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ORIGINAL RESEARCH ARTICLE

Use of lures with a mix of sweet and fetid odors for catching *Musca domestica* L. in domestic environments

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Abstract

The housefly, *Musca domestica* L. (Diptera; Muscidae), is an insect closely associated with human activities in urban and rural environments and is thus a crucial factor in the transmission of various fecal–oral pathogens. The use of traps for monitoring and controlling these species in indoors is often limited by the fetid nature of the chemical attractants commonly used. A recent study demonstrated the attraction of houseflies to terpenoids, which are perceived by humans as a sweet odor. The aim of this study was to test pleasant smelling compounds such as terpinolene, α-terpinene and linalool mixed with others (acetic, butyric, isovaleric and hexanoic acid, indole and dimethyl trisulfide) known to attract houseflies to obtain a lure that could be acceptable in domestic environments. Experiments were carried out in the laboratory, using olfactometer, and in two rooms of 32 m^3 and 108 m^3, each resembling domestic environments using trap bioassays. The results showed that the volatile blend tested elicited attraction in the olfactometer and increased the number of flies captured by the traps. In the smaller room, the lure demonstrated efficacy for two weeks from the start of the experiment, while in the larger room the number of captured flies was higher than in the control traps only during the first week. The results confirmed the attraction of the flies to the traps baited with the blend, the application of the lures in domestic environments can be taken into consideration as a new alternative tool for trapping this pest.

Keywords Housefly · Terpenes · Carboxylic acids · Nitrogen compounds · Sulfur compounds · Attractant

Introduction

The housefly [*Musca domestica* L. (Diptera: Muscidae)] is a well-known insect pest closely associated with human activities in urban and rural environments (Malik et al. 2007). The occurrence of this insect in domestic environments originates from hygiene failures and increases the potential for transmission of fecal–oral pathogens (Colacci et al. 2020). The housefly can develop on different decaying organic matters such as feces, urban wastes and animal carcasses (Quinn et al. 2007) and can, therefore, carry and transmit several pathogens (protozoa, bacteria, viruses) of numerous diseases including dysentery, typhus fever and cholera (Fotedar et al. 1992; Junqueira et al. 2017; Bahrndorff et al. 2017; Khamesipour et al. 2018). *Musca domestica* is usually controlled by application of chemical insecticides, leading to negative drawbacks including development of resistance (Walsh et al. 2001; Kaufman et al. 2010) and environmental contamination, the latter being particularly undesirable in the domestic environment. In this context, a crucial constituent of a successful housefly management program is the use of traps for catching *M. domestica* adults (Gerry 2020). The using of traps, baited with an attractant effective and suitable, is a tool of key importance for achieving optimal results in monitoring/controlling houseflies (Upakut et al. 2017).

Since the life cycle of *M. domestica* is strictly related to animal carcasses, feces and other substrates in decomposition, this insect is generally attracted to unpleasant odors. The possibility of using chemicals with these odors for trapping houseflies is limited by the "fetid" nature of these compounds (Cossè and Baker 1996; Zito et al. 2014) strongly

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undesired in domestic environments and other urban contexts. These fetid components exclude the indoor use of such baits, which are mainly restricted to outdoors environments (Quinn et al. 2007). Studies on the chemical ecology of *M. domestica* highlighted that volatile organic compounds (VOCs) emitted from sapromyiophilous plants can attract houseflies as pollinators (Zito et al. 2013, 2015). In particular, Zito et al. (2013) demonstrated that *M. domestica* can be attracted not only by "fetid" odors but also to "sweet" ones determined by terpenoids (e.g. linalool, α-terpinene and terpinolene) produced from the flowers of *Caralluma europaea* (Guss.) N.E. Br. Therefore, the potential use of these terpenes, which are not unpleasant/noxious for people (Sharmeen et al. 2021), for trapping *M. domestica* in domestic environments has opened a new route for the control/monitoring of this species. In particular, these attractants could be used to bait traps already used in domestic environments (e.g. those that exploit the attraction of houseflies for light or particular colors) (Diclaro et al. 2012) by synergizing the visual stimuli with a semiochemical-based attractant as observed in other studies (Geden 2006; Geden et al. 2009).

The objective of this study was to evaluate in both laboratory and domestic environments the efficacy of an attractant consisting of such "sweet" volatile organic compounds, i.e. linalool, α-terpinene and terpinolene, when mixed with other chemicals reported from scientific literature as carboxylic acids and nitrogen and sulfur compounds typical of organic decay (Cossè and Baker 1996; Zito et al. 2014; Upakut et al. 2017). This will enable the creation of an effective lure for *M. domestica* adults with sensory acceptability by general public, thus making it suitable for use in domestic environments. To achieve this the candidate attractant was tested initially in laboratory bioassays and then in trapping experiments in two rooms resembling domestic/industrial environments where such traps would most likely be used.

Materials and methods

Insect

The colony of houseflies used for experiments was established and restocked regularly in Gea S.r.L. (Settimo Milanese, Milan, Italy) in a climatic room at the 23 ± 1 °C with a relative humidity (RH) of $40 \pm 10\%$ and a photoperiod of 12:12 (L:D). Housefly larvae were kept in rectangular containers of 740 ml and were fed with a standard fly rearing medium made with an 8:2 mixture of bran:powdered milk soaked with water. Pupae were collected and put in plastic containers ($7 \times 7 \times 10$ cm) inside wooden cages ($25 \times 25 \times 40$ cm) with two mesh-covered holes for ventilation. Cages were kept until the emergence of the adult flies in an environmentally controlled room (23 ± 1 °C, $70 \pm 10\%$ R.H., photoperiod 16:8 h). Adult houseflies (50 individuals per cage) were fed using a 1:1 mixture of sugar and dry powdered milk. Water was supplied as needed. For the experiments newly emerged unsexed flies were used. Insects were collected through a small entrance present in the side of the cage and placed in 10 mL plastic jars until used for the experiments.

Attractant

The attractant tested in this study was made from a mixture of nine compounds belonging to the chemical classes of monoterpenes (linalool, terpinolene and α-terpinene), carboxylic acids (acetic, butyric, isovaleric and hexanoic acid), nitrogen (indole) and sulfur compounds (dimethyl trisulfide). All chemicals (> 99% pure) were provided by Sigma-Aldrich (Milan, Italy). The chemicals were serially diluted in 1:10 (v/v) solutions of acetone and gently pipetted on the brown rubber septa (10 mm O.D.) (Sigma-Aldrich, Milan, Italy) with a precision pipette (Gilson, Limburg-Offheim, Germany) to reach the desired doses tested (see below). Dispensers were kept half an hour under the vacuum cabin for solvent evaporation before the experiments. In olfactometer experiments compounds were tested at the two doses of 0.01 mg and 0.1 mg per component. In room experiments compounds were tested at 1 mg per component. We decided to use these doses as for some of the compounds (e.g. terpinolene, linalool and α-terpinene) of the blend were found to elicit the highest EAG-activities (Zito et al. 2013). In both experiments, dimethyl trisulfide, a strong attractant but characterized by an unpleasant odor, was added to the dispenser test at a lower dose (0.001 mg). To achieve this a series of acetone serial dilutions (1:10 v/v) were provided and pipetted to achieve the desired dose. As test, 10 µl of each solution of the nine compounds tested were pipetted in a rubber septum releaser (90 µl total); as control, 90 µl of acetone were used in the other releaser. After preparation, the dispensers were left in the vacuum cabin for 2 h to allow the complete solvent evaporation.

Two choice bioassays

Laboratory experiments were carried out using a two-choice olfactometer schematized in Fig. 1. The device used for the behavioral experiments was based on that

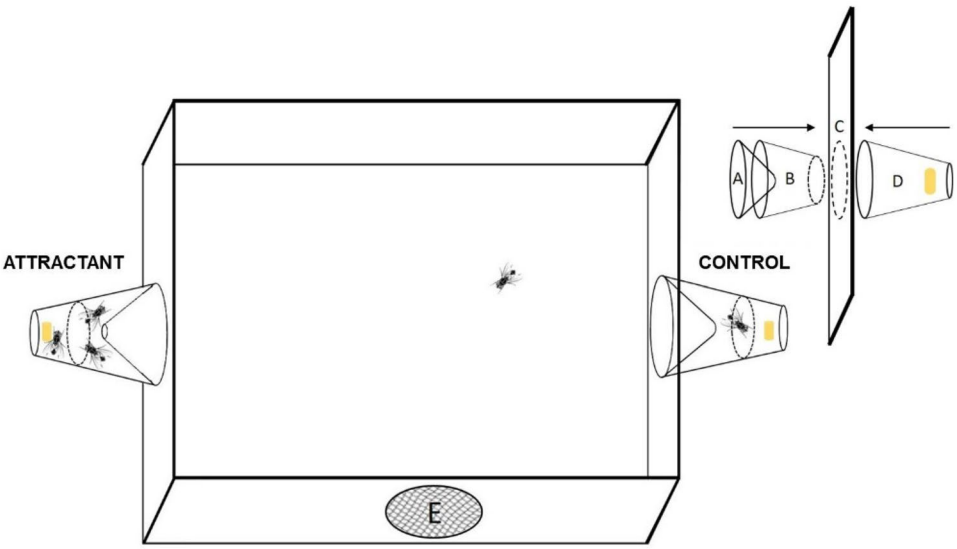

Fig. 1 Schematic drawing of the two-choice olfactometer used for laboratory bioassays (modified from Zito et al. 2015); **A** paper cone; **B** plastic cup; **C** olfactometer wall; **D** plastic cup containing the releaser; **E** entrance hole)

described by Zito et al. (2015). It consisted of a glass chamber (26 × 17 × 13 cm) covered by a glass lid. Each external side of the chamber was covered with white printer paper to eliminate potential distractions to the flies and to diffuse the light coming from the lamp positioned 1 m above the top of chamber, as described in wind tunnel experiments by Cossè and Baker (1996). Two pairs of white plastic cups (diameter 1 = 6.5 cm; diameter 2 = 4 cm, height = 8 cm) were used as olfactometer arms. For each arm, a white cone (entrance diameter = 6.5 cm, exit diameter = 0.6 cm, height = 3 cm, 60° slope) made of printer paper was placed in a plastic cup cut at the bottom and held in place using adhesive tape. The cup and the cone assemblage was connected to the inner short side of the chamber. The second plastic cup was placed over the extruding open end of the first cup to prevent the insects escaping. A rubber septum dispenser with the attractant was placed inside the bottom of one plastic cup (test), while the one loaded with solvent was placed in the other (control). The position of test and control dispensers was switched after each replication.

Five houseflies were used for each replication with the number of choices scored after 24 h, as from preliminary observation we decided this as optimal time for an adequate numbers of responses. Before the start of the experiments, the flies were collected from the cage and kept in the olfactometer room for one hour to acclimatize. Eight replications were carried out for each dose. Insects present in the central chamber (approx. 15% of the total) were scored as non-responders and not included in the statistics. At the end of each replication the apparatus was gently cleaned with a rag soaked with water and alcohol and dried using a hair-drier. Plastic cups were replaced after each replication.

Bioassays in domestic environments

In order to test the attractant in a manner resembling domestic conditions, trap catch experiments were carried out in two different rooms of 32 m^3 and 108 m^3, hereafter called respectively 'room A' and 'room B', schematically drawn in Fig. 2 (A and B). Room A was 4 m long, 4 m wide and 2 m high. Inside this room there were two windows (70 × 100 cm) with the same exposition, and oriented south-west (Fig. 2A). Pairs of traps (test and control) were put on each window, centred from the sides and at an altitude of 15 cm from the bottom of the window.

Room B was 9 m long, 4 m wide and 3 m high. Inside this room there was one window (120 × 130 cm) oriented at north-west (Fig. 2B). The test and control traps were put on each section of the window, at an altitude of 15 cm from the bottom of the window. The distance from the traps was 1.40 m in room A and 0.35 m in room B. Experiments were carried out at room temperature (22 ± 2 °C) and relative humidity (45 ± 10%). The rooms were not artificially illuminated, only natural light from windows was provided. The wooden entrance door was immediately closed after the houseflies' release and the operator's exit. Notwithstanding the different size of the two rooms where the bioassays were conducted, we decided to keep the same dosage of the lure of 1 mg per component in both environments, also to evaluate how a larger room can impact on the attractant performance.

The test and control dispensers, after loading and solvent evaporation, were placed with the use of metal forceps at the center of a window fly blade trap, European patent n. EP1331847 (Gea S.r.l.; Settimo Milanese, Italy) (Fig. 3). This trap consists of a transparent plastic rectangle with adhesive on one side (14 × 6,5 cm) and with a further adhesive border (1 cm) to glue the trap on the window as

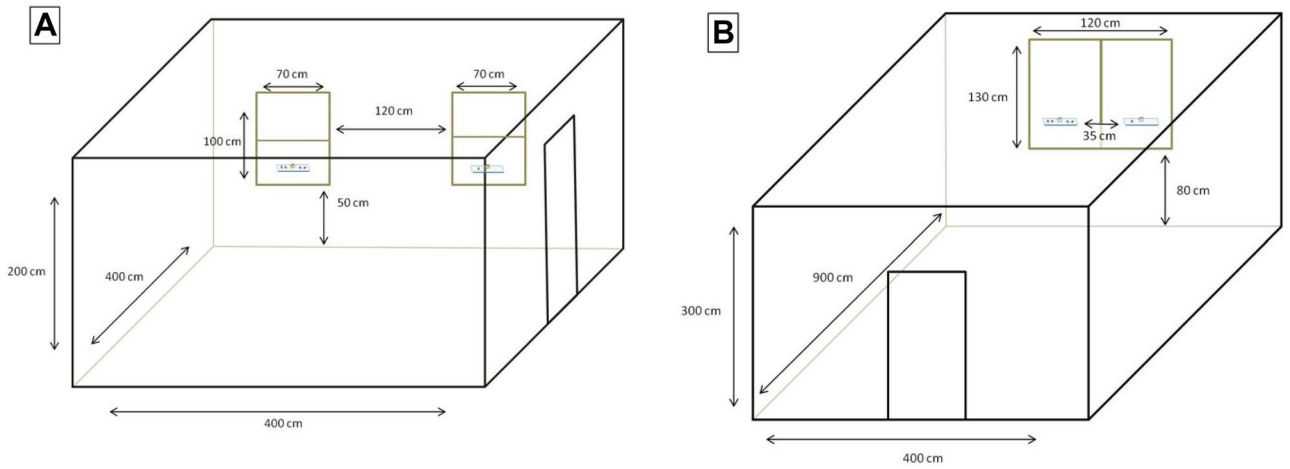

Fig. 2 (A) Room A: size 4×4×2 m. The room contained two windows (70×100 cm) facing south-west. On each window the team placed a Window Fly Blade loaded with the attractant (test) and the control (acetone). (B) Room B: size 9×4×3 m. The room contained a single window (120×130 cm) which faced north. On each side of the window the team placed a Window Fly Blade loaded with the attractant (test) and the control (acetone). Traps were positioned at a distance of 15 cm from the lower border of the window and with an angle of 45° between the adhesive surface of the trap and the window glass

designed to exploit natural light as visual stimuli. Each trap was oriented with the adhesive upper part and an inclination of 45° on the window glass. For every replication in each room were used approximately 100 newly emerged adult flies, released from the center of the room at the beginning of each week of experiment (300 adults in total). After each 24 h the adhesive rectangles were replaced daily and the captured houseflies in test and control traps were scored. The positions of the traps (test and control) were inverted after each replication to avoid position bias. To evaluate the effectiveness of the attractant over time each test was conducted over three weeks, with the experiment replicated three times, resulting in a total of nine weeks of tests carried out from 16th February 2016 to 15th April 2016. Data were pooled each week to establish the efficacy of the attractant during the first, second and third weeks of the experiment.

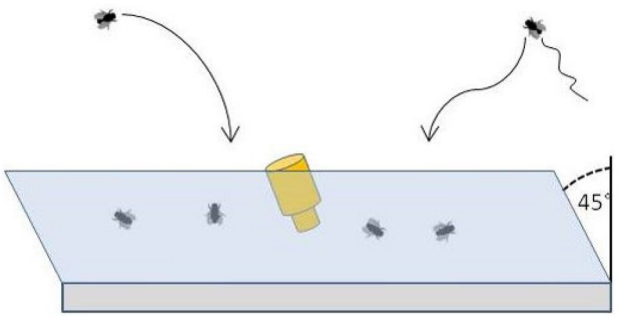

Fig. 3 Schematic drawing of the window fly blade trap loaded with a rubber septum releaser placed on the adhesive part

Statistical analysis

For laboratory experiment, the total number of choices, i.e. adult flies in test and control arm was statistically analysed using the chi-square (χ^2) test. Similarly, the data obtained from the bioassays in domestic environments, i.e. the number of adults flies captured in test and control traps, were statistically analysed using the chi-square (χ^2) test. The software used for the analysis was STATISTICA 10.0 (Statsoft, Vigonza, PD, Italy).

Results

Two choice bioassays

The results of the bioassays are shown in Fig. 4. Housefly trap catches were significantly higher at the test dose of 0.1 mg per component compared with the control dose ($\chi^2 = 6.08$; $p = 0.013$). The dose of 0.01 mg per component did not demonstrate any increased attraction when compared with the control ($\chi^2 = 2.13$; $p = 0.144$).

Bioassays in domestic environments

The results for total captures (expressed as a percentage of choice) in the window fly blades baited with attractant or control are shown in Fig. 5. Generally, the traps loaded with the attractant demonstrate a better number of catches in both rooms and in every week of the experiment.

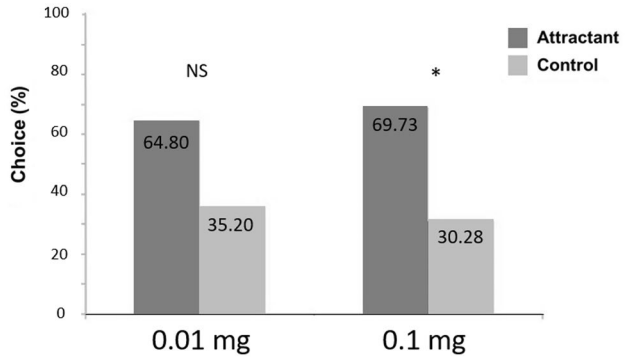

Fig. 4 Results of the two choice olfactometer bioassays expressed as a percentage of choice between test and control arm. Attractant: linalool, terpinolene, α-terpinene, acetic, butyric, isovaleric and hexanoic acid, indole and dimethyl trisulfide 0.001 mg. Asterisk indicates a significant statistic difference ($p < 0.05$); NS = not significant

In room A, the trap loaded with the attractant captured a higher number of catches and significantly different in comparison with the control during the first ($\chi^2 = 5.72$; $p = 0.016$) and the second ($\chi^2 = 4.78$; $p = 0.028$) week of the experiment, while in the third week no statistical differences were recorded ($\chi^2 = 1.92$; $p = 0.165$). In room B the traps loaded with the attractant saw a higher number of catches than the control trap during the first week ($\chi^2 = 8.44$; $p = 0.003$), while no statistical differences were observed during the second ($\chi^2 = 2.25$; $p = 0.133$) and third week ($\chi^2 = 0.64$; $p = 0.422$) of experiments.

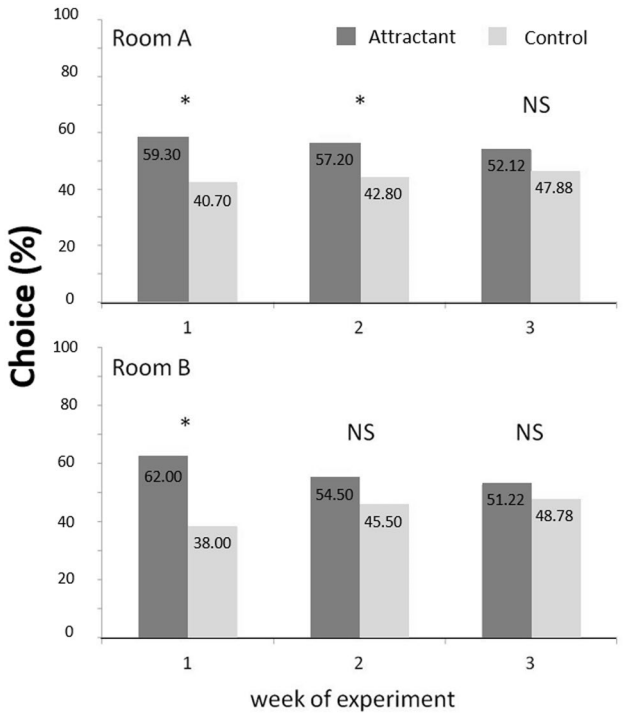

Fig. 5 Bioassays in domestic environments; room A, 32 m^3; room B, 108 m^3. Percentage of choice (captures) in the weeks of experiments between the window fly blade trap baited with the attractant (test compounds) and control (acetone). Asterisks indicate statistically significant results ($p < 0.05$); NS = not significant

Discussion

The results obtained in both laboratory and domestic environments indicated a positive response of the *M. domestica* adults toward the tested attractant and candidate this lure as useful tool for houseflies' capture in domestic environments. Two choice olfactometer data showed positive adult responses to the tested attractant at the dose of 0.1 mg per component of the blend, while at the dose of 0.01 mg, the data were not statistically significant. The trapping test carried out in domestic environments confirmed attraction of the houseflies toward the attractant in both rooms used for the experiments. The efficacy of the attractant was statistically significant for two weeks in small room (i.e., room A) and for one week in large room (i.e., room B).

The positive response to the formulated blend is related to the nature of its chemical components, already reported as attractants for houseflies. For example, indole and dimethyl trisulfide have been reported to attract houseflies and are present in the volatile blend of pig manure (Cossè and Baker 1996; Zito et al. 2014). The majority of studies on *M. domestica* attractants have been carried out in open environments where the use of lures with unpleasant odors, mimicking the houseflies' food and oviposition sites is suitable (Qian et al. 2013; Landolt et al. 2015). Such studies evidenced the response of muscid flies to carboxylic acids, such as acetic acid, butyric acid, isovaleric acid or hexanoic acid, typical components of fermenting products (Qian et al. 2013; Landolt et al. 2015). Recent studies about oviposition sources exploited by houseflies evidenced that some carboxylic acids and esters elicit attractive responses particularly in *M. domestica* females (Tang et al. 2016). The other compounds present in the attractant tested (i.e. linalool, *α*-terpinene and terpinolene) determining housefly attraction toward flowers of a *C. europaea* (Guss.), they are generally considered "sweet" volatiles rather than "fetid" (Zito et al. 2013). These volatiles can play an important role in improving not only the effectiveness of such attractants but also the acceptability of this for the consumer in domestic conditions.

The results obtained in our study evidenced a decrease of the captures observed during the final weeks of the experiments is probably linked with the decrease of the amount of the attractants emitted due to the releaser aging, or to a change in the blend of the compound emitted due to the different volatility of the chemicals. Furthermore, the stronger decrease of the captures observed in the larger room (108

m^3) rather than in the smaller one (32 m^3) suggests that also the size of the environment can influence the attractant efficacy in terms of duration. In a larger space it is probable that the same amount of attractant is more diluted, reducing with time its ability to attract houseflies (Pickens et al. 1973; Pickens and Miller 1975; Tang et al. 2016).

It should be highlighted that the type of adhesive trap used in our study recorded a relatively high number of captures also in unbaited traps, due to their advantageous position on the window that permit the exploitation of the natural light attraction elicited commonly the houseflies. The combination of chemical and visual stimuli integrated in these traps loaded with the attractive blend tested could be further explored to evaluate possible synergic effect that can be successfully exploited for capturing houseflies more quickly. In addition, the use of such a mixture of synthetic chemicals is more practical than other types of attractants based on food sources containing simple sugars or proteins such as molasses, milk, yeast, grain, blood, rotten eggs, vinegar and banana extract (Willson and Mulla 1973; Pickens et al. 1973; Pickens and Miller 1975; Qian et al. 2013; Kannan et al. 2020). Finally, this tool can have better performance than traps commonly baited with *M. domestica* pheromone, (*Z*)-9-tricosene (Chapman et al. 1998) (mainly a short-range attractant), that had in some cases exhibited insufficient captures rates to provide adequate control (Hanley et al. 2004).

The data obtained in this study suggest that the use of such traps baited with an attractant (made by a mixture of attractive compounds already known in the scientific literature) can be considered a useful tool for monitoring *M. domestica* in domestic environments and can find application for mass trapping where other control methods, such as the use of chemical sprays, is particularly undesirable. We understand that the restricted number of combinations of chemicals tested determines some limitations in this study. To fill this gap, our efforts will concentrate on testing the attractant assayed in this study at different doses or in different chemical ratios, in order to enhance its effectiveness in wider environments and further reduce the fraction of fetid components. Contextually, more chemicals with pleasant odors could be incorporated in such blend; recent studies in fact, indicated promising attractant properties of the good smelling volatiles benzaldehyde and (*Z*)-3-hexenyl acetate versus *M. domestica* (Hung et al. 2020).

Knowledge of the most suitable bait to attract adult houseflies is essential in designing an efficient control strategy (Gerry 2020). These tools can find application together with other common practices that include sanitation and hygiene maintenance to achieve a reliable IPM control program of these pests (Colacci et al. 2020). Further studies are in progress to assess the possibility of using this attractant in different trap devices and/or in other conditions such as in artificially illuminated indoor environments. Finally, future studies will focus on comparing the efficacy of the lure tested in this study with traditional lures, based on fetid odors.

Conclusions

This study demonstrated the possibility of using an attractant for houseflies made by a mix of odors (sweet and fetid) that could be acceptable in domestic environments. This trap, using a combination of visual (natural light) and olfaction stimuli can be considered as a potential tool for monitoring and mass trapping of *M. domestica*. This tool can find application in locations as hospitals, schools or houses where other control methods such as the use of insecticides or traditional houseflies' traps baited with unpleasant odors are not suitable. Future studies will be carried out to evaluate the effectiveness of this attractant in comparison with the traditional housefly commercial lures based on fetid attractants and to investigate the possibility of using a lure based only on sweet terpenoids.

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Author contributions Conceptualization, S.G., P.Z., M.C.; methodology, S.G., P.Z.; formal analysis, S.G., P.Z.; investigation S.G., M.A.A., M.C., P.Z.; data curation, S.G., P.Z.; writing—original draft preparation, S.G.; All the authors have visualized and reviewed the manuscript.

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Declarations

Conflicts of interest The author Mokhtar Abdulsattar Arif declare he has no financial interests. The author Salvatore Guarino is associate editor for International Journal of Tropical Insect Science and might have a potential conflict of interest. The authors Marco Caimi and Pietro Zito declare that they are or have been employed in Gea SrL and might have a potential conflict of interests.

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