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Research

Lethal and Repellent Effects of the Botanical *p*-Anisaldehyde on *Musca domestica* (Diptera: Muscidae)

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Abstract

The house fly, *Musca domestica* L., is a globally distributed nuisance and disease-carrying urban and livestock pest. Control mostly relies on synthetic insecticides but resistance to them has become problematic. *p*-Anisaldehyde, a compound found in many edible plants, was assessed for its effects on different life stages of *M. domestica*. Whereas *p*-anisaldehyde, applied as an adult contact spray, caused >80% mortality by 30 min at a 30% concentration, egg mortality on treated substrate was complete at 0.1%, and the LC_{90} was 0.024%. Only 0.5 and 1 ml of 1.5% *p*-anisaldehyde mixed into 100 g of cow manure curtailed pupation. When the amount of *p*-anisaldehyde was increased to 2 ml, 0.75% *p*-anisaldehyde reduced pupation by 95.5%. In static air olfactometer tubes, 0.075% *p*-anisaldehyde repelled substantial numbers of adult *M. domestica* within 30 min. Repellency of 60–78% was maintained throughout the 4-h bioassay. This study demonstrates that *p*-anisaldehyde is strongly bioactive against *M. domestica* in terms of lethal and nonlethal effects.

Key words: fumigant, house fly, natural product, organic, toxicity

The house fly, *Musca domestica* L., is a highly prolific species that can occur in great densities (e.g., ≈3,220 pupae/1 kg of manure) (James and Harwood 1969). The insect is a nuisance to humans particularly in residential areas near livestock facilities, sometimes resulting in lawsuits (Miller 1993, Hung and Gerry 2013), and it is also associated with mechanical transmission of human and animal pathogens involving bacteria, viruses, fungi, worms, and protozoa, including *Escherichia coli* and *Shigella* (Levine and Levine 1991, Braverman et al. 1999, Chakrabarti et al. 2008, Vazirianzadeh et al. 2008).

Control of *M. domestica* mainly relies on synthetic insecticides, but resistance has become common (Cilek and Greene 1994, Kunz et al. 1995, Marçon et al. 1997, Oyarzún et al. 2008). Botanical products containing bioactive compounds are desirable for pest management when they are effective and complement natural enemy activity (Schmutterer 1990, 1995; Ascher 1993). Plant-derived insecticidal compounds, in general, are considered to pose minimal environmental and safety risks and they are often exempt from Environmental Protection Agency registration under section 25(b) of the Federal Insecticide and Rodenticide Act (Cloyd et al. 2009). *p*-Anisaldehyde (4-methoxybenzenaldehyde, $C_8H_8O_2$) is a naturally occurring fragrant phenolic compound that is soluble in acetone (Boulogne et al. 2012, Science Lab 2013). The compound occurs in many plant species including anise, *Pimpinella anisum* L.; cumin, *Cuminum cyminum* L.; fennel, *Foeniculum vulgare* Miller; and garlic, *Allium sativum* L. (Boulogne et al. 2012). *p*-Anisaldehyde affects arthropod pests in different ways. For example, it deters larval lone star tick, *Amblyomma americanum* (L.), movement and sublethal concentrations halt reproduction by gravid females (Showler and Harlien 2017a). Also, *p*-anisaldehyde is lethal to adult horn flies, *Haematobia irritans irritans* (L.), exposed to droplets and volatiles, to larvae in cow manure, and to eggs (Showler and Harlien 2017b). The compound is also toxic to the mushroom sciarid fly, *Lycoriella ingenua* (Dufour) (Park et al. 2006). *p*-Anisaldehyde is more toxic to house dust mites, *Dermatophagoides farina* Hughes and *D. pteronyssinums* (Trouessart), than the synthetic acaricides benzyl benzoate, *N*,*N*-diethyl-*m*-toluamide (DEET), 3-carene, and estragol (Lee 2004). Bean weevil, *Callosobruchus maculatus* (F.), and bean bruchid, *Acanthoscelides obtectus* (Say), adults were killed using *p*-anisaldehyde sprayed on legume seeds (Ndomo et al. 2010). Yellow water traps baited with *p*-anisaldehyde, on the other hand, increased capture of western flower thrips, *Frankliniella occidentalis* (Pergande), and onion thrips, *Thrips tabaci* Lindeman, over nonbaited traps (Hollister et al. 1995, Belder et al. 2001). Traps baited with *p*-anisaldehyde also collected more varied carpet beetles, *Anthrenus verbasci* (L.), than nonbaited traps (Imai et al. 2002). The purpose of this study was to determine lethal and repellent effects of *p*-anisaldehyde on *M. domestica*.

Materials and Methods

House Flies

Musca domestica of all life stages used in this study were obtained from a colony maintained at the USDA-ARS Knipling Bushland

United States Livestock Insects Research Laboratory in Kerrville, TX. Adult *M. domestica* used in the bioassays were 3- to 5-d old.

p-Anisaldehyde

We used 98% *p*-anisaldehyde (Sigma-Aldrich, St. Louis, MO) for the bioassays in this study. All dilutions of *p*-anisaldehyde were accomplished using acetone as the solvent.

Adult Contact Mortality

p-Anisaldehyde was diluted with acetone to 1, 5, 10, 30, and 50% concentrations, and a blank control and an acetone control were used as comparators. The solutions were vortex-mixed for 30 s.

Adult *M. domestica* were temporarily immobilized on a custom-built chilling table (5°C) (Harris et al. 1965, Gjullin and Bevill 1972) for 5 min. The flies were separated into groups of 25 flies per treatment replicate, and there were six replicates. Each group of 25 flies was placed on two stacked 9-cm-diam Whatman #1 filter paper discs (GE Healthcare, Little Chakfont, Buckinghamshire, England) set inside a 10-cm-diam Petri dish. A plastic funnel with a 10-cm-diam wide end and a 1.8-cm-diam end on the 2.5-cm-long stem was placed, wide end downward, on the Petri dish over the flies. An 89-ml mist bottle (Sprayco, Livonia, MI) was used to apply each treatment by inserting the nozzle into the stem end of the funnel and actuating the trigger four times, producing a total of 0.92 ml of mist. The filter papers became completely moistened, indicating that each fly resting on the surface received a substantial amount. The flies in each Petri dish were immediately moved, using blunt featherweight forceps (Bioquip, Rancho Dominguez, CA) to a 5 × 5 × 4-cm (l by w by h) cage with Plexiglass on four sides and 1-mm^2 plastic mesh covering the two open sides for ventilation. A 12-mm-diam hole in one of the Plexiglass sides permitted insertion of the flies into the cages and the hole was sealed with a plastic plug. The cages were set on a wire stand to ensure maximum ventilation at room temperature in the laboratory. Numbers of flies that were immobilized (some movement in legs and wings but unable to walk or fly) and dead were recorded at 30 min and at 1, 2, 3, and 4 h after the treatments were applied. None of the flies were observed to have recovered from the moribund state.

Egg Mortality

p-Anisaldehyde was mixed with acetone to obtain concentrations of 0.0001, 0.001, 0.01, and 0.1%. A 4.25-cm-diam filter paper disc was wetted with 100 µl of each dilution applied from a micropipette. The filter paper discs were allowed to dry for 30 min before placing each in a separate 5-cm-diam Petri dish. The dried filter paper discs were moistened with deionized water. Twenty-five *M. domestica* eggs were placed, using a #5 1.6-cm camel hair paint brush (Charles Leonard, Glendale, NY), on each filter paper disc, and a lid was placed on each Petri dish. After 24-h storage at 27°C, 50% RH, and a photoperiod of 12:12 (L:D) h, numbers of hatched fly eggs were counted under a dissecting microscope.

Larval and Pupal Mortality

p-Anisaldehyde was mixed with 100-ml acetone to 0.075, 0.15, 0.375, 0.75, and 1.5%, and 0.5, 1, and 2 ml of each *p*-anisaldehyde concentration was homogenized with 100-g cow manure accessed from cows pastured at KBUSLIRL that have not received experimental medications and pesticides. The manure was frozen for at least 24 h before it was used in this study to kill other insects that might have been in and on it. One of the two controls did not include any additives, and the other control involved only the acetate solvent. The manure was placed in 500-ml plastic cups. Batches of 50 *M. domestica* eggs were moved from oviposition substrate using a #5 1.6-cm camel hair paint brush to separate 4.25-cm-diam filter paper discs. All of the eggs were washed off the filter paper with 2 ml of water and onto the surface of the manure. On hatching, the larvae developed to the pupal stage in the manure. The cups were placed in a room at 27°C, 50% RH, and and a photoperiod of 12:12 (L:D) h for 1 wk so that hatched larvae could pupate. Pupae were separated from the manure by soaking the manure in each cup overnight, after which the manure in the cup was poured through a #7 sieve (any pupae that passed through the sieve was caught on a no. 20 sieve; U.S.A. Standard Test Sieve, W. S. Tyler, Mentor, OH) where the pupae were collected and counted. Forceps were used to move the pupae from each treatment replicate onto a filter paper covering the bottom of a 9-cm-diam Petri dish. The Petri dishes were lidded and stored under the same conditions as before. A week later emerged adult *M. domestica* were counted. Each of the controls and the treatments were replicated six times. Percentages of pupae and emerged adults were based on the number of eggs used.

Repellency

Static air olfactometers used in this assay were made of 58.5-cm-long and 9-cm-diam glass tubes open on both ends and with a 2-cm-diam hole in the upper side of each tube at its midpoint for releasing adult *M. domestica* into the tubes after the two ends were sealed. Treatments for *p*-anisaldehyde assays were dilutions with acetone to 0.075, 0.15, 0.375, 0.75, and 1.5%. For controls, six filter paper discs were moistened with 1 ml of acetone only, and nothing was applied to six others. The filter papers were set aside for 30 min for the acetone to evaporate. Following that, each filter paper was placed in a 9-cm-diam Petri dish. The Petri dish was appressed to one end of a static air olfactometer tube such that the filter paper covered the open end, and the dish was fastened to the tube using Parafilm. The same was done using nontreated filter paper at the other end of the tube. Treatments were arranged in a completely randomized design under an acrylic pane that filtered out 98% of ultra violet radiation (acrylic OP-3, 48 mm thick, Plastic Supply of San Antonio, San Antonio, TX) and pilot tests were conducted to ensure that the flies within the tubes were not orienting toward the room lights. Twenty-five adult *M. domestica* of mixed sex were released through the tube midpoint hole after which the hole was plugged with a wad of cotton. The tubes were randomly turned 180° between replications to avoid possible position effects. The location of each fly at 5, 15, and 30 min and at 1, 2, 3, and 4 h was recorded as being in the half of the tube nearest the treated end or in the far half. We regarded significantly more flies on the end of the static air olfactometer tube farthest from the *p*-anisaldehyde repellency than in the static air olfactometer tube without *p*-anisaldehyde (the control).

Statistical Analyses

Each assay was analyzed to detect treatment differences using one-way analysis of variance (ANOVA), and means were separated using Tukey's HSD (Analytical Software 2008). For bioassays involving more than one factor (i.e., *p*-anisaldehyde concentration and time or quantity), factorial analysis was also conducted (Analytical Software 2008). Because normality and homogeneity of variance assumptions were not violated, data were not $\log(x + 1)$-transformed. Percentage data were arcsine-square root-transformed before analysis. For determining LC_{50}s and LC_{90}s of *p*-anisaldehyde on contact adult mortality, probit analysis (LeOra Software 2005) was conducted. Probit analysis could not be used for accurately calculating LC

information on egg mortality, and on development of pupae and on adult emergence.

Results

Adult Contact Mortality

Factorial analysis detected treatment (F = 486.80, df = 6, 209, P < 0.0001) and time (F = 19.04, df = 4, 209, P < 0.0001) effects. Mortality was not observed in the blank and the acetone solvent controls, whereas percentage mortality increased by 2.5-, 3.8-, 5.1-, and 5.5-fold in response to the 5, 10, 30, and 50% concentrations compared against the 1% concentration (Fig. 1A). Factorial analysis (includes the control) showed that mortality increased moderately, by 7.6–12.9%, between each consecutive sampling time (Fig. 1B).

One-way ANOVA detected many differences between means (F = 101.84, df = 34, 209, P < 0.0001). The 1% *p*-anisaldehyde concentration killed <5% of the adult *M. domestica* at 30 min and mortality increased by 7.8-fold at 4 h (Table 1). Although the 5 and 10% concentrations induced more mortality than the 1% concentration, percentage kill did not rise above 78% throughout the bioassay (Table 1). The 30 and 50% concentrations, however, induced substantial mortality at 30 min, which reached nearly 95% by 2 h in the 30% concentration treatment, and in the 50% concentration treatment, mortality was 96% by 30 min and 100% by 2 h (Table 1). The LC_{50}s and LC_{90}s declined at each successive sampling time (Table 2).

Egg Mortality

Egg hatch was not affected by the 0.0001 and 0.001% *p*-anisaldehyde concentration treatments in relation to the blank and acetone controls (Fig. 2). Hatching declined by only 8.7% compared with the controls in response to 0.01% *p*-anisaldehyde, but the 0.1% concentration completely curtailed hatching (F = 226.74, df = 5, 35, P < 0.0001; Fig. 2).

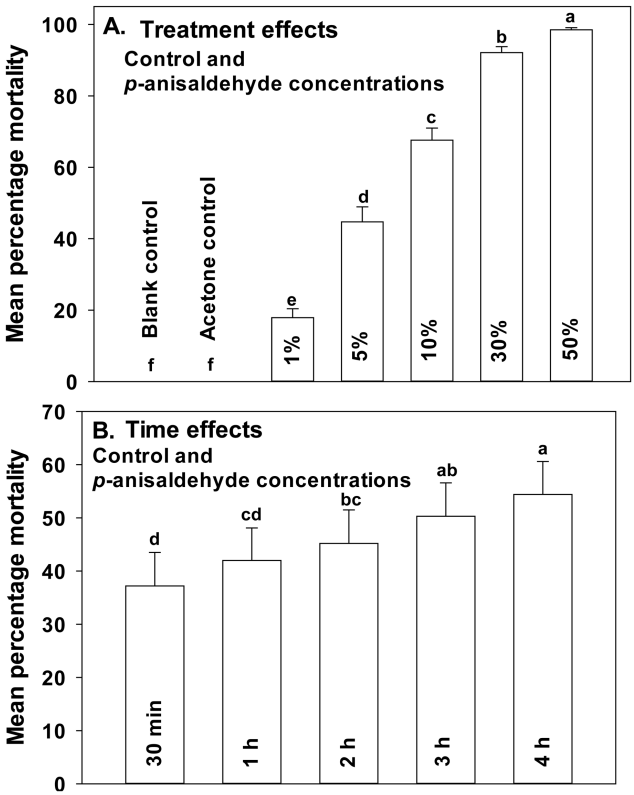

Fig. 1. Mean (±SE) percentages of adult *M. domestica* killed by contact with (A) different concentrations of *p*-anisaldehyde, and (B) at different exposure times; factorial analysis, Tukey's HSD, bars accompanied by different letters are significantly different (P < 0.05).

Larval and Pupal Mortality

In terms of percentage pupation in cow manure, factorial analysis detected *p*-anisaldehyde concentration (F = 96.58, df = 6, 125, P < 0.0001) and quantity (F = 20.93, df = 2, 125, P < 0.0001) effects. Percentage of observed pupae was reduced by 33.0% in response to the acetone solvent, but it was not reduced by the 0.075% *p*-anisaldehyde concentration (Fig. 3A). The 0.75% concentration reduced the percentage of pupae by 56.7% below the acetone solvent control, and the 1.5% concentration did not yield any pupae (Fig. 3A). Factorial analysis indicated that, while doubling the amount of *p*-anisaldehyde from 0.5 to 1.0 ml did not affect percentage pupation, doubling the amount from 1.0 to 2.0 ml reduced percentage pupation by 29.7% (F = 20.93, df = 2, 125, P < 0.0001; Fig. 3B).

One-way ANOVA detected differences (F = 46.82, df = 20, 125, P < 0.0001) between *p*-anisaldehyde concentration and quantity treatment combinations. Pupation was not reduced when 0.5 and 1.0 ml of *p*-anisaldehyde concentrations were applied, but the 1.5% concentration curtailed pupation (Table 3). When 2 ml was mixed into the cow manure, pupation was reduced by 96.3% in the 0.75% *p*-anisaldehyde treatment compared to the acetone control and pupation did not occur in the 1.5% treatment (Table 3). Although 0.5 and 1.0 ml quantities of *p*-anisaldehyde did not reduce pupation at any of the concentrations, increasing the quantity to 2 ml reduced pupation by 44.3 and 95.5% in 0.075 and 0.75% concentration treatments, respectively. Pupation did not occur in the 1.5% concentration treatment regardless of the quantity applied (Table 3).

In terms of adult emergence in relation to the number of eggs, factorial analysis detected *p*-anisaldehyde concentration (F = 90.57, df = 6, 125, P < 0.0001) and quantity (F = 16.49, df = 2, 125, P < 0.0001) effects. The acetone solvent control was associated with a reduction of 33.5% in comparison with the blank control, which was not different from the 0.075, 0.15, and 0.3% *p*-anisaldehyde concentration treatments (Fig. 4A). The 0.75% *p*-anisaldehyde concentration, however, reduced adult emergence by 61.3% and the 1.5% concentration had already completely prevented pupation (Fig. 4A). Whereas 1.0 ml of the treatment concentrations did not reduce adult emergence below that of the 0.5 ml amount, the 2 ml amount reduced adult emergence by 27.9% relative to the 1 ml amount (Fig. 4B). One-way ANOVA detected differences (F = 38.40, df = 20, 125, P < 0.0001) that occurred between the 1.5% concentrations and lower concentrations for the 0.5 and 1.0 ml amounts, and between the 0.375 and 0.75% concentrations where 2 ml was applied (Table 4). The 2 ml quantity was only more potent, by ≥94.4% than the lower amounts, where the 0.75% concentrations were used (Table 4).

Repellency

Although *M. domestica* were well distributed in the blank control and in the acetone control, they were largely (75–84%) clustered on the nontreated filter paper opposite to the treated filter paper in the *p*-anisaldehyde treatments, and not flying or moving about the static air olfactometer tube particularly as concentrations increased beyond the lowest (F = 42.10, df = 6, 293, P < 0.0001), and time effects were also detected (F = 15.58, df = 6, 293, P < 0.0001). The 0.075% *p*-anisaldehyde concentration resulted in 30% more flies in the half of the static air olfactometer tube farthest from the treatment

Table 1. Mean (±SE) percentages of adult *M. domestica* mortality in response to direct contract with *p*-anisaldehyde applied as a mist, six replicates, 25 flies per treatment replicate, one-way ANOVA, Tukey's HSD

	Percentage mortality				
Treatment[a]	30 min	1 h	2 h	3 h	4 h
Blank[b]	0 k	0 k	0 k	0 k	0 k
Acetone[b]	0 k	0 k	0 k	0 k	0 k
1%	4.7 ± 3.2 jk	13.3 ± 2.9 ij	11.3 ± 2.4 ij	23.3 ± 4.1 hi	36.7 ± 3.6 g–i
5%	20.0 ± 6.0 ij	29.3 ± 6.1 hi	45.3 ± 7.9 f–h	59.3 ± 5.0 e–g	69.3 ± 3.5 d–f
10%	53.3 ± 12.3 f–h	68.0 ± 5.0 d–f	65.3 ± 4.5 d–g	73.3 ± 6.3 d–f	78.0 ± 5.2 c–e
30%	86.7 ± 4.8 b	86.7 ± 3.2 bc	94.7 ± 2.5 a–c	96.0 ± 4.0 ab	96.7 ± 3.3 ab
50%	96.0 ± 1.5 a–c	96.7 ± 2.2 ab	100 a	100 a	100 a

Values followed by different letters are significantly ($P < 0.05$) different.
[a]Percentages are for *p*-anisaldehyde in acetone solvent.
[b]Controls.

Table 2. LC_{50}s and LC_{90}s for adult *M. domestica* in response to direct contact with *p*-anisaldehyde applied as a mist, six replicates, 25 flies per treatment replicate

			95% confidence limits	
Sampling time	LC_{50} or LC_{90}	LC %	Lower	Upper
30 min	LC_{50}	15.59	11.6	20.1
	LC_{90}	34.46	28.3	45.0
1 h	LC_{50}	11.69	7.8	15.6
	LC_{90}	33.02	27.2	42.9
2 h	LC_{50}	8.55	6.3	10.9
	LC_{90}	22.55	18.7	29.0
3 h	LC_{50}	4.23	0.2	7.2
	LC_{90}	20.51	15.9	30.2
4 h	LC_{50}	2.15	0	5.5
	LC_{90}	18.88	14.4	28.1

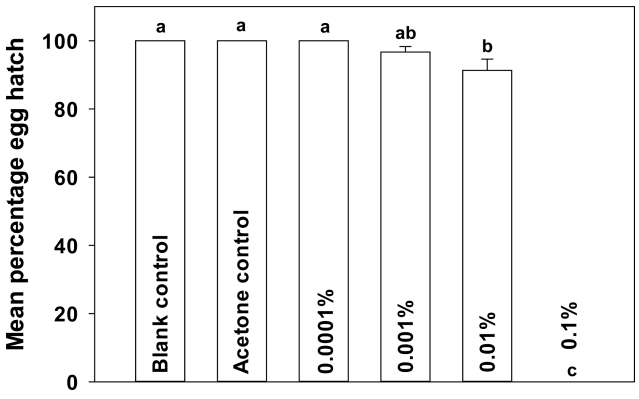

Fig. 2. Mean (±SE) percentages of *M. domestica* eggs that hatched on filter paper treated with different concentrations of *p*-anisaldehyde; 25 eggs per replicate, one-way ANOVA, Tukey's HSD, bars accompanied by different letters are significantly different ($P < 0.05$).

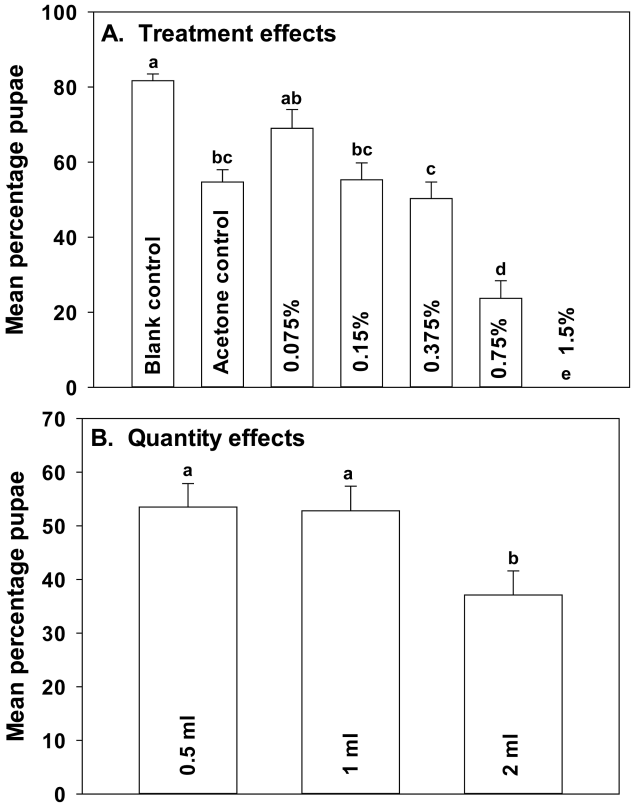

Fig. 3. Mean (±SE) percentages of *M. domestica* eggs ($n = 50$) that hatched, developed through the larval stage to pupae in response to (A) *p*-anisaldehyde concentrations, and (B) quantities of *p*-anisaldehyde; factorial analysis, Tukey's HSD, bars accompanied by different letters are significantly different ($P < 0.05$); percentages of pupae and emerged adults are based on the number of eggs used.

than was observed in the acetone control, and the four highest concentrations were associated with 51.2–65.2% more flies in the far half of the tube (Fig. 5A). Repellency was observed by the 30 min sampling time and it reached a statistical maximum by 1 h, where it remained for the duration of the 4-h-long bioassay (Fig. 5B).

One-way ANOVA detected differences between means ($F = 10.22$, df = 48, 293, $P < 0.0001$). While repellency was not observed in either of the controls, the 0.075% *p*-anisaldehyde concentration was associated with repellency by the 1 h sampling time which reached a statistical maximum by 2 h and it increased numerically at the 3 and 4 h sampling times (Table 5). The 0.15 and 0.375% *p*-anisaldehyde concentrations also did not immediately repel adult *M. domestica*, but repellency was evident by only 15 min and reached statistically maximum levels by 1 h and 30 min, respectively (Table 5). The 0.75% concentration was associated with repellency by the 5 min sampling time and repellency increased numerically until >87% of the flies were in the far half of the tube by 1 h; that level of repellency was maintained throughout the rest of the 4-h-long bioassay (Table 5).

Table 3. Mean (±SE) percentages of *M. domestica* that pupated after 25 eggs were placed on a 4.25-cm-diam filter paper disc set on 100 mg of *p*-anisaldehyde-treated cow manure which the larvae inhabited, six replicates, one-way ANOVA, Tukey's HSD

	Percentage pupae		
	Quantity *p*-anisaldehyde applied (ml)		
Treatment[a]	0.5	1	2
Blank[b]	81.7 ± 3.2 a	82.1 ± 3.0 a	81.4 ± 3.0 a
Acetone[b]	54.7 ± 6.0 b–d	55.0 ± 6.4 b–d	54.4 ± 6.0 b–d
0.075%	81.3 ± 3.6 a	80.3 ± 4.1 ab	45.3 ± 7.9 c–e
0.15%	57.0 ± 9.4 b–d	68.0 ± 4.3 a–c	41.0 ± 5.1 c–e
0.375%	55.7 ± 6.9 b–d	60.0 ± 7.2 b–d	35.3 ± 5.6 de
0.75%	44.0 ± 5.8 c–e	25.0 ± 3.2 e	2.0 ± 2.0 f
1.5%	0 f	0 f	0 f

Values followed by different letters are significantly ($P < 0.05$) different.
[a]Percentages are for *p*-anisaldehyde in acetone solvent.
[b]Controls.

Table 4. Mean (±SE) percentages of *M. domestica* adult emergence after 25 eggs were placed on a 4.25-cm-diam filter paper disc set on 100 mg of *p*-anisaldehyde-treated cow manure inhabited by the larvae, six replicates, one-way ANOVA, Tukey's HSD

	Percentage adult emergence		
	Quantity *p*-anisaldehyde applied (ml)		
Treatment[a]	0.5	1	2
Blank[b]	67.7 ± 3.6 a	65.0 ± 3.0 a	66.9 ± 3.1 a
Acetone[b]	45.0 ± 6.0 a–d	45.4 ± 6.4 a–d	44.7 ± 5.8 a–d
0.075%	58.7 ± 1.8 a–c	61.0 ± 3.9 ab	36.3 ± 7.5 b–e
0.15%	46.0 ± 8.1 a–d	52.3 ± 3.2 a–d	32.3 ± 4.9 de
0.375%	45.3 ± 5.5 a–d	49.7 ± 7.7 a–d	29.0 ± 3.5 de
0.75%	33.7 ± 5.2 c–e	17.7 ± 2.0 e	1.0 ± 1.0 f
1.5%	0 f	0 f	0 f

Values followed by different letters are significantly ($P < 0.05$) different.
[a]Percentages are for *p*-anisaldehyde in acetone solvent.
[b]Controls.

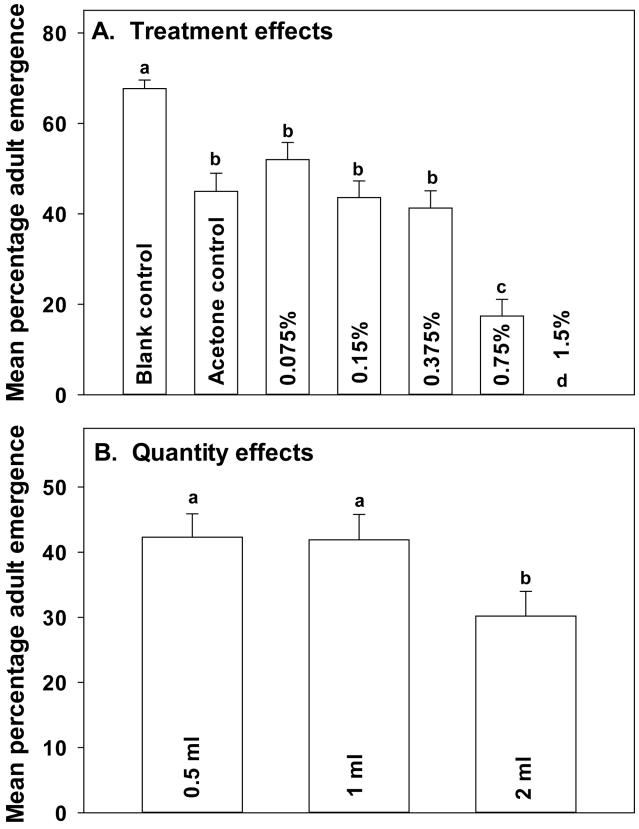

Fig. 4. Mean (±SE) percentages of *M. domestica* eggs ($n = 25$) that hatched, developed through the larval and pupal stages, and emerged as adults in response to (A) *p*-anisaldehyde concentrations, and (B) quantities of *p*-anisaldehyde; factorial analysis, Tukey's HSD, bars accompanied by different letters are significantly different ($P < 0.05$).

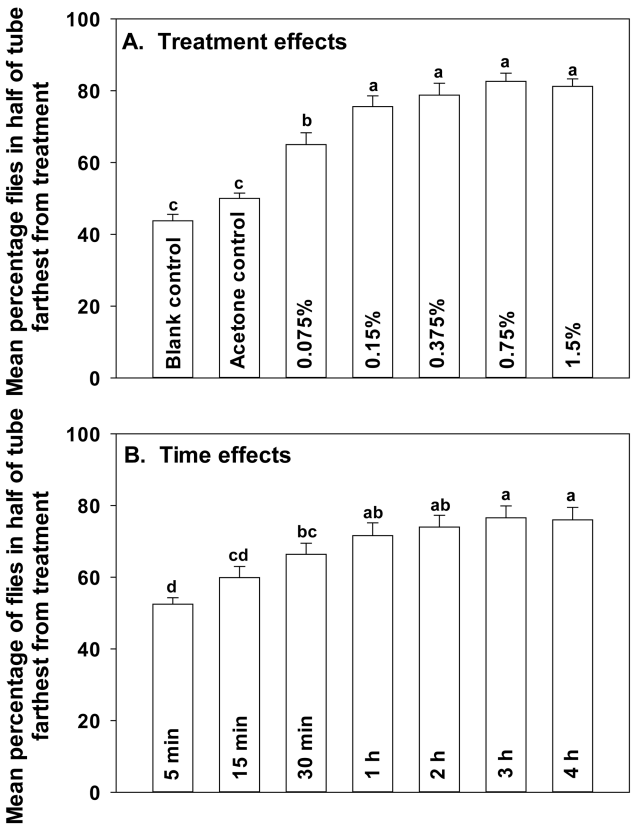

Fig. 5. Mean (±SE) percentages of adult *M. domestica* observed in the half of static air olfactometer rubes farthest from the treatment in response to (A) different concentrations of *p*-anisaldehyde, and (B) time; dashed lines indicate an equal distribution of flies expected in the absence of a repellent factor, factorial analysis, Tukey's HSD, bars accompanied by different letters are significantly different ($P < 0.05$).

The greatest concentration, 1.5%, repelled >80% of *M. domestica* by 15 min and throughout the rest of the bioassay (Table 5).

Discussion

This study shows that *p*-anisaldehyde has multiple effects on *M. domestica* and that it can afflict different developmental stages. In terms of muscid species, *p*-anisaldehyde was toxic to adult *H. irritans* as a result of contact and fumigant exposures, and contact exposure was lethal to eggs (Showler and Harlien 2017b).

Other botanically based substances are toxic to *M. domestica* eggs through direct contact, including cassumunar ginger, *Zingiber cassumunar* Roxb.; peppermint, *Mentha piperita* (Ehrh.) Briq.;

Table 5. Mean (±SE) percentages of 25 adult *M. domestica*, released into static air olfactometer tubes with *p*-anisaldehyde-treated filter paper discs affixed to the other end that were in the nontreatment half of the tube, six replicates, one-way ANOVA, Tukey's HSD; the flies in the treatment tubes were mostly clustered on the nontreated filter paper

	Percentage flies in nontreated half of tube						
Treatment[a]	5 min	15 min	30 min	1 h	2 h	3 h	4 h
Blank[b]	48.7 ± 5.3 e–g	41.3 ± 4.2 g–h	46.7 ± 3.8 f–h	44.0 ± 3.7 f–h	45.3 ± 6.0 f–h	38.0 ± 5.8 h	42.7 ± 5.5 gh
Acetone[b]	52.0 ± 1.0 d–f	48.0 ± 3.3 e–g	49.3 ± 1.3 e–g	47.3 ± 3.8 fg	50.0 ± 3.8 e–g	56.7 ± 3.6 ef	46.7 ± 7.6 f–h
0.075%	46.7 ± 1.3 f–h	46.0 ± 4.6 f–h	54.0 ± 6.2 d–f	60.0 ± 11.2c–e	74.0 ± 7.9 bc	84.0 ± 1.5 a–c	90.0 ± 2.5 ab
0.15%	48.0 ± 2.1 f–h	62.7 ± 8.0 c–e	68.7 ± 8.0 bc	86.7 ± 4.5 a–c	84.0 ± 5.8 a–c	89.3 ± 3.7 ab	90.0 ± 3.8 ab
0.375%	46.0 ± 4.6 f–h	62.0 ± 10.9 c–e	82.7 ± 8.0 a–c	89.3 ± 3.2 ab	90.7 ± 4.5 a	93.3 ± 2.5 a	87.3 ± 4.6 ab
0.75%	66.7 ± 6.6 bc	76.7 ± 1.9 bc	83.3 ± 5.0a–c	87.3 ± 7.0 ab	88.0 ± 6.9 ab	88.0 ± 6.0 ab	88.0 ± 4.1 ab
1.5%	59.3 ± 4.1 d–e	82.7 ± 5.8 a–c	80.0 ± 5.0 a–d	86.7 ± 4.0 a–c	86.0 ± 2.5 a–c	86.7 ± 3.5 a–c	87.3 ± 3.6 ab

Values followed by different letters are significantly ($P < 0.05$) different.
[a]Percentages are for *p*-anisaldehyde in acetone solvent.
[b]Controls.

orange, *Citrus sinensis* L. Osbeck; lemongrass, *Cymbopogon citratus* D.C.) Stapf; star anise, *Illicium verum* Hook.f.; Canada goldenrod, *Lavandula angustifolia* L., and lantana, *Lantana camata* L. (Kawazu et al. 1977, Sinthusiri and Soonwera 2014, Ordanza-Cortes 2015). The only study on the effects of *p*-anisaldehyde against the eggs of another muscid species demonstrated that 100% of *H. irritans* eggs placed on a treated filter paper substrate failed to hatch when concentrations were as low as 0.00001% (Showler and Harlien 2017b). Although *M. domestica* eggs were not as vulnerable to contact exposure as *H. irritans* eggs, complete egg mortality was achieved at a relatively low concentration (0.1%). The potency of *p*-anisaldehyde against *M. domestica* eggs suggests that the compound might be as useful for control of *M. domestica* as synthetic growth regulators and that it is superior to botanically based substances that have been assessed for reducing adult fecundity (Wright and Harris 1976) and for contact toxicity (Kawazu et al. 1977, Sinthusiri and Soonwera 2014, Ahmed et al. 2015, Ordanza-Cortes 2015).

In addition to synthetic insecticides, such as diflubenzuron (da Silva and Mendes 2002), numerous botanically based substances are larvicidal against *M. domestica*, such as the essential oil of *M. piperita*; scented thorn, *Acacia nilotica* (L.) Wild. ex Delile (seed powder); nutgrass, *Cyperus rotundus* L. (whole plant powder); geranium, *Pelargonium zonale* L'Her. (leaf powder); Monterey cypress, *Cupressus macrocarpa* Hartw. ex Gordon (leaf powder); bitter almond, *Amygdalus communis* L. (essential oil); thyme, *Thymus vulgaris* L.; and jojoba, *Simmondsia chinensis* (Link) C.K. Schneid (Pavela 2008, Elbermawy et al. 2011, Kumar et al. 2011, Chauhan et al. 2016).

In terms of *p*-anisaldehyde effects on the larvae of other muscids, the compound strongly reduced *H. irritans* larval development to pupae at 5% and prevented it at 10% (Showler and Harlien 2017b). Because *p*-anisaldehyde exerted strong larvicidal effects against *M. domestica* when only 2 ml of 0.75% solution was mixed into 100 g of cow manure and the 1.5% solution caused complete control, *p*-anisaldehyde at relatively low concentrations is more lethal to *M. domestica* larvae than most of the botanically based substances that have been tested, and *M. domestica* larvae appear to be more vulnerable to *p*-anisaldehyde than *H. irritans* larvae.

Many synthetic insecticides are available for contact use against adult *M. domestica* (Khalequzzaman et al. 2002). In terms of botanically based substances, crude *A. indica* leaf acetone extracts were not strongly toxic, with an LD_{50} of ≈170 µg/fly, when compared against an LD_{90} of 3.6 µg per fly for 2,2-dicholovinyldimethyl phosphate (DDVP) (Khan and Ahmed 2000). Among 33 terpenes tested for contact effects, thymol and pulegone were the most bioactive with LD_{50}s of 29 and 39 µg per fly, respectively (Rice and Coats 1994). Singh and Singh (1991) tested 31 essential oils for contact toxicity against adults, but the most potent among them caused only ≈40% mortality after 24 h in contrast to malathion and pyrethryum, which provided 100% kill by 2 h. Petroleum ether extracts of griffonia, *Griffonia simplicifolia* Baill., and Senegal prickly ash, *Zanthoxylum zanthoxyloides* (Lam.), had 24 h contact LD_{50}s of 0.28 and 0.35 µg per fly, respectively (Bisseleua et al. 2008). LD_{50}s for topically applied essential oils of *Pelargonium* sp., mint, *Mentha* sp.; lavender, *Lavandula* sp.; *Eucalyptus* sp., and *C. sinensis* were 0.07, 0.09, 0.13, 0.14, and 0.16 µg per fly, respectively, and for the monoterpenes linalool, menthyl acetate, limonene, menthone, and eucalyptol LD_{50}s are 0.04, 0.09, 0.1, 0.11, and 0.13 µg per fly, respectively (Tarelli et al. 2009). Some other botanically based substances that are relatively effective against adult *M. domestica* include essential oils of rosemary, *Rosemarinus officinalis* L.; pennyroyal mint, *Mentha pulegium* L.; and cuerno de cabra, *Haplopappus foliosus* DC (Geden 2012).

We found *p*-anisaldehyde to be a relatively weak contact toxin against *M. domestica* adults, because it did not result in >80% control even after 4 h and at concentrations of up to 10%. The 30 and 50% concentrations provided >85% control within only 30 min, but this is likely not economical. The LD_{50}s for *p*-anisaldehyde against adult *M. domestica* showed that, at the short exposure times, concentrations were relatively great and the values did not decline to more acceptable levels until 4 h had elapsed, which is likely to be too slow-acting. The LC_{90} values, which are more indicative of acceptable levels of control than LC_{50}s, were relatively great regardless of exposure time, suggesting that *p*-anisaldehyde might not be useful as a contact pesticide against *M. domestica*. It is, however, possible that contact lethality could be heightened by adding other toxins, synergists, and carriers that might enhance efficacy.

Fumigant action of botanically based compounds against adult *M. domestica* has been reported for a number of substances that include knockdown caused by essential oils (10% in acetone) of *Eucalyptus* sp., *C. sinensis*, *Mentha* sp., *Lavandula* sp., and *Pelargonium* sp., which had knockdown time (KTs) of 3.3, 10.1, 10.4, 10.9, and 17.7 min, respectively (Tarelli et al. 2009). Pavela (2008) found that volatiles of essential oils of *R. officinalis* and *M. pulegium* were effective for killing adults. The essential oils from leaves of peperita, *Minthostachys verticillata* (Griseb.) Epling; tomillo del campo, *Hedeoma multiflora* Benth.; and sweet wormwood, *Artemisia annua* L. had LD_{50}s of 0.5, 1.3, and 6.5 mg/dm^3,

respectively (Palacios et al. 2009b). The botanically based volatile terpenoids citronella, menthol, and *l*-fenchone had LD_{50}s of 2, 3.6, and 3.8 mg/dm^3, respectively (Palacios et al. 2009a). Rossi and Palacios (2015) determined that *E. cinerea* volatiles were comprised of 1,8-cineole (88.5%), α-pinene (2%), α-terpineol (9%), and 2,3-dehydro-1,8-cineole (percentage not specified) and that adult *M. domestica* were killed within 15 min. 1,8-cineole (74%), α-pinene (0.1%), and α-terpineol (24.7%) had LD_{50}s of 3.3, 11.5, and 36.8 mg/dm^3, respectively (Rossi and Palacios 2015).

Unlike the immobilization and lethal effects of volatilized *p*-anisaldehyde reported on *H. irritans* (Showler and Harlien 2017b), we did not detect notable immobilization and mortality responses in *M. domestica*. This demonstrates that *p*-anisaldehyde acts differently against species within the same taxonomic family; hence, it is possible that the compound will affect other muscids, such as stable flies, *Stomoxys calcitrans* (L.), and little house flies, *Fannia* spp., in ways that are not the same as those observed for *M. domestica* and *H. irritans*.

In terms of repellency, DEET has been regarded as an effective synthetic repellent against blood-feeding ectoparasitic arthropods but concerns have arisen about its toxicity to nontarget organisms (Qiu et al. 1998, Sudakin and Trevathan 2003, Singh et al. 2010, Kim et al. 2011), which has prompted renewed investigations into less toxic repellent substances (Nerio et al. 2010). The ability of DEET to prevent hematophagous dipterans from detecting the attractive odors of hosts (Dogan and Rossignol 1999, Ditzen et al. 2008, Lee et al. 2010) might also fail to deter nonhematophagous flies, such as *M. domestica* (Haselton et al. 2015).

Some botanically based substances that have been assessed as repellents against *M. domestica* include the terpene (1*S*)-(−)-α-pinene in constant air flow laboratory conditions. The terpene repelled flies at concentrations as low as 0.11% (Haselton et al. 2015). At a concentration of 25%, *L. camara* was a strong deterrent against oviposition (Ahmed et al. 2013). Kumar et al. (2011) found that the concentration of *M. piperita* crude essential oil that repelled 84% of adult *M. domestica* was 61 µg/cm^2 of treated substrate and that the RT_{50} of 69.7 µg/cm^2 was 16.2 min. The volatile extracts of pepper tree, *Schinus molle* L., leaves showed repellent and feeding deterrent activity in laboratory bioassays (Wimalaratne et al. 1996), and 20 mg of catnip, *Nepeta cataria* L., essential oil provided 79% repellency against *M. domestica* (Zhu et al. 2009). Oils andiroba, *Carapa guianensis* Aublet, and copaiba, *Copaifera reticulata* Ducke, diluted to 5%, sprayed in pens with sheep, *Ovis aires* L., strongly reduced *M. domestica* numbers by >90% for up to 24 h (Zortéa et al. 2017). Singh and Singh (1991) reported that essential oils of clove basil, *Ocimum gratissimum* L.; Breckland thyme, *Thymus serpyllum* L; star anise, *I. verum* Hooks. f.; nutmeg, *Myristica fragrans* Houtt.; and mango ginger, *Curcuma amada* Roxb., showed 100% repellent activity for 5 h. Petroleum ether seed extracts of *G. simplicifolia* and stem extracts of *Z. zanthoxyloides* had RD_{50}s (repellent action) of 1 and 1.3 µg/cm^2, respectively (Bisseleua et al. 2008). Adler and Jacobson (1982) found that *Calamus* sp. root oil completely repelled *M. domestica* for 30 min. The commercial herbal fly repellent, Keetguard (5%), comprised of the oils of *E. globulus*; deodar cedar, *Cedrus deodara* (Roxb.) G. Don f.; chir pine, *Pinus longifolia* Roxb. ex Lamb; and others, repelled 79% of *M. domestica* adults at 4 h post-treatment on a poultry farm (Bharkad et al. 2013).

In comparison, *p*-anisaldehyde's strong repellency against *M. domestica* might offer a range of commercial applications, including potential use in livestock production areas, stored products, and households. The relatively low concentrations of *p*-anisaldehyde that induced repellency against *M. domestica* offer greater potential than the commercial available herbal repellent, Keetguard, which was less effective at a concentration 66.7-fold greater.

Some feed-through products in cattle have been tested for muscid population management, but organic compounds for such use are not commercially available. While acetone mixed into cow manure decreased *M. domestica* development from larvae to pupae in our study, low amounts failed to reduce numbers of pupae further, excluding the highest concentration which was effective. Doubling the low quantity of *p*-anisaldehyde was moderately more effective by reducing pupation at half the concentration (0.75%). Strong reduction of pupation, however, was accomplished at the 0.75% concentration by quadrupling the quantity applied. Our results show that the development of larvae was more impaired by *p*-anisaldehyde than pupal development to the adult stage, which was a possible artifact of the experimental approach: larvae were directly exposed to *p*-anisaldehyde, whereas the pupae were removed from the treated manure until adults emerged.

Disclaimer

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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References Cited

Adler, V. E., and M. Jacobson. 1982. Evaluation of selected natural and synthetic products as house fly repellents. J. Environ. Sci. Health. 17: 667–673.

Ahmed, S., H. Malik, M. A. Riaz, and M. Akthar. 2013. Influence of plant extracts on the life history and population development of house fly, *Musca domestica* L. (Diptera: Muscidae). Pakistan J. Zool. 45: 345–349.

Ahmed, S., A. S. Khan, M. S. Nisar, and M. M. Shakir. 2015. Effect of aqueous extracts of different plants on life cycle and population build up parameters of house fly, *Musca domestica* L. (Diptera: Muscidae). J. Agric. Sci. Technol. 4: 1–6.

Analytical Software. 2008. Statistix for windows. Analytical Software, Tallahassee, FL.

Ascher, K. R. S. 1993. Nonconventional insecticidal effects of pesticides available from the neem tree, *Azadirachta indica*. Arch. Insect Biochem. Physiol. 22: 433–449.

Belder, E. den, J. Elderson, and I. Agca. 2001. Is olfactory orientation of *Thrips tabaci* disrupted by a non-host plant? Proc. Exper. Appl. Entomol. 21: 61–64.

Bharkad, G., M. Saxena, K. Ravikanth, A. Thakur, and S. Maini. 2013. Efficacy of herbal fly repellent product (Keetguard liquid) to control *Musca domestica* population in poultry egg layer farm. Global J. Anim. Sci. Res. 1: 66–75.

Bisseleua, H. B. D., S. W. K. Gbewonyo, and D. Obeng-Ofori. 2008. Toxicity, growth regulatory and repellent activities of medicinal plant extracts on *Musca domestica* L. (Diptera: Muscidae). Afr. J. Biotechnol. 7: 4635–4642.

Boulogne, I., P. Petit, H. Ozier-Lafontaine, L. Desfontaines, and G. Loranger-Merciris. 2012. Insecticidal and antifungal chemicals produced by plants: a review. Environ. Chem. Lett. 10: 325–347.

Braverman, Y., A. Chizov-Ginzburg, A. Saran, and M. Winkler. 1999. The role of houseflies (*Musca domestica*) in harbouring *Corynebacterium pseudotuberculosis* in dairy herds in Israel. Rev. Sci. Tech. 18: 681–690.

Chakrabarti, S., D. J. King, C. J. Cardona, and A. C. Gerry. 2008. Persistence of exotic Newcastle Disease Virus (ENDV) in laboratory infected *Musca domestica* and *Fannia canicularis*. Avian Dis. 52: 375–379.

Chauhan, N., A. Malik, S. Sharma, and R. C. Dhiman. 2016. Larvicidal potential of essential oils against *Musca domestica* and *Anopheles stephensi*. Parasitol. Res. 115: 2223–2231.

Cilek, J. E., and G. L. Greene. 1994. Stable fly (Diptera: Muscidae) insecticide resistance in Kansas cattle feedlots. J. Econ. Entomol. 87: 275–279.

Cloyd, R. A., C. L. Galle, S. R. Keith, N. A. Kalscheur, and K. E. Kemp. 2009. Effect of commercially available plant-derived essential oil products on arthropod pests. J. Econ. Entomol. 102: 1567–1579.

Ditzen, M. M. Pellegrino, and L. B. Vosshall. 2008. Insect odorant receptors are molecular targets of the insect repellent DEET. Science. 319: 1838–1842.

Dogan, E. B., and P. A. Rossignol. 1999. An olfactometer for discriminating between attraction, inhibition, and repellency in mosquitoes (Diptera: Culicidae). J. Med. Entomol. 36: 788–793.

Elbermawy, S. M., N. A. I. Elkattan, K. S. Ahmed, and R. M. Abdel-Gawad. 2011. Screening for bioactive plant materials against house fly, *Musca domestica* L. (Diptera: Muscidae). Funct. Plant Sci. Biotechnol. 5: 45–61.

Geden, C. J. 2012. Status of biopesticides for control of house flies. J. Biopest. 5(suppl.): 1–11.

Gjullin, C. M., and V. D. Bevill. 1972. Insect chilling table. J. Med. Entomol. 9: 266–267.

Harris, R. L., E. D. Frazar, and R. A. Hoffman. 1965. Chilling vs. other methods of immobilizing flies. J. Econ. Entomol. 58: 379–380.

Haselton, A. T., A. Acevedo, J. Kuruvilla, E. Werner, J. Kiernan, and P. Dhar. 2015. Repellency of α-pinene against the house fly, *Musca domestica*. Phytochemistry. 117: 469–475.

Hollister, B., E. A. Cameron, and D. A. J. Teulon. 1995. Effect of *p*-anisaldehyde and a yellow color on behavior and capture of western flower thrips, pp. 571–574. *In* B. C. Parker, M. Skinner, and T. Lewis (eds.), Thrips biology and management, series, vol. 276. Springer, New York.

Hung, K., and A. Gerry. 2013. House fly, (*Musca domestica* L.). Insect pests of animals. Available from http://veterinaryentomology.ucr.edu/house_fly.html

Imai, T., M. Maekawa, and S. Tsuchiya. 2002. Attractiveness of *p*-anisaldehyde to the varied carpet beetle, *Anthrenus verbasci* (L.) (Coleoptera: Dermestidae). Appl. Entomol. Zool. 37: 505–508.

James, M. T., and R. F. Harwood. 1969. Herms's medical entomology. McMillan, New York.

Kawazu, K., M. Ariwa, and Y. Kii. 1977. An ovicidal substance, *cis*-dehydromatricaria ester from *Solidago altissima*. Agric. Biol. Chem. 41: 223–224.

Khalequzzaman, M., H. Ara, F. Zohura, and J. Naher. 2002. Toxic, repellent and attractant properties of some insecticides towards the housefly (*Musca domestica* L.). Online J. Biol. Sci. 2: 672–676.

Khan, M. F., and S. M. Ahmed. 2000. Toxicity of crude neem leaf extract against housefly *Musca domestica* L. adults as compared with DDVP, dichlorvos. Turk. J. Zool. 4: 219–223.

Kim, I. S., X. Ren, J.-S. Chang, J. W. Lee, S.-J. Kim, J. S. Heo, A. Jang, and J. J. Han. 2011. The effect of environmental micropollutant (DEET) on the expression of cell cycle and apoptosis regulatory proteins in human cells. Biotechnol. Bioprocess Eng. 16: 400–406.

Kumar, P., S. Mishra, A. Malik, and S. Satya. 2011. Repellent, larvicidal and pupicidal properties of essential oils and their formulations against the housefly, *Musca domestica*. Med. Vet. Entomol. 25: 302–310.

Kunz, S. E., M. O. Estrada, and H. F. Sanchez. 1995. Status of *Haematobia irritans* (Diptera: Muscidae) insecticide resistance in northeastern Mexico. J. Med. Entomol. 32: 726–729.

Lee, H.-S. 2004. *p*-Anisaldehyde: acaricidal component of *Pimpinella anisum* seed oil against the house dust mites *Dermatophagoides farina* and *Dermatophagoides pteronyssinums*. Planta Medica. 70: 279–281. Available from https://www.thieme-connect.de/products/ejournals/html/10.1055/s-2004–818925

Lee, Y., S. H. Kim, and C. Montell. 2010. Avoiding DEET through insect gustatory receptors. Neuron. 67: 555–561.

LeOra Software. 2005. POLO-plus, POLO for windows. LeOra Software, Petaluma, CA.

Levine, O. S., and M. M. Levine. 1991. Houseflies (*Musca domestica*) as mechanical vectors of shigellosis. Rev. Infect. Dis. 13: 688–696.

Marçon, P. C., G. D. Thomas, B. D. Siegfried, and J. B. Campbell. 1997. Susceptibility of stable flies (Diptera: Muscidae) from southeastern Nebraska beef cattle feedlots to selected insecticides and comparison of 3 bioassay techniques. J. Econ. Entomol. 90: 293–298.

Miller, R. 1993. The influence of dairy operations on the urban fly problem, pp. 25–29. *In* G. D. Thomas and S. R. Skoda (eds.), Rural flies in the urban environment? Res. Bull. No. 317, University of Nebraska, Lincoln, NB.

Ndomo, A. F., L. A. Tapondjou, and F. M. Tchouanguep. 2010. Insecticidal effect of anisaldehyde against *Acanthoscelides obtectus* and *Callosobruchus maculatus* (Coleoptera: Bruchidae), pp. 735–740. *In* 10th International Working Conference on Stored Product Protection 27 June–2 July 2010. Estoril, Portugal.

Nerio, L. S., J. Olivero-Verbel, and E. Stashenko. 2010. Repellent activity of essential oils: a review. Bioresour. Technol. 101: 372–378.

Ordanza-Cortes, A. 2015. Insecticidal effects of leaves, flowers and fruits of *Lantana camara* Linn. (Philippine kantutay) on *Musca domestica* L. (Philippine houseflies). J. Nat. Prod. Plant Resour. 5: 26–35.

Oyarzún, M. P., A. Quiroz, and M. A. Birkett. 2008. Insecticide resistance in the horn fly: alternative control strategies. Med. Vet. Entomol. 22: 188–202.

Palacios, S. M., A. Bertoni, Y. Rossi, R. Santander, and A. Urzúa. 2009a. Efficacy of essential oils from edible plants as insecticides against the house fly, *Musca domestica* L. Molecules. 14: 1938–1947.

Palacios, S. M., A. Bertoni, Y. Rossi, R. Santander, and A. Urzúa. 2009b. Insecticidal activity of essential oils from native medicinal plants of Central Argentina against the house fly, *Musca domestica* (L.). Parasitol. Res. 106: 207–212.

Park, I. K., K. S. Choi, D. H. Kim, I. H. Choi, L. S. Kim, W. C. Bak, J. W. Choi, and S. C. Shin. 2006. Fumigant activity of plant essential oils and components from horseradish (*Armoracia rusticana*), anise (*Pimpinella anisum*) and garlic (*Allium sativum*) oils against *Lycoriella ingenua* (Diptera: Sciaridae). Pest Manag. Sci. 62: 723–728.

Pavela, R. 2008. Insecticidal properties of several essential oils on the house fly (*Musca domestica* L.). Phytother. Res. 22: 274–278.

Qiu, H., H. W. Jun, and J. W. McCall. 1998. Pharmacokinetics, formulation, and safety of insect repellent *N*,*N*-diethyl-3-methylbenzamide (deet): a review. J. Am. Mosq. Control Assoc. 14: 12–27.

Rice, P. J., and J. R. Coats. 1994. Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 87: 1172–1179.

Rossi, Y. E., and S. M. Palacios. 2015. Insecticidal toxicity of *Eucalyptus cinerea* essential oil and 1,8-cineole against *Musca domestica* and possible uses according to the metabolic response of flies. Indust. Crops Products. 63: 133–137.

Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol. 35: 271–297.

Schmutterer, H. 1995. The neem tree, *Azadirachta indica* A. Juss., and other meliaceous plants: sources of unique natural products for integrated pest management, medicine, industry and other purposes. VCH Publishers, Weinheim, Germany.

Science Lab. 2013. Material safety data sheet *p*-anisaldehyde MSDS. Available from www.sciencelab.com

Showler, A. T., and J. L. Harlien. 2017a. Botanical compound *p*-anisaldehyde deters larval lone star tick, *Amblyomma americanum* (L.) (Acari: Ixodidae), and halts reproduction by gravid females. J. Med. Entomol. 55: 200–209.

Showler, A. T., and J. L. Harlien. 2017b. Effects of the botanical compound *p*-anisaldehyde on horn fly, *Haematobia irritans irritans* (L.) (Diptera: Muscidae) repellency, mortality, and reproduction. J. Med Entomol. 55: 183–192.

da Silva, J. J., and J. Mendes. 2002. Effect of diflubenzuron on immature stages of *Haematobia irritans* (L.) (Diptera: Muscidae) in Uberlandia, State of Minas Gerais, Brazil. Mem. Inst. Oswaldo Cruz. 97: 679–682.

Singh, D., and A. K. Singh. 1991. Repellent and insecticidal properties of essential oils against housefly, *Musca domestica* L. Insect Sci. Applic. 12: 487–491.

Singh, S. P., A. Azua, A. Chaudhary, S. Khan, K. L. Willett, and P. R. Gardinali. 2010. Occurrence and distribution of steroids, hormones and selected pharmaceuticals in South Florida coastal environments. Ecotoxicology. 19: 338–350.

Sinthusiri, J., and M. Soonwera. 2014. Oviposition deterrent and ovicidal activities of seven herbal essential oils against female adults of housefly, *Musca domestica* L. Parasitol. Res. 113: 3015–3022.

Sudakin, D. L., and W. R. Trevathan. 2003. DEET: a review and update of safety and risk in the general population. J. Toxicol. Clin. Toxicol. 41: 831–839.

Tarelli, G., E. N. Zerba, and R. A. Alzogaray. 2009. Toxicity to vapor exposure and topical application of essential oils and monoterpenes on *Musca domestica* (Diptera: Muscidae). J. Econ. Entomol. 102: 1383–1388.

Vazirianzadeh, B., S. S. Solary, M. Rahdar, R. Hajossein, and M. Mehdinejad. 2008. Identification of bacteria which are transmitted by *Musca domestica* (Diptera: Muscidae) in the region of Ahvaz, Iran. Jundishapur J. Microbiol. 1: 28–31.

Wimalaratne, P. D., K. N. Slessor, J. H. Borden, L. J. Chong, and T. Abate. 1996. Isolation and identification of house fly, *Musca domestica* L., repellents from pepper tree,*Schinus molle* L. J. Chem. Ecol. 22: 49–59.

Wright, J. E., and R. L. Harris. 1976. Ovicidal activity of Thompson-Hayward TH 6040 in the stable fly and horn fly after surface contact by adults. J. Econ. Entomol. 69: 728–730.

Zhu, J. J., X. P. Zeng, D. Berkebile, H. J. DU, Y. Tong, and K. Qian. 2009. Efficacy and safety of catnip (*Nepeta cataria*) as a novel filth fly repellent. Med. Vet. Entomol. 23: 209–216.

Zortéa, T., D. Baretta, A. Volpato, W. R. Lorenzetti, J. C. Segat, A. P. Maccari, R. C. Santos, R. A. Vaucher, L. M. Stefani, and A. S. da Silva. 2017. Repellent effects of andiroba and copaiba oils against *Musca domestica* (common house fly) and ecotoxicological effects on the environment. Acta Sci. Vet. 45: 1439.