

Comparative repellent activity of plant-derived preparations against four insect species representing distinct ecological groups*

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Received 10.09.2026

Accepted 03.10.2026

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Summary

This study evaluated 48 preparations, including essential oils, alcoholic macerates, hydrolates and formulated mixtures, against *Drosophila melanogaster*, *Plodia interpunctella*, *Calliphora vomitoria* and *Culex pipiens* using a two-choice laboratory assay. Repellency was quantified using a repellency index after 60 min of exposure, with five independent replicates per preparation and species. Significant differences among preparations were detected for all four species. The highest observed mean RI was recorded for X5 against *D. melanogaster*, Y1 against *P. interpunctella*, X5 and X6 against *C. vomitoria*, and O1 against *C. pipiens*. Cross-species assessment identified several preparations with relatively high and comparatively consistent RI values across multiple species; X6a had the highest descriptive mean RI across the four species (66.50%). The results demonstrate pronounced species-dependent variation in behavioral avoidance. The study provides preliminary laboratory evidence for further investigation, including potential veterinary applications against arthropod vectors, but does not establish protection against biting, pathogen transmission, infestation or field efficacy.

Keywords: plant-derived preparations, repellency, essential oils, *Culex pipiens*

Blood-feeding arthropods locate their hosts using combinations of chemical and physical cues. In mosquitoes, carbon dioxide, body odors and thermal stimuli contribute to host-seeking behavior, whereas in ticks host-associated odors, carbon dioxide and heat are detected by specialized sensory structures, including Haller's organ located on the forelegs (10, 13). *Culex pipiens* is a blood-feeding mosquito of vector-control and veterinary relevance (4), and its host-seeking behavior is mediated by chemical and physical cues (9,

16). Other insects use olfactory cues associated with resources characteristic of their ecological niches. Fruit flies, particularly *Drosophila* spp., are attracted to volatile compounds associated with fermenting fruits and other plant substrates. Fermentation-derived compounds, including acetic acid, may influence both attraction and oviposition behavior (10). In contrast, blow flies respond to volatile compounds released during animal decomposition, and changes in the volatile profile of carcasses can influence their orientation and landing behavior (13). These examples illustrate the strong relationship between insect sensory ecology and the localization of suitable food, breeding or reproductive resources.

Stored-product moths represent an important group in which chemical cues are also relevant to resource exploitation. The Indian meal moth, *Plodia interpunc-*

*The research was conducted as part of the project „Development of innovative products based on essential oils for use as animal feed additives and as repellents for non-food animals,” (PL – Opracowanie innowacyjnych produktów bazujących na olejkach eterycznych stosowanych jako dodatek do pasz dla zwierząt oraz jako repelent dla zwierząt niekonsumpcyjnych) co-financed by the European Union under the Regional Operational Programme for the Lubelskie Voivodeship for 2014-2020, Priority Axis 1: Research and Innovation, Measure 1.2: Targeted Research.

tella, is a widespread pest of stored and processed food commodities and can infest cereals and cereal products, flour, rice, nuts, dried fruits and other food materials (19). Larvae may leave infested commodities and move to protected sites where pupation occurs, which can contribute to the persistence and spatial distribution of infestations (19). The Mediterranean flour moth, *Ephestia kuehniella*, is another important pyralid pest associated with stored food products (20). Chemical communication is particularly important in the management of these insects, as pheromone-based traps are widely used for monitoring adult populations and semiochemical-based approaches, including mating disruption, have been investigated as complementary control methods (5, 14). Experimental application of mating disruption against *E. kuehniella* has been shown to reduce adult captures and larval infestation, demonstrating the potential of manipulating insect behavior as part of integrated pest management (1). These species are therefore relevant to hygiene and the protection of stored food and feed materials, although the present study evaluates behavioral avoidance in a laboratory arena rather than protection of commodities under practical conditions.

The economic importance of stored-product insects and limitations associated with conventional control methods have increased interest in alternative management strategies. One of the major challenges is the development of resistance to phosphine, an important fumigant used for stored-product protection (20). At the same time, concerns regarding pesticide residues, environmental effects and the sustainability of conventional pest-control practices have stimulated research on plant-derived substances as potential alternatives or complementary tools (16, 21).

Essential oils are among the most extensively investigated botanical preparations. They consist mainly of volatile secondary metabolites, including terpenes and phenolic compounds, and have demonstrated insecticidal, fumigant, repellent and behavioral effects against a wide range of arthropods (16, 21, 28). Their biological activity may involve several compounds acting simultaneously, and the overall effect of an essential oil cannot necessarily be attributed to its predominant constituent alone. Monoterpenes, oxygenated terpenoids and phenylpropanoids such as limonene, linalool, eugenol, thymol, carvacrol and terpinen-4-ol have been associated with biological activity against insects, including stored-product pests (17, 18). However, the composition and efficacy of essential oils may vary according to plant species, chemotype, developmental stage, environmental conditions and extraction method (18, 21).

The repellent activity of plant-derived preparations is particularly relevant because behavioral modifi-

cation may prevent insects from reaching suitable hosts or resources without necessarily causing direct mortality. Numerous plant-derived preparations and essential oils have demonstrated repellent activity against mosquitoes and other arthropods, although their effectiveness varies considerably among species and experimental conditions (9, 16). Such variation may reflect differences in sensory systems, ecological requirements and behavioral responses to individual volatile compounds. Consequently, activity demonstrated against one arthropod species cannot necessarily be extrapolated to another. This is particularly relevant for stored-product moths, for which behavioral responses to complex botanical volatile mixtures may differ from those observed in blood-feeding or other host-seeking arthropods.

Plant secondary metabolites have evolved as important components of plant interactions with herbivores and other organisms. Many of these compounds exhibit toxic, deterrent or repellent properties and may contribute to plant defense (18). Essential oils and other plant-derived preparations therefore represent potential sources of biologically active substances for pest management. Their practical application, however, depends on the chemical composition of the preparation, concentration, volatility, persistence and characteristics of the target organism (16, 17, 21). In addition, complex botanical preparations may contain multiple compounds whose interactions contribute to the observed biological effect (16, 21). Experimental evaluation under standardized conditions is therefore necessary to determine the activity of individual preparations against particular target species.

The differences in ecological requirements among pest species provide an opportunity to compare behavioral responses to the same plant-derived preparations. Fruit flies, blow flies, blood-feeding arthropods and stored-product moths rely on different combinations of chemical and physical cues to locate their respective resources (10, 13, 16, 19). Comparative assessment of these organisms may therefore provide information on the extent to which the activity of botanical preparations depends on the sensory and ecological characteristics of the target species.

Against this background, the present study evaluated the repellent activity of 48 plant-derived preparations, including essential oils, alcoholic macerates, hydrolates and formulated mixtures, against four insect species representing different ecological groups: *Drosophila melanogaster*, *Plodia interpunctella*, *Calliphora vomitoria* and *Culex pipiens*. The tested organisms were associated with blood feeding, fermentation, decomposition and stored food products. The study aimed to identify preparations showing high repellency for each target species and to assess the consistency of their activity across species.

Material and methods

The study evaluated the repellent activity of selected plant-derived preparations against adult insects representing different ecological groups. The assays were performed under laboratory conditions using a two-choice arena assay.

Essential oils, macerates, hydrolates and repellent formulations. The following essential oils (sample group O) were obtained: tansy (*Tanacetum vulgare* L.) – O1, geranium (*Pelargonium graveolens* L'Hér.) – O2, citronella (*Cymbopogon winterianus* Jowittex Bor) – O3, lavender (*Lavandula angustifolia* Mill.) – O4, catnip (*Nepeta cataria* L.) – O5, tea tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel) – O6, proprietary herbal blend – O7, oregano (*Origanum vulgare* L.) – O8, peppermint (*Mentha piperita* L.) – O9, thyme (*Thymus vulgaris* L.) – O10, sage (*Salvia officinalis* L.) – O11. Essential oils were produced by Biolive Innovation using a Pilotech YC-050 multi-extractor under steam-distillation conditions, based on the relevant pharmacopoeial procedure (8). The essential oil of tansy (*Tanacetum vulgare* L.) was obtained using a steam-distillation procedure (15).

The following alcoholic macerates (sample group M) were prepared: tansy (*Tanacetum vulgare* L.) – M1, geranium (*Pelargonium graveolens* L. L'Hér.) – M2, lemongrass (*Cymbopogon* spp.) – M3, St. John's wort (*Hypericum perforatum* L.) – M4, lavender (*Lavandula angustifolia* Mill.) – M5, catnip (*Nepeta cataria* L.) – M6, oregano (*Origanum vulgare* L.) – M7, peppermint (*Mentha × piperita* L.) – M8, thyme (*Thymus vulgaris* L.) – M9, and sage (*Salvia officinalis* L.) – M10. Alcoholic macerates were prepared from the respective herbal materials by maceration with 70% (V/V) ethanol at a herbal material-to-extraction solvent ratio of 1 : 5, following the general pharmacopoeial approach used for the preparation of tinctures. Maceration was carried out for 24 h at room temperature with continuous stirring.

The following hydrolates (sample group H) were obtained: mountain germander (*Teucrium montanum*) – H1, tansy (*Tanacetum vulgare* L.) – H2, lemongrass (*Cymbopogon* spp.) – H3, geranium (*Pelargonium graveolens* L. L'Hér.) – H4, lavender (*Lavandula angustifolia* Mill.) – H5, catnip (*Nepeta cataria* L.) – H6, oregano (*Origanum*

vulgare L.) – H7, thyme (*Thymus vulgaris* L.) – H8, sage (*Salvia officinalis* L.) – H9, and peppermint (*Mentha × piperita* L.) – H10.

The following proprietary herbal-blend preparations were also evaluated: P1, an alcoholic tincture prepared from the proprietary herbal blend (with alcohol); P2, an alcoholic tincture prepared from the proprietary herbal blend with alcohol removed; and P5h, a hydrolate obtained from the proprietary herbal blend. All preparations were tested in their as-prepared form unless otherwise stated.

Based on the statistical analysis described below, preparations belonging to the primary top group were used to select formulations for subsequent testing. Formulation X5 was based on the selected preparations and was emulsified with Tween 20; it contained proprietary herbal extract with ethanol (10 g), Tween 20 (2 g), tansy oil (2 g), citronella oil (2 g), and geranium oil (1 g), giving a total essential oil concentration of 29.4%. X5a, X5b, X5c and X5d corresponded to twofold, fourfold, eightfold and 16-fold dilutions of X5, respectively, with essential oil concentrations of 14.70%, 7.35%, 3.67% and 1.84%. The available preparation record specifies these concentrations but does not document the dilution vehicle. Formulation X6 was based on "MIX1", a predefined essential-oil blend, and contained distilled water (10 g), Tween 20 (2 g), and 5 g of MIX1 essential oils, giving a total essential oil concentration of 29.4%. X6a, X6b, X6c and X6d corresponded to twofold, fourfold, eightfold and 16-fold dilutions of X6, respectively, with essential oil concentrations of 14.70%, 7.35%, 3.67% and 1.84%. The available preparation record does not provide the composition of MIX1 or the dilution vehicle. Additional blends were Y1 (lavender + MIX1), Y2 (lavender + geranium), Y3 (lavender + tea tree), and Y4 (tea tree + geranium); each Y mixture contained the two listed essential oils in a 1 : 1 ratio. Comprehensive descriptions and sample coding are provided in Table 1.

Insects. Adult *Drosophila melanogaster* were obtained from larvae developing in fermenting grapes. Adult *Plodia interpunctella* were obtained from naturally infested dried mushrooms. Adult *Calliphora vomitoria* were obtained from larvae purchased from a commercial fishing-tackle

supplier and reared to adulthood. *Culex pipiens* were obtained from larvae collected from natural water bodies and maintained until adult emergence. The available experimental records did not contain standardized information on adult age, sex, or acclimatization period; these parameters therefore cannot be specified retrospectively.

Repellent activity against *D. melanogaster*, *P. interpunctella*, *C. vomitoria* and *C. pipiens* was evaluated using a two-choice arena

Tab. 1. Codes and descriptions of the 48 tested preparations

Group	Codes	Preparation/formulation
O	O1-O11	Essential oils: tansy, geranium, citronella, lavender, catnip, tea tree, proprietary herbal blend, oregano, peppermint, thyme and sage
M	M1-M10	Alcoholic macerates (70% ethanol): tansy, geranium, lemongrass, St. John's wort, lavender, catnip, oregano, peppermint, thyme and sage
H	H1-H10	Hydrolates: mountain germander (<i>Teucrium montanum</i>), tansy, lemongrass, geranium, lavender, catnip, oregano, thyme, sage and peppermint
P	P1, P2, P5h	Proprietary herbal-blend preparations: P1 – alcoholic tincture; P2 – alcoholic tincture with alcohol removed; P5h – hydrolate
X5	X5, X5a-d	Formulation containing proprietary herbal extract, Tween 20, tansy, citronella and geranium oils; X5 = 29.4% essential oils; X5a-d = 14.70, 7.35, 3.67 and 1.84% essential oils
X6	X6, X6a-d	Formulation based on MIX1 essential-oil blend and Tween 20; X6 = 29.4% essential oils; X6a-d = 14.70, 7.35, 3.67 and 1.84% essential oils
Y	Y1-Y4	Essential-oil blends: Y1 – lavender + MIX1; Y2 – lavender + geranium; Y3 – lavender + tea tree; Y4 – tea tree + geranium (1 : 1 ratio for each blend)



Fig. 1. Experimental chamber used in the behavioral avoidance assay. The terrarium measured 100 × 50 cm, was 15 cm high, and contained two 40 × 50 cm compartments connected by a 5 × 5 cm central corridor

assay. The experimental chamber (Fig. 1) consisted of a terrarium measuring 100 × 50 cm, with a height of 15 cm, covered with a transparent glass lid. The chamber was divided into two equal compartments (40 × 50 cm each) connected by a central corridor measuring 5 × 5 cm and 15 cm in height. A 5 × 5 cm filter paper impregnated with 0.5 mL of the tested preparation was placed in one compartment, while the opposite compartment remained untreated and served as the control. Approximately 20 adult insects were introduced into the chamber and allowed to move freely between the compartments for 60 min, after which the number of insects in each compartment was recorded. The position of the treated compartment was alternated between assays to minimize positional effects. No vehicle-matched control was included; therefore, the untreated compartment served as a reference but did not allow the effects of botanical preparations to be separated from those of ethanol, Tween 20, or other formulation components. The available records also did not specify a separate scoring rule for insects remaining in the central corridor or becoming immobile.

Five independent experimental replicates were performed for each preparation and insect species. Each replicate was conducted as a separate assay using a new group of approximately 20 adult insects and a separate experimental arena. Therefore, the five observations recorded for each preparation (test.01–test.05) represented five independent experimental replicates ($n = 5$). This experimental design was applied consistently to all four insect species, including *P. interpunctella*.

Repellency was expressed as the repellency index (RI), calculated as follows:

$$RI (\%) = [(C - T)/(C + T)] \times 100$$

where C is the number of insects recorded in the untreated compartment and T is the number recorded in the compartment containing the tested preparation. Positive values indicate avoidance of the treated compartment, whereas negative values indicate attraction (8, 9).

Statistical analyses were performed separately for each insect species. All statistical analyses were performed in R. For each preparation, five independent experimental replicates were analyzed. Mean, standard deviation, and median repellency index (RI) values were calculated. Normality

was assessed using the Shapiro-Wilk test and Q-Q plots, while homogeneity of variances was evaluated using the Brown-Forsythe test (9, 16).

For species for which homogeneity of variances was not rejected, one-way ANOVA followed by Tukey's HSD test was applied (27). When heterogeneity of variances was detected, Welch's one-way ANOVA followed by the Games-Howell post hoc test was used (11, 29). Games-Howell pairwise comparisons were implemented using `rstatix::games_howell_test`; the adjusted p-values returned by this procedure were used for pairwise inference. A significance level of $p < 0.05$ was adopted. The primary top group was defined as preparations whose mean RI did not differ significantly from that of the preparation with the highest mean RI. This classification indicates a lack of detected difference from the preparation with the highest observed mean; it does not demonstrate equivalence or identical efficacy.

For cross-species assessment, the individual RI measurements obtained for each preparation were compared among the four insect species using Welch's ANOVA and Games-Howell post hoc comparisons, with Holm adjustment applied across the 48 preparation-level tests. In addition, a descriptive cross-species assessment was performed using the number of species for which each preparation belonged to the primary top group, the mean RI across species, the minimum species-specific mean RI, and the mean within-species rank. These descriptive measures were not used to infer equivalence or overall efficacy across species.

Results and discussion

Statistical analysis demonstrated significant differences in repellency among the 48 preparations for all four insect species (all global $p < 2.2 \times 10^{-16}$). For *Drosophila melanogaster*, one-way ANOVA was significant ($F_{47, 192} = 189.51$, $p < 2.2 \times 10^{-16}$). The highest mean RI was recorded for X5 ($76 \pm 3.81\%$), followed by X5b ($72 \pm 7.81\%$), X5a ($70 \pm 5.00\%$), X6a ($70 \pm 6.04\%$) and X5c ($68 \pm 6.60\%$); these five preparations constituted the primary top group according to Tukey's HSD test.

For *Plodia interpunctella*, one-way ANOVA also showed a significant effect of preparation ($F_{47, 192} = 106.39$, $p < 2.2 \times 10^{-16}$). The highest mean RI was observed for Y1 ($72 \pm 6.16\%$), followed by O4 ($68 \pm 6.08\%$), O2 ($65 \pm 5.70\%$) and O6 ($65 \pm 5.29\%$). These four preparations constituted the primary top group.

For *Calliphora vomitoria*, heterogeneity of variance led to the use of Welch's ANOVA, which showed significant differences among preparations ($F_{47, 66.65} = 201.83$, $p < 2.2 \times 10^{-16}$). The primary top group comprised 18 preparations, with the highest mean RI recorded for X5 and X6 ($71 \pm 7.62\%$ and $71 \pm 6.96\%$, respectively), followed by X6c ($68 \pm 5.48\%$), X6a and X6b ($66 \pm 5.52\%$ each), X5a ($65 \pm 5.39\%$), X5b and Y1 ($60 \pm 5.05\%$ each), X6d ($59 \pm 2.35\%$), O1 ($58 \pm 5.79\%$), Y2 ($57 \pm 4.85\%$), O3 and Y4

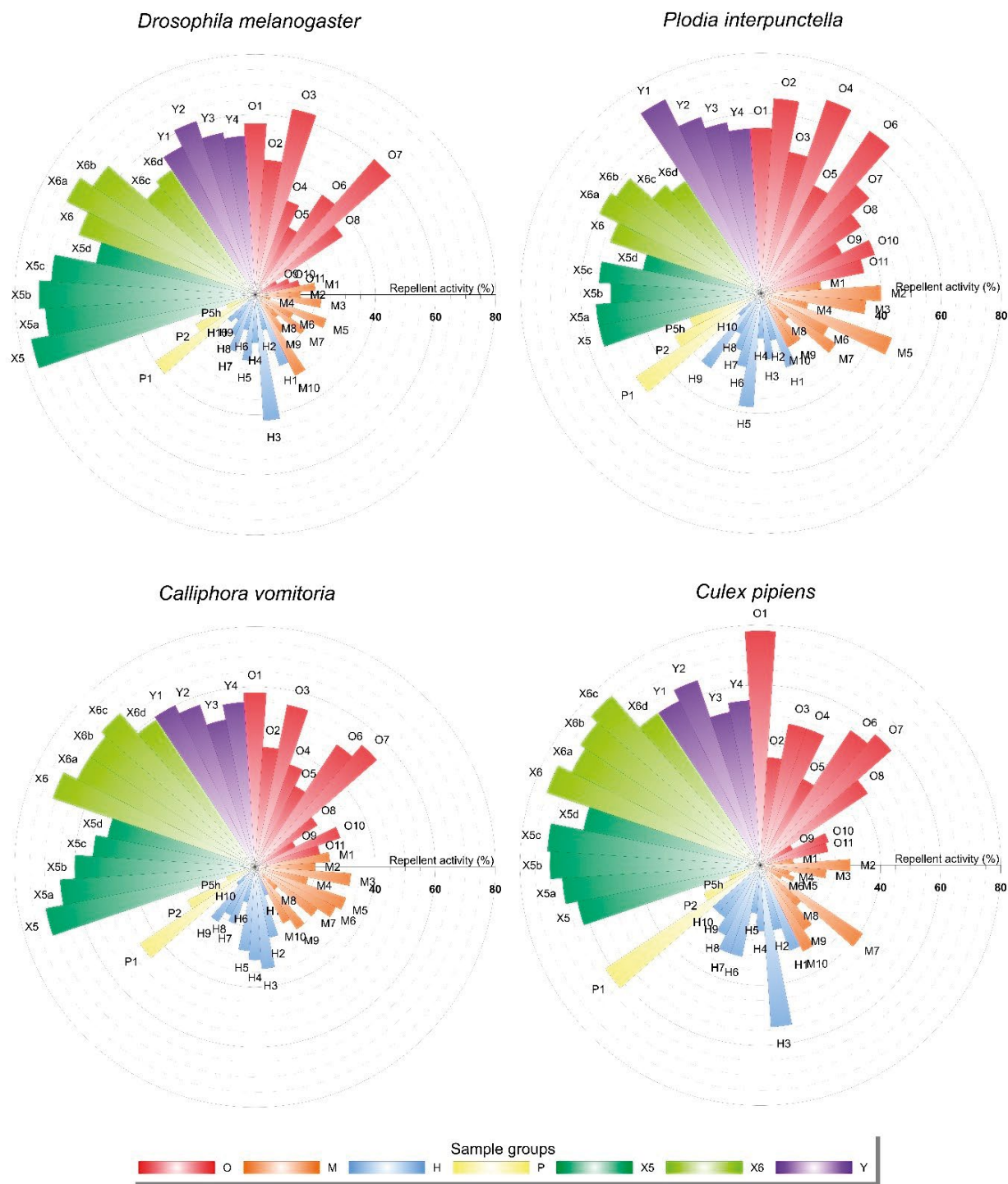


Fig. 2. Mean repellency index (RI, %) of the tested samples against *Drosophila melanogaster*, *Plodia interpunctella*, *Calliphora vomitoria* and *Culex pipiens*. Each radial bar represents the mean value obtained from five independent experimental replicates. Colors indicate the respective sample groups (O, M, H, P, X5, X6 and Y).

($55 \pm 3.67\%$ each), O7 and X5c ($54 \pm 5.15\%$ each), and X5d, Y3 and O6 (50%, 50% and 49%, respectively).

For *Culex pipiens*, Welch's ANOVA likewise indicated significant differences among preparations ($F_{47,66.64} = 160.38$, $p < 2.2 \times 10^{-16}$). The primary top group comprised 18 preparations. O1 showed the highest mean RI ($78 \pm 8.00\%$), followed by X6 ($75 \pm 8.12\%$) and X6c ($75 \pm 6.67\%$), X6b ($72 \pm 6.32\%$), X5c ($71 \pm 7.68\%$), X5b ($70 \pm 6.96\%$) and X6a ($70 \pm 7.31\%$), X5a ($66 \pm 4.53\%$), Y2 ($65 \pm 6.04\%$), P1 and X5

($62 \pm 4.18\%$ each), X6d and Y1 ($61 \pm 5.34\%$ each), X5d ($60 \pm 4.64\%$), O7 ($58 \pm 5.70\%$), Y4 ($55 \pm 6.04\%$), and H3 ($54 \pm 5.79\%$) and O6 ($54 \pm 5.15\%$). Analysis of the original replicate-level data confirmed that the Games-Howell comparisons of O1 with H3 ($p = 0.071$) and O1 with O6 ($p = 0.066$) were not statistically significant at $\alpha = 0.05$.

The Kruskal-Wallis sensitivity analysis also showed significant differences among preparations for all four species (all $p < 2.2 \times 10^{-16}$). The Dunn-Holm analysis

supported the stability of the primary top-group classification: X5b, X5a, X6a and X5c were not significantly different from X5 for *D. melanogaster*, while O4, O2 and O6 were not significantly different from Y1 for *P. interpunctella*. For *C. vomitoria* and *C. pipiens*, 17 preparations in each primary top group were not significantly different from the respective preparation with the highest observed mean RI. These non-significant comparisons should be interpreted as a lack of detected difference rather than evidence of equivalence.

Marked differences among species were also reflected in the cross-species analysis. Seven preparations belonged to the primary top group for three of the four species: X6a, X5, X5a, X5b, Y1, X5c and O6. No preparation belonged to the primary top group for all four species. X6a had a mean within-species rank of 5.25, a minimum species-specific mean RI of 60% and an overall descriptive mean RI of 66.50%.

The formal cross-species analysis further showed that RI differed significantly among species for 40 of the 48 preparations after Holm adjustment. This finding confirms that the response to many preparations was species-dependent and supports the use of species-specific analyses rather than relying only on a pooled measure of repellent activity.

The descriptive mean RI across the four species showed the following values among the preparations with the highest overall means. X6a had the highest overall mean RI (66.50%), followed by X5 (66.00%), X6b and X6 (65.25% each), X5a (64.00%), X5b (63.00%), O1 and Y1 (62.00% each), X5c (61.75%) and Y2 (61.25%). X6a belonged to the primary top group for three species and had a minimum species-specific mean RI of 60%. X6b and X6 had high overall means but belonged to the primary top group for only two species, illustrating that the overall mean alone does not capture species-specific classification.

The results demonstrate substantial species-specific variation in the response to plant-derived preparations. The preparation with the highest observed mean RI differed among species: X5 for *D. melanogaster*, Y1 for *P. interpunctella*, X5 and X6 for *C. vomitoria*, and O1 for *C. pipiens*. This pattern indicates that repellent activity depended strongly on the target insect and supports the use of species-specific comparisons when evaluating plant-derived repellents. Similar species- and formulation-dependent variation has been reported for plant-derived repellents, supporting the need to evaluate repellency against defined target species rather than assuming uniform activity across insects (2, 15).

Multi-component formulations, particularly X5 and X6 and several of their variants, occurred repeatedly among the primary top groups across the tested species. X5 belonged to the primary top group for *D. melanogaster*, *C. vomitoria* and *C. pipiens*, while X6 did so for *C. vomitoria* and *C. pipiens*. Several related formulations, including X5a, X5b, X5c, X6a, X6b, X6c and X6d, also occurred repeatedly in the

primary top groups. These findings indicate that several multi-component formulations showed comparatively consistent activity across the tested species. However, the present data do not allow the mechanism of this performance to be attributed specifically to synergistic interactions, because the individual constituents and their interactions were not experimentally separated. The biological activity of essential oils and plant-derived mixtures is associated with complex volatile compositions, and differences in composition can contribute to differences in repellent performance (21, 22). Experimental work in *D. melanogaster* has also shown that multiple odorant receptors can contribute to avoidance responses to a plant-derived repellent, illustrating the complexity of olfactory responses to multicomponent repellents (28).

X6a had the highest overall mean RI (66.50%) and a minimum species-specific mean RI of 60% among the seven preparations that occurred in the primary top group for three species. These descriptive measures indicate comparatively consistent RI values across the tested insects. O6 also belonged to the top group for three species, with a mean RI across species of 52.00% and a minimum species-specific mean of 40%. Such cross-species differences are consistent with the broader literature, in which repellency varies with both the target species and the formulation tested (2, 16).

Essential oils and other preparation types also showed substantial variation in activity. The present dataset supports the conclusion that several essential-oil-based preparations achieved high RI values, but it does not justify treating all essential oils as uniformly superior to hydrolates, macerates or other formulations. The strongest evidence concerns individual preparations and their statistical classification rather than preparation type as a whole. Previous reviews likewise emphasize substantial variation among essential oils and preparations, rather than a uniform level of efficacy across all plant-derived products (21, 22).

Similarly, differences observed among diluted or modified formulations should be interpreted descriptively. Higher RI values for a diluted preparation do not by themselves demonstrate an optimal concentration or a concentration-dependent behavioral mechanism. Such conclusions would require a dedicated dose-response experiment with controlled concentrations.

From an applied perspective, several preparations reached mean RI values above 70% for at least one species, including X5 for *D. melanogaster*, Y1 for *P. interpunctella*, X5 and X6 for *C. vomitoria*, and O1, X6 and X6c for *C. pipiens*. Nevertheless, laboratory performance should not be directly extrapolated to field efficacy. Environmental conditions, volatility, persistence and the composition of the tested matrices may alter repellent activity outside the experimental arena. Comparable laboratory studies have reported high repellency for individual essential oils against *C. pipiens*, but efficacy depends on the oil, concen-

tration and test conditions (10, 17). In particular, the *C. pipiens* findings provide a laboratory basis for considering selected preparations in future veterinary arthropod-vector studies (4), while the *C. vomitoria* and *P. interpunctella* models may provide potential hygiene and feed-protection contexts, respectively; these applications were not directly tested in the present study.

A limitation of the present analysis is that the original numbers of insects in the treated and control compartments were not available; therefore, statistical analyses were performed on the recorded RI values rather than on the underlying counts. In addition, the experiments were conducted under controlled laboratory conditions. Further studies should characterize the chemical composition of the most promising preparations, examine concentration-response relationships and duration of protection, and evaluate the most effective formulations under semi-field and field conditions. This caution is consistent with reviews emphasizing the need for standardized laboratory and field testing before laboratory repellency results are translated into practical protection claims (2, 16). The use of five independent replicates per preparation also limits the information available for assumption diagnostics. In addition, vehicle-matched controls were not included; therefore, when ethanol, Tween 20 or other formulation components were present, the observed RI values cannot be attributed exclusively to the botanical constituents.

In conclusion, the tested plant-derived preparations showed pronounced and statistically significant differences in repellent activity, with a clear species-specific component. X6a and X5 showed relatively high and comparatively consistent RI values across several tested species, while other preparations showed high activity against particular species. The combination of species-specific inferential analyses and a descriptive cross-species assessment provides information that complements a pooled mean and identifies formulations that warrant further chemical and efficacy evaluation. The findings do not establish protection against biting, pathogen transmission, infestation or field efficacy.

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