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Avoidance Behavior of *Rattus rattus* to Odors of Eucalyptus and Citronella Oils under Maze Experiments

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Abstract: Substances that make animals move away from the source are called repellents. It may be an object, a device or a chemical substance which by touch, sound, color, taste or odor will ward off the animal or prevent an animal from feeding or gnawing. In multi-choice T-maze experiments, the behavior of male and female house rat (*Rattus rattus*) was recorded in response to the odors of 5% eucalyptus and 5% citronella oils released through encapsulated wax blocks. A significant (P ≤0.05) difference in values of different parameters related to distance moved, velocity and frequency of movement was observed between untreated zone and the zones treated with eucalyptus and citronella oils indicating avoidance behavior of rats in response to the odors from eucalyptus and citronella oils. The record of animal tracks in different zones of T-maze also revealed reduced movement and activity of the rats in treated zones. No significant difference was observed between behavior of male and female rats. These experiments suggested the use of two oils in repelling the rats away from enclosed store houses.

Key words: Citronella oil, encapsulated wax blocks, eucalyptus oil, *Rattus rattus*, repellent, maze.

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The continued survival and proliferation of rodent populations in the urban environment owes much to their capacity to adapt to life in close association to man (Blasdell *et al*., 2019). Rodent species of the genus, *Rattus* have probably been responsible for more human sufferings than any other group of vertebrates, not only through their destructive impact on food crops, but also through their role in the transmission of fatal or debilitating diseases such as plague, leptospirosis and typhus. The house rat, *Rattus rattus* (Rodentia: Muridae), is one of the most commonly encountered and economically important commensal rodents.

Rodent populations are becoming resistant to anticoagulants (Garg *et al*., 2017) and their numbers will continue to rise as the proportions of those with heritable resistance continue to increase. Natural products are an excellent alternative to synthetic pesticides as a means to reduce their negative impacts on human health and the environment. Plant essential oils are volatile natural complex secondary metabolites characterized by a strong odor and generally have the density lower than that of water (Dhifi *et al*., 2016). Moreover, these essential oils are

easily extractable, ecofriendly and biodegradable thus, get easily catabolized in the environment (Zygadlo and Grosso, 1995), do not persist in soil and water (Misra and Pavlostathis, 1997; Isman, 2000; Isman 2006), possess low or no toxicity against vertebrates such as fishes, birds and mammals (Enan *et al*., 1998) and play an important role in crop protection against pests (Isman and Machial, 2006; Bakkali *et al.,* 2008; Sachdeva and Singla, 2018). Among the plant families with promising essential oils used as repellents include *Cymbopogon* spp., *Ocimum* spp., *Thymus* spp., *Eucalyptus* spp. etc. (Koul *et al*., 2008). These can be useful for the prevention of rodent damage to grains in stores and seeds and seedlings in crop fields and nurseries. Unpleasant taste and odor cues function as initial deterrents against ingestion of food (Kaur *et al*., 2017, 2018) that contain toxins leading to primary food aversion. Eucalyptus (EO) and citronella oils (CO) are essential oils obtained from the leaves and stems of different species of *Eucalyptus* and *Cymbopogon*, respectively.

Eucalyptus oil is the generic name for distilled oil from the leaf of *Eucalyptus*, a genus of the plant family Myrtaceae native to Australia and cultivated worldwide. Its chief constituent is eucalyptol (cineole), a colorless liquid with camphor-like odor and cooling taste. Essential oils of eucalyptus appear particularly potent as repellents against mosquitoes, rice weevils, pine processionary moths and mushroom flies (Choi *et al*., 2002; Batish *et al.,* 2008; Singla *et al*., 2014).

The main components of CO are citronellal and citronellol. It is found in many familiar insect repellent products like candles, lotions, gels, sprays and towelette wipes for use on clothing and people. CO is also present in some pellets and tablet products for use around home lawns and gardens to repel dogs and cats. Animal collars and tags containing CO are used on pets and other domestic animals to repel fleas and ticks.

Both the oils are renowned plant-based insect repellents and have been registered for this use in the United States since 1948. However, the volatile nature of the oils is the limiting factor. To increase the stability of such compounds, it is necessary to formulate them so that they are protected from degradation by UV light and oxygen. Moreover, the formulation must ensure a controlled release of the compound. Several formulations and dispensers have been developed and commercialized with various slow-release capacities including the use of paraffin wax formulations (Atterholt *et al*., 1999). Relatively little work has been carried out on the use of plant derived repellents for control of rodents. The aim of present study was to evaluate the behavior of house rat, *R. rattus* towards the odors of EO and CO released through encapsulated wax blocks in maze experiments.

Materials and Methods

Collection and maintenance of animals

The house rat, *R. rattus* of both sexes were live trapped from poultry farms and brought to the laboratory for acclimatization individually in cages for 15-20 days before the commencement of the experiment. Food and water were provided *ad libitum*. Food consisted of a mixture of cracked wheat, powdered sugar and groundnut oil (WSO) in a ratio 96:2:2. Animals were used and maintained as per the guidelines of the Institutional Animal Ethics Committee. After acclimatization, healthy and mature rats of both sexes were weighed and selected for experimentation.

Experimental set up and treatment

Commercially available (EO)was procured from SD Fine Chemicals Pvt. Ltd., whereas, the CO was obtained from the New Crops Laboratory, Department of Agronomy, Punjab Agricultural University, Ludhiana. In multi-choice maze experiments, the behavior of rats (three of each sex) was recorded in response to their exposure to 5% EO and CO encapsulated in wax blocks. Oils were diluted in isopropyl alcohol. Each paraffin wax block contained 3 drops (0.5 mL) of a particular oil. Every time blocks were prepared fresh. T-maze having three arms (each of length 47 cm and at an angle of 90^{o} from each other) and a central hub was used for the purpose. During treatment, in one arm (called treated zone EO) of the T-Maze, a wax block containing EO was placed, in the second arm (called treated zone CO), a wax block containing CO was placed, whereas no wax block was kept in the third arm (called untreated zone). Observations were recorded through camera operated Ethovision Pro Software of Netherlands. One rat was released at a time in the maze in the central hub initially.

Table 1. Behavior of male Rattus rattus in response to odors of 5% eucalyptus and citronella oils encapsulated in wax blocks and exposed in multi-choice in T-Maze experiment

Parameter	Treatment days	Untreated Zone (n = 3)	Treated Zone EO (n = 3)	Treated Zone CO (n = 3)
Latency of first occurrence (s)	No treatment	2.88±3.73	148.00±182.50	29.44±28.46
	Day 1 of Treatment	14.08±19.91	3315.20±4688.40	492.80±593.64
	Day 2 of Treatment	3.52±3.42	1027.36±1452.90	178.72±199.38
	Day 3 of Treatment	16.03±22.51	3733.76±3110.64	24.32±24.63
In zone frequency	No treatment	179.66±132.32	68.00±87.68	68.66±23.11
	Day 1 of Treatment	86.66±109.25	0.66±0.94	10.00±5.71
	Day 2 of Treatment	14.33±16.11	1.66±2.35	4.00±4.32
	Day 3 of Treatment	269.00±368.40	27.35±37.95	4.33±1.24
Total distance moved (cm)	No treatment	13378.70±3748.43	1471.33±1331.62	2080.89±1565.65
	Day 1 of Treatment	5668.30±2342.16 [a]	81.36±115.06 [b]	515.37±645.48 [b]
	Day 2 of Treatment	8520.76±6860.37 [a]	18.82±26.61 [b]	49.05±44.51 [b]
	Day 3 of Treatment	6738.02±5195.48 [a]	1379.58±1928.85 [b]	64.72±43.14 [b]
Maximum distance moved (cm)	No treatment	99.23±58.78	52.03±37.42	39.98±14.57
	Day 1 of Treatment	15.95±4.96 [a]	2.28±3.22 [b]	6.81±2.84 [b]
	Day 2 of Treatment	32.93±31.66 [a]	2.29±3.24 [b]	6.20±4.43 [b]
	Day 3 of Treatment	44.91±32.62 [a]	18.98±23.22 [b]	5.99±2.22 [b]
Mean velocity (cm/s)	No treatment	15.83±4.54	12.50±10.22	5.88±4.72
	Day 1 of Treatment	3.16±1.78 [a]	1.45±2.05 [b]	1.71±1.17 [b]
	Day 2 of Treatment	4.92±3.92 [a]	0.24±0.34 [b]	0.56±0.42 [b]
	Day 3 of Treatment	6.61±2.04 [a]	1.52±2.12 [b]	2.23±1.52 [b]
Frequency of rearing	No treatment	431.66±201.09	82.00±109.62	131.66±80.10
	Day 1 of Treatment	364.00±194.43 [a]	4.33±6.12 [b]	41.00±55.87 [b]
	Day 2 of Treatment	508.00±449.69 [a]	1.00±1.41 [b]	2.66±2.05 [b]
	Day 3 of Treatment	442.66±283.70 [a]	24.33±33.70 [b]	0.66±0.47 [b]
Frequency of mobility	No treatment	777.00±398.14	68.66±86.79	169.66±104.93
	Day 1 of Treatment	705.33±362.83 [a]	8.00±11.31 [b]	55.66±78.72 [b]
	Day 2 of Treatment	1044.66±900.61 [a]	0.66±0.94 [b]	2.33±3.29 [b]
	Day 3 of Treatment	989.66±847.04 [a]	13.33±18.85 [b]	1.33±0.94 [b]

-Values are mean ± SD, n = number of rats, EO = Eucalyptus oil, CO = Citronella oil
-Values with different superscripts in a row differ significantly at $P \leq 0.05$

For each rat, the experiment was continued for 4 days a week (Monday to Thursday) and the wax block was kept once on the second day of the week (i.e. on Tuesday). On day 1, the behavior of the rat was recorded in T-maze without keeping the wax block in any of the arms. Each day, the movement of the rat in T-maze was recorded for 3 h in the form of tracks and the quantitative data on in zone frequency, the latency of first occurrence (s), total distance moved (cm), maximum distance moved (cm), velocity (cm s^{-1}), frequency of rearing and frequency of mobility.

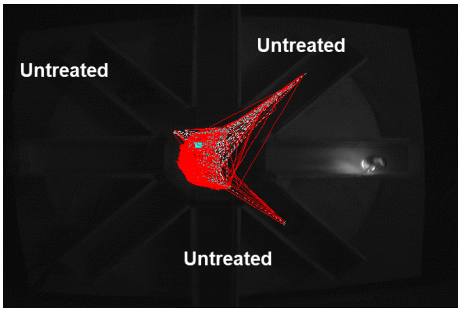
Statistical analyses

Values were determined as mean ± SD. The significance of difference in various quantitative parameters used for the evaluation of two oils was determined by analysis of Variance through the software SAS 9.3. All pair-wise treatment comparisons were made using Tukeys' test at 5% level of significance using SAS 9.3 software. Different treatments intersecting each other at solid lines indicate significant differences.

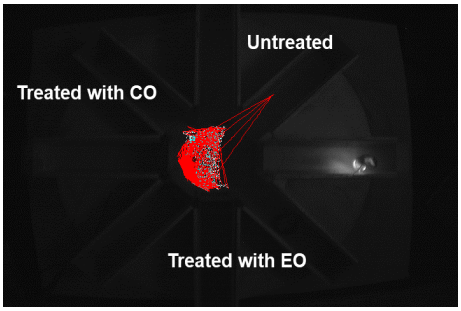
Results and Discussion

Behavior of male rats

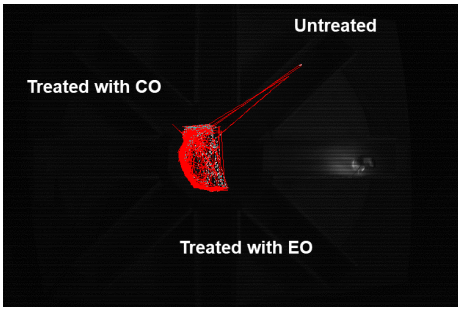
Quantitative data indicating behavior of male *R. rattus* towards 5% EO and CO kept as encapsulated wax blocks in T-maze is presented in Table 1. Though the value for latency of first occurrence was apparently high in zone treated with EO but statistically there was no significant



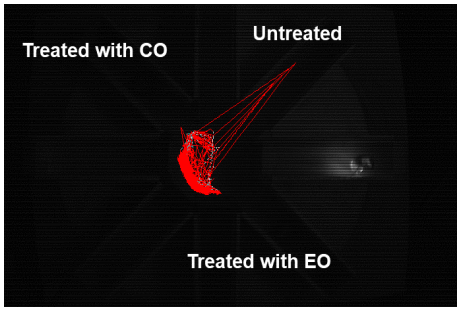
Male rat in multichoice day with no treatment



Male rat in multichoice day 1 of treatment



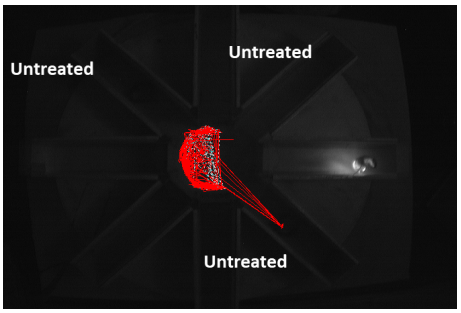
Male rat in multichoice day 2 of treatment



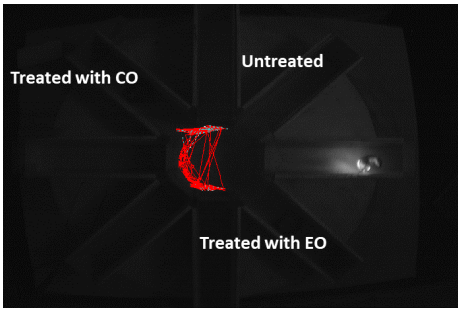
Male rat in multichoice day 3 of treatment

Fig. 1. Animal tracks showing behavior of male rats in response to wax blocks encapsulated with EO and CO in T-maze experiment.

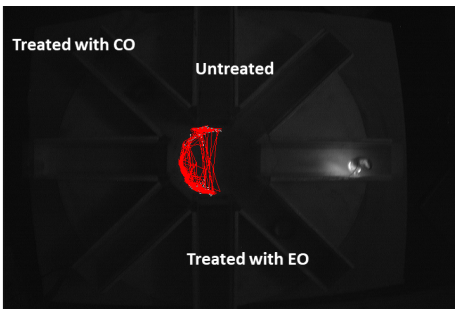
difference among the treated and untreated zones. Similarly, frequency was higher in the untreated zone but the difference in values was non-significant among the treated and untreated zones. The values for other parameters such as total distance moved, maximum distance moved, mean velocity, frequency of rearing and frequency of mobility were found to be significantly (P ≤0.05) less in zones treated with EO and CO as compared to the untreated



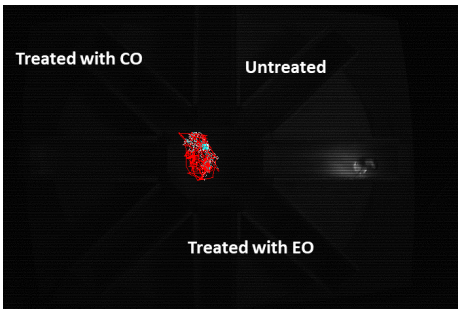
Female rat in multichoice day with no treatment



Female rat in multichoice day 1 of treatment



Female rat in multichoice day 2 of treatment



Female rat inmultichoice day 3 of treatment

Fig. 2. Animal tracks showing behavior of female rats in response to wax blocks encapsulated with EO and CO in T-maze experiment.

Table 2. Behavior of female Rattus rattus in response to odors of 5% eucalyptus and citronella oils encapsulated in wax blocks and exposed in multi-choice in T-Maze experiment

Parameter	Treatment days	Untreated Zone (n = 3)	Treated Zone EO (n = 3)	Treated Zone CO (n = 3)
Latency of first occurrence (s)	No treatment	0.00±0.00	78.88±111.55	0.00±0.00
	Day 1 of Treatment	4.96±7.01 [a]	0.00±0.00 [b]	4028.64±2214.05 [b]
	Day 2 of Treatment	2.72±2.15 [a]	0.00±0.00 [b]	0.00±0.00 [b]
	Day 3 of Treatment	0.00±0.00	0.00±0.00	0.00±0.00
In zone frequency	No treatment	2.33±1.88	1.33±1.88	0.00±0.00
	Day 1 of Treatment	2.33±1.24 [a]	0.00±0.00 [b]	3.66±2.49 [ab]
	Day 2 of Treatment	1.00±0.00 [a]	0.00±0.00 [b]	0.00±0.00 [b]
	Day 3 of Treatment	1.00±0.00 [a]	0.00±0.00 [b]	0.00±0.00 [b]
Total distance moved (cm)	No treatment	2082.96±1937.58	81.26±114.92	0.00±0.00
	Day 1 of Treatment	3784.42±2491.08 [a]	0.00±0.00 [b]	501.08±700.57 [b]
	Day 2 of Treatment	8675.32±5809.92 [a]	0.00±0.00 [b]	0.00±0.00 [b]
	Day 3 of Treatment	6750.45±4965.39 [a]	0.00±0.00 [b]	0.00±0.00 [b]
Maximum distance moved (cm)	No treatment	18.74±15.19	14.76±20.87	0.00±0.00
	Day 1 of Treatment	11.56±1.46 [a]	0.00±0.00 [b]	3.56±2.70 [b]
	Day 2 of Treatment	12.14±0.94 [a]	0.00±0.00 [b]	0.00±0.00 [b]
	Day 3 of Treatment	12.99±1.66 [a]	0.00±0.00 [b]	0.00±0.00 [b]
Mean velocity (cm/s)	No treatment	0.57±0.55	0.20±0.28	0.00±0.00
	Day 1 of Treatment	1.23±0.28	0.00±0.00	5.48±6.80
	Day 2 of Treatment	3.16±1.24	0.00±0.00	0.00±0.00
	Day 3 of Treatment	2.47±1.41	0.00±0.00	0.00±0.00
Frequency of rearing	No treatment	122.66±87.24	6.66±9.42	0.00±0.00
	Day 1 of Treatment	238.00±144.97 [a]	0.00±0.00 [b]	3.66±2.49 [b]
	Day 2 of Treatment	571.33±292.91 [a]	0.00±0.00 [b]	0.00±0.00 [b]
	Day 3 of Treatment	532.33±389.11 [a]	0.00±0.00 [b]	0.00±0.00 [b]
Frequency of mobility	No treatment	197.66±160.75	11.75±20.35	0.00±0.00
	Day 1 of Treatment	652.00±434.71 [a]	0.00±0.00 [b]	0.00±0.00 [b]
	Day 2 of Treatment	1067.33±592.77 [a]	0.00±0.00 [b]	0.00±0.00 [b]
	Day 3 of Treatment	643.33±353.84 [a]	0.00±0.00 [b]	0.00±0.00 [b]

-Values are mean ± SD, n = number of rats, EO = Eucalyptus oil, CO = Citronella oil, -Values with different superscripts in a row differ significantly at P ≤0.05

zone indicating avoidance behavior of rats in response to the odors of two oils. No significant difference was observed among the three days of treatment for all parameters recorded. The latency of first occurrence was non-significantly more in the zone treated with EO than zone treated with CO whereas the values of the other parameters recorded were non-significantly more in the zone treated with CO than zone treated with EO in first two days indicating higher repellent effect of EO than CO. The values of all the parameters recorded were comparatively high in zone treated with EO on day 3 of treatment indicating reduction in effect of the oil encapsulated in wax block by third day. However, in zone treated with CO, there was gradual reduction in effect during all the three days. The record of animal tracks in the maze also revealed reduced movement and activity of the rats in treated zones (Fig. 1).

Behavior of female rats

Quantitative data indicating behavior of female *R. rattus* in response to odors of 5% EO and CO kept as encapsulated wax blocks in T-maze is presented in Table 2. Data revealed no significant difference in mean velocity among the untreated and treated zones. A significant difference (P ≤0.05) among treated and untreated zones was, however, observed for latency of first occurrence, in zone frequency, total distance moved, maximum distance

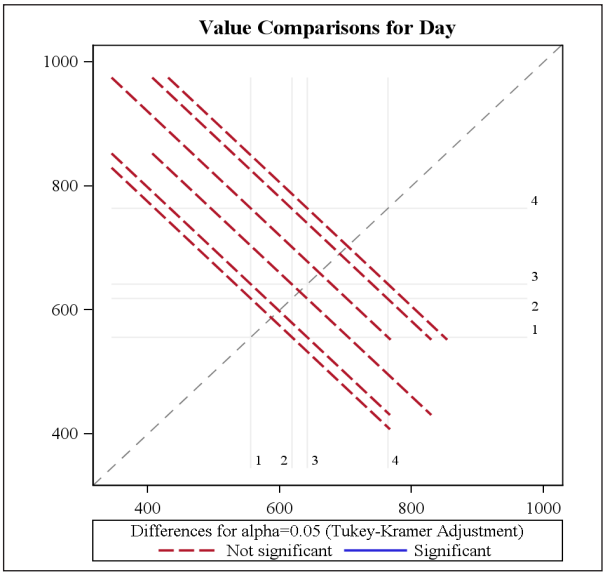


Fig. 3. Day wise comparison of behavior of rats exposed to odors of eucalyptus and citronella oils encapsulated in wax blocks (1-No treatment, 2-Day 1 of treatment, 3-Day 2 of treatment, 4-Day 3 of treatment) indicating no significant difference among days.

moved, frequency of rearing and frequency of mobility. The movement of the rats was found to be almost nil in the zone treated with EO, whereas some movement was shown by the rats in zone treated with CO on day 1 of treatment. The record of animal tracks in the maze revealed reduced movement and activity of the rats in treated zones (Fig. 2).

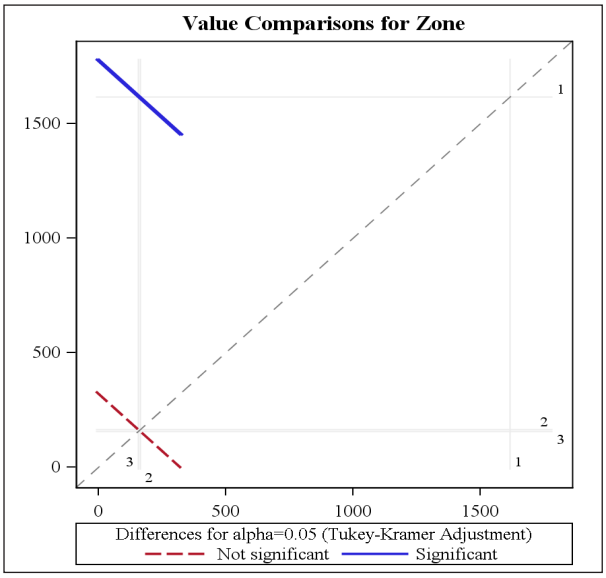


Fig. 5. Comparison of behavior in response to odors of eucalyptus and citronella oils encapsulated in wax blocks among different zones (1-Untreated zone, 2-Treated zone EO, 3-Treated zone CO) indicating significant differences between zones 1& 2 and zones 1& 3.

In overall, multi-choice experiment conducted in T-maze using 5% EO and CO revealed statistically no significant difference among the three days of treatment (Fig. 3) and different rats used in an experiment (Fig. 4). A significant (P≤0.05) difference in different parameters was, however, observed between untreated zone and zones treated with EO and CO (Fig. 5). Though from data it appears that

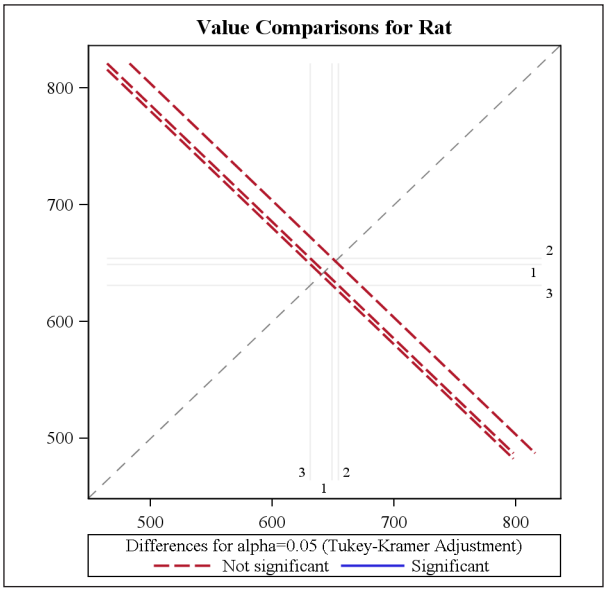


Fig.4. Comparison of behavior in response to odors of eucalyptus and citronella oils encapsulated in wax blocks among different rats indicating no significant difference.

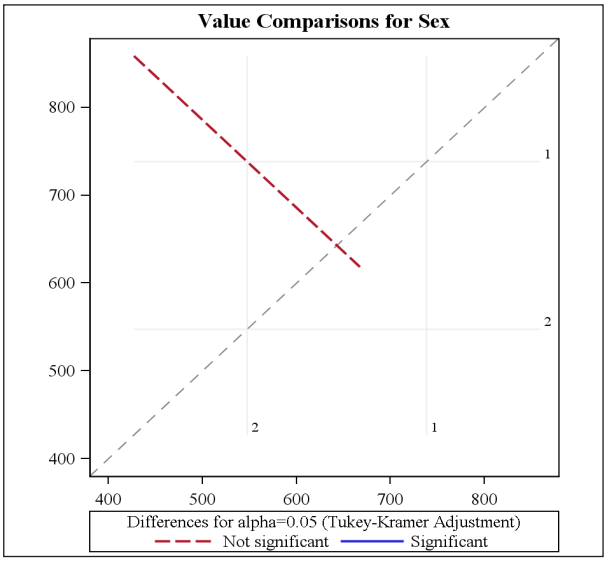


Fig. 6. Comparison of behavior in response to odors of eucalyptus and citronella oils encapsulated in wax blocks between male and female rats (1-Male rats, 2-Female rats) indicating no significant difference between sexes.

females are more sensitive towards EO and CO as the movement of females to treated zones was almost nil, but statistically no significant difference was observed between behavior of male and female rats towards these two oils (Fig. 6).

These essential oils have been used against insects in a number of studies (Asadollahi *et al*., 2019; Sheikh *et al*. 2021), however, studies on the effect of these oils on rodents or other vertebrates are limited. Hile (2004) has reported the repellent effect of CO against European starlings. Kalandakanond-Thongsong *et al.* (2010) evaluated the efficacy of chilli, wintergreen oil, bergamot oil, peppermint oil and geranium oil as repellents in the circular open field against adult male Wistar rats. Rats spent less time in the inner zone of the circular field exposed to repellents compared to the control zone indicating that the rats were most likely trying to avoid the close contact to these substances. They further suggested that these potential repellents may be further applied on packaging and shipping supplies to prevent destruction by rats. Potential of different concentrations of EO applied as paint (Singla *et al*., 2013) and as spray (Singla *et al.,* 2014) in laboratory pens for repellent effect against *R. rattus* was evaluated revealing higher repellent effect of the oil when was applied daily as compared to that when applied on alternative days or once a week due to volatile nature of the oil. Further studies were suggested to enhance the persistence of repellent effect of the oil for longer period of time. Singla and Kaur (2014) also observed maximum repellent effect of CO applied as paint daily as compared to that applied on alternative days or once a week. Similar to present study, no significant difference in behavior of male and female rats in response to EO was observed in these studies (Singla *et al.,* 2013, Singla and Kaur, 2014; Singla *et al*., 2014). This may be due to large variations observed in replicated rats leading to higher standard deviation values.

Sachdeva and Singla (2018) studied the persistence of repellent effect of EO encapsulated in sodium alginate-based microcapsules against *R. rattus*. The microcapsules containing different concentrations of oil were mixed in food at different concentrations. A significant repellent effect of the oil was observed for 7 days at 5% concentration. Similar to present studies, maze experiments conducted by Sachdeva and Singla (2018) also revealed reduced activities of rats in the zone treated with microcapsules containing 5% oil compared to the untreated zone. Further, under simulated store conditions, microcapsules containing 5% oil showed significant repellent and antifeedant effects which persisted for all the 15 days of the experiment.

Conclusion

Quantitative data recorded in multi-choice experiments and animal tracks obtained during present studies revealed the avoidance behaviour of both male and female *R. rattus* in response to controlled release of the odor of 5% EO and CO encapsulated in wax blocks. The present studies thus suggest the use of two oils in repelling away rats from enclosed store houses.

Acknowledgments

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