioassay: Oviposition punctures per 100 cm² differed significantly among vegetables ($F = 54.75$; df = 4, 45; $P < 0.001$). The highest normalised puncture counts were recorded on cucumber (17.62 ± 1.49 per 100 cm²), followed by ash gourd (13.76 ± 1.30), bitter gourd (11.83 ± 1.30) and ridge gourd (11.15 ± 1.47), while the lowest were on muskmelon (8.00 ± 0.39). Cucumber received 2.20 times as many punctures as muskmelon. Bitter gourd and ridge gourd did not differ significantly from each other (Figure 4).

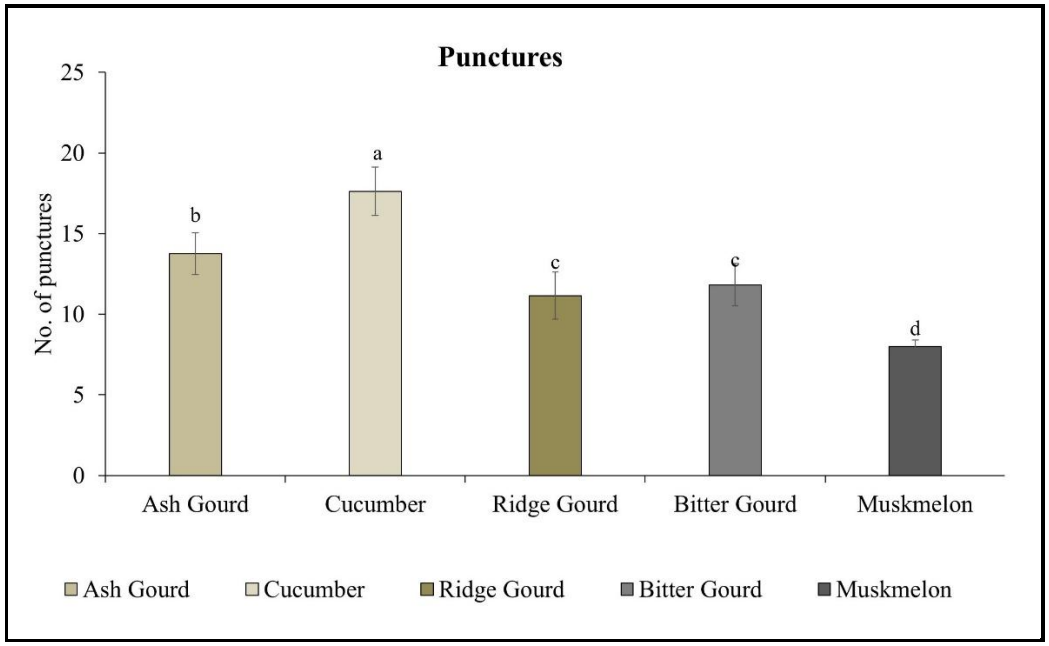

Figure 4. Mean (± SE) number of oviposition punctures per 100 cm^2 of fruit surface made by female *Z. cucurbitae* on different vegetable hosts over 24 h. Bars with the same letter are not significantly different (F = 54.75, df = 4, 45, P < 0.001). Error bars indicate SE.

Experiment 2: Attraction to protein sources: No-choice test: In the no-choice test, the numbers of visits differed significantly among the four protein sources. For males, the numbers of visits differed significantly among the sources (F = 167.86; df = 3, 116; P < 0.001, Mean ± SE = 13.11 ± 0.51), with the most visits to the commercial protein (CA) and the fewest to the vegetable protein (VP), with FPH and DS in between. For females, the pattern was the same (F = 209.17; df = 3, 116; P < 0.001, Mean ± SE = 16.71 ± 0.61): CA received the most visits, followed by FPH and DS, and VP the fewest. All four sources differed from each other in both sexes (Figure 5).

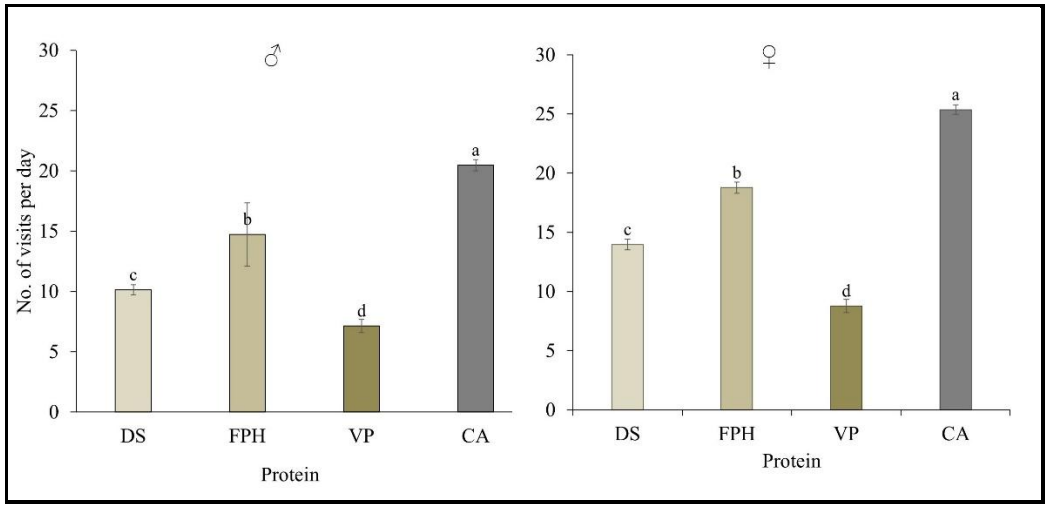

Figure 5. Mean (± SE) number of visits per cage by male (a) and female (b) *Z. cucurbitae* to different protein sources in the no-choice test over 8 h. Bars sharing the same letter are not significantly different (F = 209.17, df = 3, 116, P < 0.001, LSD test). DS, defatted soybean; FPH, fish protein hydrolysate; VP, vegetable protein; CA, commercially available protein. Error bars indicate SE.

Choice test: In the pairwise choice tests, CA received significantly more visits than FPH (females: t = –28.96, df = 59, P < 0.001, Mean ± SE = 21.91 ± 0.64; males: t = –29.51, df = 59, P < 0.001, Mean ± SE = 18.26 ± 0.51) and than DS (females: t = –17.50, df = 59, P < 0.001, Mean ± SE = 19.68 ± 1.10; males: t = –19.68, df = 59, P < 0.001, Mean ± SE = 15.98 ± 0.76). Also, DS received significantly more visits than FPH (females: t = –25.41, df = 59, P < 0.001, Mean ± SE = 16.16 ± 0.51; males: t = –44.40, df = 59, P < 0.001, Mean ± SE = 15.85 ± 0.32). In the three-way choice test, CA received the most visits, followed by FPH and then DS (males: F = 82.89; df = 2, 87; P < 0.001, Mean ± SE = 16.17 ± 0.51; females: F = 228.96; df = 2, 87; P < 0.001, Mean ± SE = 19.42 ± 0.66) (Figures 6–7). Overall, CA received the highest number of visits in all no-choice and choice comparisons.

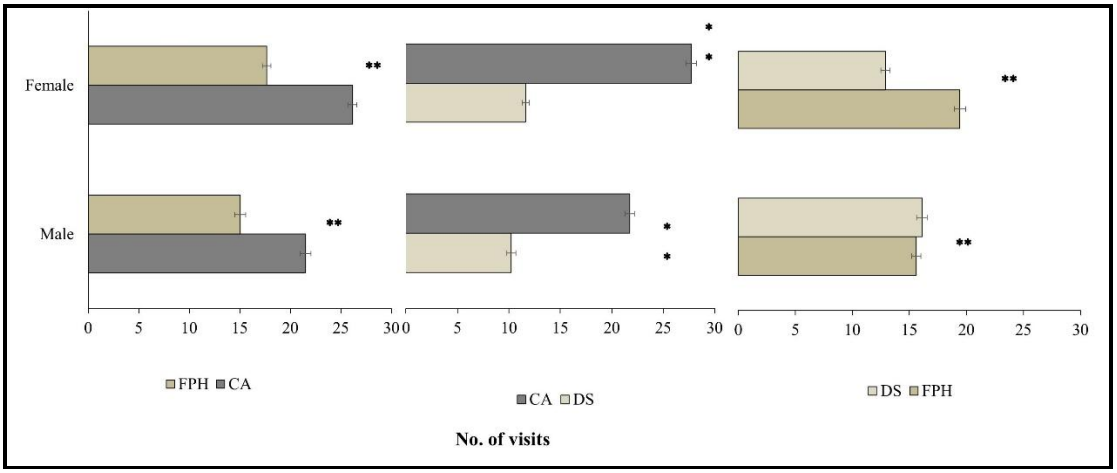

Figure 6. Mean (± SE) number of visits per cage by male and female *Z. cucurbitae* to CA and FPH (a), CA and DS (b) and DS and FPH (c) in paired choice tests. ** indicates a significant difference at $P < 0.01$ (paired t-test). Error bars indicate SE.

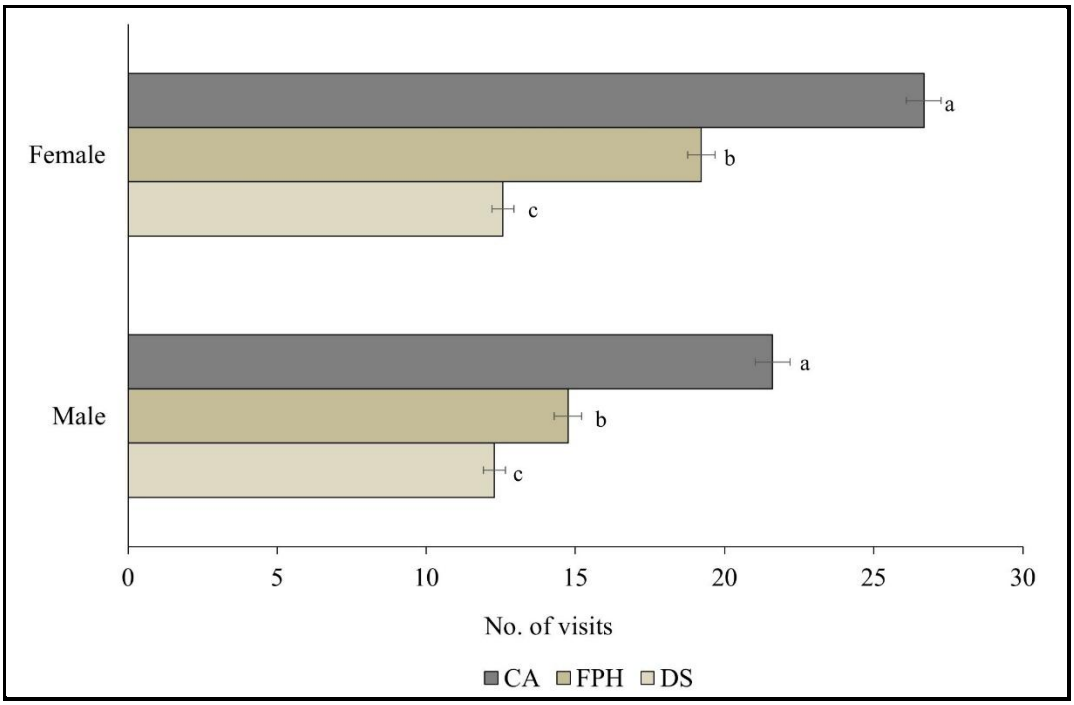

Figure 7. Mean (± SE) number of visits per cage by male and female *Z. cucurbitae* to CA, FPH and DS in the three-way choice test. Bars sharing the same letter are not significantly different (Tukey HSD, $P < 0.05$). Error bars indicate SE.

DISCUSSION

These two experiments provide preliminary laboratory evidence for identifying candidate host-fruit and protein components for future field evaluation in a bait-based approach for the control of *Z. cucurbitae*. In the no-choice test, cucumber was a highly preferred host of *Z. cucurbitae* under laboratory conditions, while ash gourd and ridge gourd were intermediate, and bitter gourd and muskmelon were visited least (Figure 1). In pairwise choice tests, ash gourd received more visits than cucumber and ridge gourd, and cucumber received more visits than ridge gourd (Figure 2), whereas in the three-way choice test, cucumber again received more visits, followed by ash gourd and ridge gourd (Figure 3). Cucumber also received the most oviposition punctures, followed by ash gourd, bitter gourd, ridge gourd and muskmelon (Figure 4). Among the protein sources, the commercially available protein (CA) received the most visits in the no-choice test, followed by fish protein hydrolysate (FPH), defatted soybean hydrolysate (DS) and vegetable protein (VP), and in the choice tests, CA also received more visits than FPH and DS (Figures 6–7). Overall, cucumber was the most visited host, and CA received the highest number of visits among the protein sources across assays under laboratory conditions.

The high ranking of cucumber agrees with earlier laboratory studies. In no-choice and free-choice tests with hosts from Pakistan, cucumber had the highest number of visits and oviposition punctures (Farooq *et*

al., 2020), and Pradhan *et al.* (2023) observed that cucumber was the most attractive host and flies oviposited most on it in both choice and no-choice tests, and development was also best on cucumber in that study. The preference may be explained by the volatiles in cucumber, although this was not tested here. Cucumber odour is highly attractive to female melon flies (Miller *et al.*, 2004; Piñero *et al.*, 2006), in field trials a nine-compound blend identified from cucumber was attractive to female fruit flies (Siderhurst and Jang, 2010), and a synthetic blend of cucumber and tomato volatiles attracted both sexes (Njuguna *et al.*, 2018). The same host ranking was obtained for males and females, which suggests that the cues that lead to visits are the same for both sexes. In this study, however, the sexes were analysed separately and sex × host interactions were not examined.

The relative position of cucumber and ash gourd changed with the assay. Cucumber received the most visits when each host was offered alone and when all three hosts were offered together, but ash gourd was preferred over cucumber when only these two hosts were offered. The reversals are unlikely to reflect differences in fly age, starvation period or observation schedule, which were identical in all visitation assays, although position effects cannot be fully excluded. A possible explanation is the influence of the other hosts on offer, for example overlapping odour plumes from two or three pulps inside the cage. This is a hypothesis only, because odour movement was not measured. Piñero *et al.* (2006) showed that female melon flies rely on host-associated visual and olfactory stimuli together to respond strongly, which fits the idea that visitation depends on the combination of cues available. Thus, cucumber and ash gourd were the preferred pair, but these data cannot show which of the two would rank first in the field.

Ridge gourd received the fewest visits of the three hosts in all choice tests. This contrasts with Shivaramu *et al.* (2022), who showed in olfactometer assays that gravid females preferred ridge gourd volatiles over those of cucumber, bitter gourd and snake gourd. The difference could be due to the physiological condition of the flies (ours were 18–22 days old and starved for 12 h), the odour source (grated pulp versus headspace volatiles of intact fruit) and the response measured (visits to pulp versus choice in an olfactometer). Physiological state is an important factor since melon fly females' response to host and food odours varies with their protein status (Miller *et al.*, 2004). Host rankings also vary among other studies: bitter gourd ranked first in the analysis of Nazir *et al.* (2024), while bitter gourd was the second-least-visited host here, and preference varied among hosts and conditions compared by Mwatawala *et al.* (2015). Cultivars, fruit maturity and region may also differ among studies, so results should not be directly extrapolated to other assays or regions. Cultivar, fruit maturity and volatile chemistry were not measured in this study.

A partial congruence was also observed between visitation and oviposition. Muskmelon was the least visited host and also received the fewest oviposition punctures, whereas bitter gourd was the second-least-visited host (Figure 1) but ranked third for oviposition punctures (Figure 4). This indicates that there may be some variation in whether a host is visited and whether it is accepted for oviposition, and that visitation data should not be used to rank hosts based on oviposition.

The ranking of protein sources was the same for both males and females, which is consistent with the need for protein for sexual development in tephritid flies (Biasazin *et al.,* 2018). In all comparisons, the commercial protein (CA) received the highest number of visits, and VP received the fewest visits in the no-choice tests (VP was not included in the choice tests, which included only the three most attractive sources). The superiority of CA is consistent with field results showing that traps baited with a commercial protein hydrolysate caught more flies than traps baited with soybean, yeast or casein (Sruthi *et al.,* 2023). Differences in attractiveness among protein baits may be related to the volatiles they emit, including ammonia and ammonia derivatives detected by tephritid antennae and used as cues to find protein-rich food; the contribution of ammonia release to the attractiveness of protein baits has also been investigated in other tephritids (Lasa and Williams, 2021). We did not measure the volatile composition, pH or protein content of the four materials, so this explanation remains a hypothesis. The rank of FPH was not consistent: it ranked second to CA in the no-choice tests and in the three-way choice test, but DS was preferred to FPH in the pairwise choice test. A hungry fly may respond to almost any protein odour when only one is offered, whereas in a choice situation small differences in odour quality or strength decide the outcome; this needs testing with standardised concentrations and degrees of hydrolysis. Since the order of FPH and DS is not settled, both are best treated as candidate low-cost alternatives to a commercial bait, without ranking them against each other.

Taken together, the results suggest that cucumber and ash gourd are candidate host-fruit components and that CA is the protein component that received the most visits under laboratory conditions, with FPH and DS as lower-cost materials that need further evaluation. Because host cues and protein cues act at different stages (host location and oviposition versus adult feeding and reproductive maturation), combining them is a logical next step. Earlier work supports this idea but also shows that the outcome depends on the condition of the flies. A cucumber volatile blend was more attractive to females than a protein bait in field trials (Siderhurst and Jang, 2010), and a cucumber-based mixture containing protein and ammonia sources was strongly attractive in olfactometer and field studies (Iqbal *et al.*, 2020). In contrast, protein-fed females in cages preferred cucumber odour to protein odour (Miller *et al.*, 2004), and in the field more wild females

responded to cucumber odour than to protein odour, except egg-free females, which responded similarly to both (Vargas *et al.*, 2018). These authors therefore discussed adding cucurbit volatiles to protein baits. Whether ash gourd odour has a similar effect is unknown, and our data do not show whether ash gourd volatiles would work in a combined lure.

There are benefits to insecticidal control. Conventional cover sprays are fast-acting, readily available and popular among farmers, and they are effective in reducing the number of adults in a short period of time. Therefore, the conventional cover spray is the major management strategy for *Z. cucurbitae* (Dhillon *et al.,* 2005; Rashmi *et al.,* 2025). However, their limitations have also been extensively documented. Because larvae feed inside the fruit, sprays control only the adults and the eggs on the fruit surface and must be applied repeatedly, and repeated use is associated with insecticide resistance, residues and impacts on non-target organisms (Rashmi *et al.,* 2025). Resistance is a real risk. In Hawaii, spinosad resistance increased 34-fold over nine years of GF-120 use on one farm (Hsu *et al.,* 2021), which led to changing the active ingredients in bait sprays (Shikano *et al.,* 2022). Protein baits help overcome some of these problems because a small quantity of insecticide is placed on roosting or border plants and is directed at hungry adults (females) instead of being sprayed on the crop (Shikano *et al.,* 2022). However, they have their own drawbacks: they rely on the attractiveness of the protein source, which was measured in the present study, and they still contain an insecticide to which resistance may build up.

Several limitations should be considered. First, grated market vegetables were used instead of intact fruit on the plant, and their volatile and moisture profiles may differ from those in the field. Whether the same host ranking holds on intact fruit in the field is therefore unknown. Second, observations were limited to fixed windows (8 h for visitation and 24 h for oviposition), and the physiological state of the flies (mating status, egg load and protein hunger), which is known to alter responses to host and food odours (Miller *et al.*, 2004; Vargas *et al.*, 2018), was not varied systematically. How mating status, egg load and protein hunger would change these rankings remains to be tested. Third, volatiles were not characterised, so the compounds that explain the rankings remain unidentified; coupled gas chromatography–electroantennographic detection, as used for food lures by Biasazin *et al.* (2018), would be a suitable approach. The compounds behind the observed preferences are therefore still unknown. Fourth, VP was not included in the choice tests. We also cannot say how VP would rank against CA, FPH and DS when flies can choose among them. Finally, the flies came from a laboratory culture established from field-collected pupae, and the rankings should be validated in the field under agro-ecological conditions in Punjab before they are used in an operational bait-based IPM programme. It is also not known whether laboratory-reared flies respond like wild ones.

The limitations of this laboratory study suggest several directions for future studies. (i) Field evaluation: Replicated bait-spray and attract-and-kill experiments should be conducted in cucurbit crops in the field to compare the commercial bait with FPH, DS and cucumber- or ash gourd-based baits at standardised concentrations in Multan and elsewhere in Punjab. (ii) Chemistry: The volatiles, pH and protein content of the host pulps and protein sources should be characterised, e.g., using GC–EAD, and correlated with behavioural responses. (iii) In addition to grated pulp, intact fruit and plants, other cultivars and other fruit maturity stages should be tested. (iv) Combined host and protein lures should be tested for effectiveness in combination with an insecticide, performance in the field under different weather conditions, non-target insect activity and cost of use compared with cover sprays.

In summary, cucumber and ash gourd were the most visited hosts (cucumber was highest in the no-choice and three-way tests, and ash gourd exceeded cucumber in the pairwise test), CA received the highest number of visits among the protein sources, and the order of the remaining hosts and of FPH relative to DS depended on the assay. These laboratory results suggest that cucumber and ash gourd pulp and CA, FPH or DS are candidate components, requiring field evaluation, for protein bait sprays and attract-and-kill stations for *Z. cucurbitae*.

Field trials in Multan are needed to see whether fish protein hydrolysate captures as many female *Z. cucurbitae* as the commercial protein. If field evaluation confirms these results, this information can be used to refine protein bait spray programmes and reduce cover sprays of broad-spectrum insecticides for cucurbit growers in Punjab.

CONCLUSION

Under laboratory conditions, cucumber received the most visits from both sexes in the no-choice and three-way choice tests and the most oviposition punctures. In the pairwise choice tests, ash gourd received more visits than cucumber, so host preference depended on assay type. The commercial protein received the most visits among the protein sources for both sexes in all tests, and vegetable protein received the fewest visits in the no-choice test. Fish protein hydrolysate and defatted soybean hydrolysate were intermediate, and their order differed between the pairwise choice test (DS > FPH) and the no-choice and three-way choice tests (FPH > DS). These laboratory results identify promising host and protein components for subsequent field evaluation in protein bait sprays and attract-and-kill stations for *Z. cucurbitae* in cucurbit production in Pakistan.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

HA: Conceptualization, methodology, investigation, data curation, and writing original draft. SS: Supervision, conceptualization, methodology, and writing review and editing. WJ: Supervision, validation, and critical revision of the manuscript. RA: Investigation and data analysis. FZAK: Data curation and formal analysis. FB: Visualization and resources. NI: Validation, project administration, and manuscript review.

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