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BRIEF REPORT

Dose-dependent responses: a preliminary investigation into the olfactory effects of essential oil concentrations on canine behavior

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Abstract

The positive impact of essential oils (EOs) on stress release has been demonstrated in both humans and dogs. Among the EOs known for their anxiety-reducing properties, including *Cananga odorata, Citrus aurantium, Cupressus sempervirens, Lavandula angustifolia, and Litsea citrata*, there is a lack of consensus on the optimal concentration for efficacy. This exploratory study sought to investigate the effects of olfactory enrichment with a blend of these EOs on dogs introduced to an unfamiliar environment. The authors sought to determine the minimum concentration required to achieve increased relaxation. In a randomized controlled crossover study design, 54 dogs were exposed to 0, 1, 5, and 10 drops of the EO blend applied to their collars before entering an unfamiliar room with their owners. Behavioral observations were employed to quantify the total duration of activity and relaxation related behaviours for each dog under each treatment condition. A significant difference in panting was identified among the treatments ($\chi2(3) = 9.88$; $p = 0.020$). Dunn-Bonferroni post-hoc tests revealed a significant reduction in panting during the 10 drops treatment compared to the control treatment ($p = 0.047$). No significant differences were observed for other behaviors. To provide a comprehensive overview of behavioral tendencies in this canine population, owners also completed the Canine Behavioral Assessment and Research Questionnaire (C-BARQ), revealing low scores for anxiety in the study group. These preliminary findings suggest that a concentration of 10 drops of the EO blend on a dog's collar induces increased relaxation, specifically reflected in decreased panting behavior. Lower concentrations did not exhibit a significant relationship with the observed behaviors. These initial findings underscore the importance of exposing dogs to an appropriate concentration of EOs when exploring their potential benefits on welfare among dogs with low anxiety levels. Further research in this area is crucial for a comprehensive understanding of the potential benefits of EOs for canine welfare.

Keywords Dog · Relaxation · Olfactory enrichment · Essential oils · Behavior

Introduction

Despite the provision of commendable care, dogs frequently encounter situations where stress becomes a pervasive factor. Given the established association of stress with negative emotional states, its influence can induce a profound impact on the overall quality of life for dogs (Hall et al. 2019). Furthermore, stress has been identified as a potential catalyst for severe health complications in dogs, encompassing cardiovascular disease, insulin insensitivity, and cancer (Rohleder 2019). To prevent emotional and physical diseases and increase dog's welfare, it becomes imperative to proactively help dealing with stressors in their living environments (Protopopova 2016).

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The assessment of stress encompasses a range of physiological parameters, including heart rate, temperature, immune responses, and hormone levels measured in saliva, blood, and urine. Alternatively, the observation of canine behavior by researchers provides a less invasive yet informative avenue for stress evaluation (Mariti et al. 2012). Recognized behavioral indicators such as vocalization, yawning, a lowered posture, and panting are widely acknowledged manifestations of stress in dogs (Horwitz and Mills 2009). Beerda et al. (1998) conducted a study revealing that dogs with moderate stress levels exhibited increased restlessness, oral behaviors, yawning, and open mouth expressions, coupled with a concurrent decrease in their overall body posture. Notably, when introduced to unfamiliar environments, dogs may exhibit signs of stress and agitation, as evidenced by reduced periods of lying down, sleeping, and resting, and an increased proportion of time spent in sitting, standing, walking, and panting (Part et al. 2014). These behavioral patterns serve as valuable indicators in understanding the nuanced responses of dogs to unfamiliar stimuli, contributing to a comprehensive assessment of stress levels.

Essential Oils (EOs) have been recognized for their mood-altering effects in both human and animal studies, demonstrating efficacy in alleviating symptoms of stress, anxiety and depression and in improving mood (Setzer 2009; De Sousa et al. 2015; Nadeem et al. 2018; Kwangjai et al. 2021). Given the exceptionally sensitive olfactory organs of dogs (Binks et al. 2018) and the volatile nature of EOs, which facilitates their airborne dispersion (Srivastava et al. 2019), these aromatic compounds can serve as effective olfactory enrichment for canines (Srivastava et al. 2019). In recent years, there has been a growing interest in investigating the anxiolytic effects of EOs in dogs, with several studies suggesting that specific EOs may alleviate acute stress (Graham et al. 2005; Uccheddu et al. 2018; Haverbeke et al. 2019). This growing body of research suggests that EOs hold promise for positively influencing the well-being of dogs. Depending on the intended purpose, EOs can be applied individually or in combination with others to create blends with diverse properties or to amplify specific effects (Srivastava et al. 2019). This versatility underscores the potential of EOs as a nuanced and customizable tool for promoting positive behavioral and emotional outcomes in dogs. The five EOs mentioned below were selected in this study because they have traditionally been used to treat a variety of ailments, including anxiousness. Some of these EOs have undergone extensive research, while others have not yet had their anxiolytic properties fully examined. These five EOs were also used in a blend of EOs as olfactory enrichment in shelter dogs in a study by Uccheddu et al. (2018). Olfactory enrichment using the blend resulted in the dogs having a more positive bias and thus potentially improved well-being (Haverbeke et al. 2019).

Cananga odorata EO is widely employed for promoting relaxation and mood modulation in humans. Its anxiolytic effects have been demonstrated in mice through various behavioral tests that gauge instinctive responses to novel environments (Zhang et al. 2016). Moreover, this EO has exhibited notable analgesic and anxiolytic-like properties in murine models with neuropathic pain, suggesting its potential therapeutic relevance in the context of pain management and anxiety-related conditions (Borgonetti et al. 2022).

Citrus aurantium EO is recognized for its sedative and relaxing effects in rat models (Leite et al. 2008) and its anxiolytic properties in mice (de Moraes Pultrini et al. 2006). Additionally, various studies have demonstrated its capacity to alleviate pre-operative anxiety in humans (Mannucci et al. 2018).

Cupressus sempervirens EO, derived from both branches and fruits, exhibits robust antioxidant and antiglycation properties. These attributes suggest a potential role in preventing complications associated with Advanced Glycation Endproducts (AGEs) as ethiopathological factors, notably in conditions like atherosclerosis, Alzheimer's disease, and diabetes (Asgary et al. 2013). Notably, the presence of AGEs is correlated with symptoms of anxiety and depression (Ljubičić et al. 2020), providing a plausible explanation for the potential anxiolytic effect associated with Cupressus sempervirens EO.

Lavandula angustifolia EO not only exhibits promising results in alleviating anxiety among dementia patients but also demonstrates anti-inflammatory and analgesic properties (Hajhashemi et al. 2003; Holmes and Ballard 2004; Karaman et al. 2016). The potential benefits of lavender extend to mood enhancement, improved sleep quality, and the mitigation of anxiety and depression (Setzer 2009; Karadag et al. 2017). Takahashi et al. (2014) have elucidated the involvement of the serotonergic system in the anxiolytic effect of lavender EO, observed in both normosmic and anosmic mice. In a study by Graham et al. (2005) investigating the impact of various EOs (including Lavandula angustifolia, Anthennis nobilis, Cymbopogon citrates, and Mentha piperita) on kennel-housed dogs, those exposed to lavender exhibited increased rest and reduced motion compared to those exposed to rosemary and peppermint or without EO application. Furthermore, Wells (2006) explored the effect of lavender on travel excitement in dogs, revealing that dogs exposed to lavender spent more time in restful and seated positions, while exhibiting less vocalization and movement compared to the control group.

Litsea citrata EO was investigated as part of a blend with other EOs in a study conducted by Haverbeke et al. (2019). The implementation of olfactory enrichment using this blend resulted in dogs assuming a high posture for a shorter duration during the study compared to instances when the blend was not employed. This observation suggests a potential indication of increased relaxation among the dogs when exposed to the EO blend.

These collective findings underscore the multifaceted positive impact of these EOs, not only in addressing anxiety-related issues but also in promoting relaxation, restfulness, and behavioral calm in diverse settings.

The current study aims to investigate relaxation-enhancing effects of a blend of EOs - specifically *Cananga odorata*, *Citrus aurantium*, *Cupressus sempervirens*, *Lavandula angustifolia*, *and Litsea citrata* - on dogs placed in an unfamiliar environment. The assessment of relaxation was conducted by observing predefined behaviors commonly associated with activity and calmness in dogs.

Additionally, the study aimed to ascertain the optimal concentration of the EO blend, quantified by the number of drops administered, that would exert the most significant influence on canine behavior.

To achieve these goals, the canine population was subjected to varying sequences of 0, 1, 5, and 10 drops of EOs. Comparative analysis was then performed to evaluate the efficacy of these treatments in relaxation associated behaviors. The composition of the EO blend, mirroring that of a previous study by Haverbeke et al. (2022), remained consistent, ensuring continuity in research methodology.

Owners of the participating dogs were required to complete a Canine Behavioral Assessment and Research Questionnaire (C-BARQ), providing a comprehensive overview of their dogs' temperaments. Additionally, the collated C-BARQ results can also facilitate future comparisons with other dog populations in subsequent studies.

By focusing on the enhancement of relaxation behaviors, the study aims to identify effective strategies for olfactory enrichment that can improve dog's welfare, particularly in unfamiliar environments. Anticipated outcomes included the expectation that increased EO concentrations would accelerate the transition towards relaxation-related behavior.

Materials and methods

Trial design

The study was conducted at a veterinary behavioral practice in Overijse, Belgium (Vet Ethology), with data collection spanning from March 2020 to October 2020. All dog owners were approached and invited to voluntarily participate in the study, resulting in the inclusion of a total of 54 dogs. To exclude potential order effects, a crossover study design was implemented, considering the owners' availability.

Table 1 Randomized controlled crossover study design ($n = 54$)

	Group 1 ($n = 10$)	Group 2 ($n = 11$)	Group 3 ($n = 13$)	Group 4 ($n = 10$)	Group 5 ($n = 10$)
Session 1	0	1	5	10	0
Session 2	10	0	1	5	10
Session 3	1	5	10	0	5
Session 4	5	10	0	1	1

The dogs were randomly assigned to one of five groups using a randomized controlled crossover design. To avoid an effect of order, each group was exposed to varying concentrations of EOs (0, 1, 5 or 10 drops) in a unique order. Each dog participated at four 20-minutes sessions, with a minimum of seven days between each session (Table 1).

The drops, comprising a blend of EOs (*Cananga odorata*, *Citrus aurantium*, *Cupressus sempervirens*, *Lavandula angustifolia*, *Litsea citrata*), were applied to a piece of cotton using a pipette. The experimental environment maintained a consistent temperature range of 18 °C to 23 °C throughout the study.

High-quality dropper bottles were utilized with consistent droplet sizes to ensure precise application of the essential oil blend. This minimized volume variations across different test subjects. Additionally, by specifying volumes associated with each drop (0.05 ml per drop), the authors aimed to mitigate potential dosage discrepancies.

The experimental protocol followed a structured format. Upon arrival, owners and dogs were conducted directly into a designated 20m2 test room, minimizing waiting times and preventing interactions between different dogs and owners. The room, equipped with a chair, had shielded windows to minimize external stimuli. Dogs were leashed to a chair by their collars, granting them freedom to move within a 2-meter radius.

The session commenced with a brief welcome, introductions to the researchers, and an explanation of the protocol. Owners were instructed to sit on the chair for 20 min with no interaction with their dogs. Literature was provided, and cell phone use was permitted, excluding phone calls. The experimental purpose was clarified at the first session. A 15 cm length piece of cotton, to which drops were applied, was secured to the bottom of the collar in the test room, even during sessions with no drops, to eliminate potential observer bias or the fabric's influence on the dog's behavior.

Following the briefing and preparation, the researcher initiated the camera, recorded the dog's name and test date, and documented its behavior for the designated 20-minute period. After completion, the recording ceased, the examination concluded, and both owner and dog were escorted back outside. This rigorous protocol ensured standardized testing conditions, contributing to the reliability and validity of the study's findings.

Dogs

The 54 dogs had ages between 1 and 12 years. A total of 24 males and 30 females participated in the study divided among the different groups. Of the 54 dogs, 25 were neutered and 29 were non-castrated. Each group in the study was representative of the dog population in terms of sex, hormonal status and age. The breed frequency of dogs in decreasing order is described in Table 2.

Essential oils

The EOs were obtained by steam distillation and purchased from the company Alanine Laboratory (EODIS, Belgium). The Biochemical composition and relative proportion of the constituents have been assessed by Gas Chromatograph with Mass Selective Detector for each EO (see Supplementary Information for the complete analyses reports).

Table 2 Breed frequency of dogs in decreasing order

Breed	Frequency
Border Collie	8
Golden Retriever	5
German Shepherd	4
Saarloos wolfhound	3
Labrador Retriever	3
English Cocker Spaniel	2
American Cocker Spaniel	2
White Swiss Shepherd	2
Nova Scotia Duck Tolling Retriever	2
French Bulldog	2
Spanish Greyhound	1
German Spitz	1
Rabbit dachshund corduroy hair	1
Teckel	1
Great Dane	1
Malinois Shepherd / Border Terrier	1
Podenco	1
Basset Hound	1
Podenco Andaluz	1
English Springer Spaniel	1
Malinois Shepherd/Cane Corso	1
German Longhaired Pointer	1
Dobberman	1
Jack Russel/ Maltese	1
Blue Bay Shepherd	1
Timber Shepherd	1
English Bulldog	1
Wirehaired Pointing Griffon	1
Pyrenean Mountain Dog	1
Pug	1
Appenzeller Sennenhund	1

The principal components of Lavender EO were linalyl acetate (34%), linalool (30%) and Lavandulyl acetate (3%).

Next to the anti-inflammatory and stress-releasing effects of linalyl acetate, (Shin et al. 2022) have discussed its utility in a stress management treatment among repeatedly stressed Ulcerative Colitis patients. Several studies have shown the anxiolytic (Harada et al. 2018) and sedative effects of linalool (de Moura Linck et al. 2009; Liu and Xu 2012). (Harada et al. 2018) examined the anxiolytic effects of linalool odor with light/dark box test and with elevated plus maze (EPM) and found that linalool odor has an anxiolytic effect without motor impairment in mice. Lavandulyl acetate has been described for its anti-repellent effects (Govindarajan and Benelli 2016), but so far, no anxiolytic effects have been reported.

The principal components of Ylang-Ylang EO were Germacrene D (21%), B-caryophyllène (17%), E, E-α-Farnesene (13%), Benzyl benzoate (6,85%) and linalool (4,42%).

Germacrenes are typically produced in a few plant species for their antimicrobial and insecticidal properties and can serve as a precursor for many other sesquiterpenes (Liu et al. 2022). Beta-caryophyllene (BCP) has been identified as one of the best terpenes to reduce anxiety. Hwang et al. (Hwang et al. 2020) observed that BCP improved chronic stress-related behavioral and biochemical changes in mice, concluding that BCP may be effective in treating depression and stress-related mental illnesses. Farnesene acts as a natural insect repellent (Cui et al. 2012), but its already field-reported sedative effects need still to be validated. Benzyl benzoate and linalool have shown individual anxiolytic effects in male mice (Zhang et al. 2016).

The most relevant components of Orange EO were linalyl acetate (53%), linalool (21%), and α-terpinéol (6%). It is interesting to note that its 2 major components are similar to those of Lavender EO.

Litsea citrata E.O. was mainly composed of geranial (41%, geranial is considered as citral), neral (32%), and d-limonene (12%). While geranial and neral are mainly known for their anti-inflammatory activities (Liao et al. 2015), stress-reducing activit*ies* were reported for citral (Dobetsberger and Buchbauer 2011) and d-limonene (Dobetsberger and Buchbauer 2011; d'Alessio et al. 2014).

Cypress EO was mainly composed of α-pinene (58%), δ-3-care (18%), and α-terpinolene (*2*%).

Weston-Green and colleagues reviewed the anti-anxiety and anti-depressant effects of α-pinene on humans (Weston-Green et al. 2021). D3-care is known to have hypnotic, anti-inflammatory, antioxidant, and anti-stress effects (Woo et al. 2019). Terpinolene is known for its sedative effects through oral administration, as well as through nasal absorption (Ito and Ito 2013).

The concentration of the 5 EOs in the blend (100% EO concentration, treatment 3) was proportionally the same, each EO was present for 20% in the blend.

It is important to acknowledge the potential side effects associated with the use of EOs. These complex EOs, each containing over 100 constituents, can pose risks, especially at high concentrations or if ingested orally. Reports have documented adverse events, including toxicity and even fatalities, linked to the ingestion of certain EOs. Furthermore, allergic reactions and dermatitis have been reported, particularly in individuals exposed to EOs through cosmetic and personal hygiene products. While our study focuses on the effects after olfactory exposure, it is essential to consider the broader context of safety concerns and weigh the potential benefits against the risks associated with their use (Posadzki et al. 2012).

Observations and ethogram

In each session, every dog underwent four 20-minute filming sessions, with continuous observation focused on behaviors outlined in the ethogram detailed in Table 3. The cumulative duration of seconds per behavior was recorded for each session. These behaviors were selected because they are indicative of a transition to relaxation. Additionally, for behaviors such as "lying with head up" and "lying with head down," the latency time was documented. Latency time reflects the duration it takes for the dog to initiate these behaviors, providing insight into the time required for the dog to exhibit rest. While both behaviors signify a state of rest in the dog, "lying with the head up" and "lying with the head down" represent different levels of repose (Ladha and Hoffman 2018).

To minimize bias, the observers remained blind to the specific treatment employed during video analysis, ensuring an impartial assessment of the recorded behaviors.

Table 3 Ethogram adapted from Walker et al. (2016)

Walking	Forward movement of the legs resulting in a shift of the entire body to a new position. Only one leg comes off the ground at a time.
Stand	All four legs remain on the ground with the legs extended to support the body.
Sit	Hindquarters on the ground with the front legs extended for support.
Lying with the head up	Ventral or lateral lying on the ground with the four legs in contact with the ground and at rest. The head is held in the air.
Lying with the head down	Ventral or lateral lying on the ground with the four legs in contact with the ground and at rest. The head rests on the ground.
Panting	Mouth open with tongue extended accompanied by rapid breathing and expansion or contraction of the chest.
Browse	Air is forcefully inhaled through the nose.

C-BARQ

The Canine Behavioral Assessment and Research Questionnaire (C-BARQ) employed in this study constitute a standardized method for evaluating dog behavior, utilizing a numerical scoring system to assess various behavioral categories (Hsu and Serpell 2003). In this investigation, an abbreviated version of the original C-BARQ, as validated by Duffy et al. (2014), was utilized, and the specific version is detailed in Appendix 2.

Owners of the participating dogs were tasked with completing the C-BARQ during the initial session of the study. For each dog a median score was calculated for each sections, including arousal, anxiety, aggression, attachment and attention-seeking behavior, and energy. While energy is not explicitly delineated as a distinct section in the C-BARQ, its quantification was derived by the median responses to questions 39 and 40 in the abbreviated version of the C-BARQ.

To synthesize a comprehensive overview of behavioral tendencies in our study group, a mean score per section was calculated.

Results

Behavioral data

The application of a Friedman test revealed a notable difference in panting between treatments, with a significant *p*-value of 0.020. Subsequent pairwise analysis was conducted to delineate the specific differences between treatments, as outlined in Table 4. To ensure the robustness of these findings, a Bonferroni correction was implemented (see Fig. 1). Notably, the comparison indicated that treatment with 10 drops elicited a statistically significant reduction in panting compared to the control treatment with 0 drops (p-value = 0.047).

According to the Friedman test, a significant difference ($p = 0.034$) was seen in panting between the treatments. A pairwise comparison was again made between the treatments using a Bonferroni correction. After the correction, there appears to be no significant difference between the different treatments. We do see a trend in less sniffing between the group with 5 drops and the control group ($p = 0.053$).

Table 4 Friedman test for panting between treatments

Sample 1-Sample 2	*p*-value (after Bonferroni correction)
10 – 5	1,000
10 – 1	0,568
10 – 0	**0,047**
5 – 1	1,000
5 – 0	1,000
1 – 0	1,000

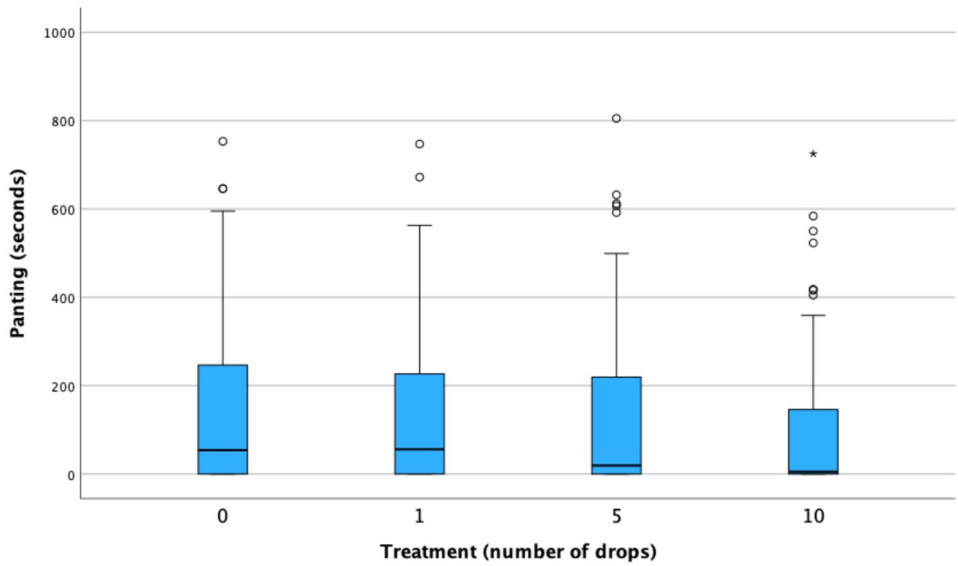

Fig. 1 Boxplot of panting compared between treatments. The bold line in the box represents the median, the box itself indicates the interquartile range (in which 50% of all data lie). The whisker shows the maximum value that is not an outlier. Circles and asterisks represent mild respectively extreme outliers.

No significant difference was seen between the different treatments in sitting ($p = 0.182$), time spent walking ($p = 0.894$), standing ($p = 0.768$), lying with the head up ($p = 0.343$) and down ($p = 0.252$) and the latency period for lying with the head up ($p = 0.250$) and down ($p = 0.101$).

C-BARQ

The calculation of medians for each dog across all questions within a section employed a scoring system where a 0 denoted the absence of the behavior, while a 4 signified frequent occurrence. In line with established conventions (Hsu and Serpell 2003), interpretations were derived from previous studies. A score below 2 indicated a low frequency (seldom or never), a score of 2 denoted occasional occurrence, and a score exceeding 2 signified a higher frequency (usually or always). Consequently, a score of 2 represented a medium level, with scores below 2 considered low and scores exceeding 2 considered high (Serpell and Duffy 2014; Hare et al. 2021). Notably, in this context, higher scores are less favorable, while lower scores are considered more desirable.

In our study group, the dogs exhibited a rather low mean score in anxious behavior (1.21). Next to this, they also exhibited elevated scores in arousal (2.27), energetic behavior (2.63), and attachment/attention-seeking behavior (2.81), as well as low scores in aggressive behavior (0.83).

Additional analyses were conducted to investigate whether there is any correlation between C-BARQ scores (specifically anxiety scores) and the effect of the EO blend on relaxation induction in dogs. Specifically, we examined whether the difference in panting between T0 and T1, T5 and T10 respectively (with lower panting at T1, T5 and T10 expected compared to T0) varied with anxiety scores.

Our statistical approach involved using Spearman rank correlation to assess the relationship between the anxiety scores and the change in panting levels from T0 to T5. Based on our analysis, we found that there was no significant correlation observed between anxiety scores and the effectiveness of the EO blend (T1 compared to T0: $p = 0.764$; T5 compared to T0: $p = 0.691$; T10 compared to T0: $p = 0.085$). This suggests that the relaxation-induced effect of the EO blend was consistent across dogs, regardless of their initial anxiety levels as measured by C-BARQ.

Discussion

The underlying hypothesis driving this study suggested that the outcomes of olfactory enrichment with EOs may exhibit substantial variability in the behavioral responses contingent upon the dosage administered (Uccheddu et al. 2018). In pursuit of this hypothesis, a total of 54 dogs were systematically exposed to varying quantities of the EO blend—ranging from no drops to 1, 5, or 10 drops—in a randomized order. This experimental design was employed to comprehensively explore potential dose-dependent effects.

A significant disparity in panting emerged among the different treatments, and a detailed examination revealed a statistically significant reduction in panting during the 10-drop treatment compared to the control treatment ($p = 0.047$). Panting is recognized as a behavioral manifestation of stress, as well as associated with decreased relaxation in dogs, often linked to thermoregulation processes triggered by stressors (Beerda et al. 1997). While it is acknowledged that dogs may pant for reasons other than stress, such as thermoregulation in response to elevated ambient temperatures, it is pertinent to remind that the experimental environment maintained a consistent temperature range of 18 °C to 23 °C throughout the study. Given this controlled environment, the authors contend that panting observed in this study is more likely to be indicative of activity-related responses rather than temperature-related thermoregulation. This interpretation enhances the significance of the observed reduction in panting during the 10-drop treatment, suggesting a potential relaxation-alleviating effect associated with the EO blend.

While panting exhibited a significant reduction during the 10-drop treatment, the lack of significant alterations in other behavioral indicators implies a nuanced yet beneficial impact on overal relaxation levels. This outcome contributes to a more comprehensive understanding of the potential relaxation -alleviating effects associated with the application of the EO blend, emphasizing its selective efficacy in addressing specific relaxation-related behaviors within the studied canine population.

The C-BARQ outcomes closely match average dog scores in comparison to the general population, indicating that, on average, dogs tend to have lower anxiety scores (1.21). This suggests that the specific EO blend in the study may mildly relax a typical dog. The idea that dogs with higher anxiety might benefit more from EO enrichment remains uncertain in this dog group, emphasizing the need for future research to explore how dogs behave based on individual C-BARQ scores. Investigating this could provide a deeper understanding of how temperament interacts with EO effects. Past research (Hawken et al. 2012) has shown that EO soothing effects may be influenced by individual temperament, making it interesting to validate this hypothesis in subsequent research projects.

While C-BARQ anxiety scores were obtained for each individual dog in our study, the observed relaxation induced effect of the EO blend was not influenced by the dog's initial anxiety level.

While exhaustive measures were taken to execute the study with the utmost objectivity, it is acknowledged that certain aspects might be approached differently in future investigations. The transparent acknowledgment of these considerations underscores the commitment to methodological refinement and the pursuit of more robust scientific outcomes in future research endeavors.

The olfactory enrichment with the blend of EOs introduces a potential variable, considering that all EOs used in this study are commonly employed in human aromatherapy for their anxiolytic effects (Setzer 2009; Nadeem et al. 2018; Kwangjai et al. 2021). It is plausible that the owners, exposed to the same blend, might have experienced a shift in their own emotional states, subsequently influencing their dogs through altered, possibly more relaxed, behavior. Indeed, existing research underscores the capability of dogs to discern and respond to human gestures, signals (Udell and Wynne 2008), and emotions, categorizing them into positive or negative emotional states (Albuquerque et al. 2016). Consequently, a dog's behavior might reflect a more positive or relaxed demeanor based on the corresponding behavior of its owner.

Despite efforts to limit the blend's impact to the immediate surrounding area by applying it solely to the dog's collar, potential effects on owners cannot be entirely excluded. Future studies should address the effective range of EOs administered in this manner. Consideration might be given to methodologies that enable blinding owners, or even exploring designs that exclude owner involvement, to better isolate the direct impact of the EO blend on canine behavior. These refinements would contribute to a more nuanced understanding of the interplay between olfactory enrichment and owner influence in similar experimental settings.

The consistent presence of the person filming, positioned approximately 2 m from the dogs throughout the study, raises the possibility of an influence on the observed behaviors. To mitigate potential effects on canine behavior, the utilization of cameras on tripods could be considered as an alternative in future studies. This approach offers the advantage of stabilizing the camera position, minimizing the potential for inadvertent influences on the dogs' behavior.

However, it's recognized that in the present study, the practicality of using tripods was constrained by the dynamic nature of some dogs, which frequently moved out of the camera's view, necessitating continuous adjustment by the person filming. While this logistical challenge precluded the use of tripods in the current study, it highlights a consideration for future research to explore innovative solutions that balance practical constraints with the aim of maintaining optimal filming conditions.

While the current study included a substantial number of dogs, increasing the sample size could enhance the representativeness of the findings. A larger pool (considering various factors as size, breed, temperament, background, the human-dog bond…) of canine participants would contribute to a broader and more comprehensive understanding of the potential effects of olfactory enrichment with EOs. This consideration acknowledges the potential variability in individual responses and allows for a more robust generalization of results to a broader canine population. In future studies, efforts to expand the sample size could further strengthen the validity and reliability of the findings.

The consistent use of the same room for all four sessions introduces a potential factor related to the familiarity of the environment. The initial session, in particular, may have induced additional stress as the dogs were not yet acclimated to the surroundings. While subsequent sessions maintained the unfamiliar environment, the novelty factor decreased over time. Nevertheless, the crossover design mitigated this potential effect, as each group experienced the same environmental conditions sequentially, minimizing any disproportionate impact.

While the 10-drop treatment demonstrated the most pronounced effect on dog behavior in this study, it is crucial to note two instances where dogs did not respond favorably to the EO post-session. Although a direct causal link between these complications and the treatments was not established, these occurrences warrant attention. One dog in the study group exhibited illness and vomited a few hours after the treatment, while another dog displayed difficulty waking up immediately after the 20-minute session and slept throughout the afternoon. Despite the overall effectiveness of the 10-drop treatment on canine behavior, it may not be universally suitable for every dog and, in some instances, could potentially be excessive.

The potential side effects mentioned in the study occurred in approximately 3.70% of cases (2 dogs out of 54). This underscores the paramount importance of considering individual responses and tailoring treatments accordingly. Future research should delve into the determination of optimal dosage levels for individual dogs, exploring potential correlations between the concentration of EO and factors such as the size and weight of the dog. Such investigations will contribute to refining guidelines for the safe and tailored use of EO treatments in diverse canine populations.

To better prevent such side effects in future studies, researchers could conduct pre-screening assessments to identify dogs with known sensitivities or medical conditions that might predispose them to adverse reactions. Exploring alternative methods of administration or dilution protocols may offer additional safety measures. Future studies should prioritize individualized approaches to dosage determination and rigorous monitoring protocols to mitigate the risk of side effects and enhance the safety profile of EO treatments for canine populations.

The authors acknowledge the limited scope of physiological and behavioural parameters. Additional physiological measures were not included at this stage for feasibility reasons in the field and for preventing a potential effect of handling on the dog's behavior. The authors are keen on incorporating them in future research designs. Moving forward, authors intend to explore feasible methods for integrating e.g. heart rate monitoring into their study protocols to enhance the depth of physiological data collected and improve the overall robustness of their findings.

Scientific literature on the effect of olfactory enrichment with EOs with anxiolytic or other consequences in dogs is still scarce. Also, in different animals, it has not yet been studied enough to generalize into practical applications. That is why further research into the effect of olfactory enrichment with EOs on the behavior of dogs and other animals has yet to be conducted.

This scarcity of research extends to various other animal species, warranting caution when attempting to generalize findings into practical applications. Given the lack of comprehensive studies in this domain, further research is imperative to delve deeper into the nuanced effects of olfactory enrichment with EOs on the behavior of dogs and, by extension, other animal species. These investigations will not only contribute to expanding our understanding of the potential benefits but will also pave the way for the development of more informed and tailored applications of EOs interventions for the well-being of diverse animal populations.

Conclusion

These preliminary findings indicate that the 10-drop treatment of this EO blend enhanced subtle relaxation behaviors, notably through a decrease in panting, among this dog's population. Future research should extend beyond this blend to assess individual EOs, their combinations, and varying concentrations to broaden our understanding of their effects on canine behavior. These preliminary insights lay the groundwork for future, more nuanced, investigations, facilitating the development of tailored olfactory enrichment strategies for dogs in various relaxation-related contexts.

Abbreviations

EO	essential oil
EOs	Essential Oils
C-BARQ	canine behavioral assessment and research questionnaire

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Author contributions The experiment was concepted and designed by AH and AS. The experiment was performed by AH, ST and NE. The analysis of data was made by ST, NE and AS Statistical analyses were performed by HA and AS. The article was written by AH, AD and ML.

Data availability The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declarations

Ethics statement The dogs in this study, not classified as laboratory animals per Belgian law, were nonetheless subject to ethical scrutiny. A report was submitted to the local ethics committee of the Salto research group at Odisee. Additionally, owners provided informed consent for the participation of their dogs in the study.

Competing interests The authors declare no competing interests.

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