

The Effect of VOCs from the Branches and Leaves of *Pistacia chinensis* Bunge and *Juniperus chinensis* cv. Kaizuka on Mouse Behavior

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To meet both landscape aesthetics and health needs, the effects of different concentrations of volatile organic compounds (VOCs) from *Pistacia chinensis* Bunge (*P. chinensis*) and *Juniperus chinensis* cv. Kaizuka (*J. chinensis*) on mouse spontaneous behavior were studied during successive six-day experiments. The results were as follows: 1) The excitability of the mice and their total moving distance increased significantly upon exposure to low volatile concentrations of *P. chinensis* ($P < 0.05$), whereas there was an opposite effect after exposure to *J. chinensis*. 2) The explorative capacity of mice was enhanced by *J. chinensis*; in contrast, *P. chinensis* treatment resulted in an opposite effect. 3) The scent of *P. chinensis* volatiles reduced mouse appetites while *J. chinensis* had the opposite effect. 4) *P. chinensis* volatiles helped enhance mouse tension. The number of fecal grains in the treatment group was always greater than that of the control group and increased with increasing volatile concentration to a number that was two times that of the controls when the volatile concentration reached a relatively high level. In contrast, in the *J. chinensis* environment, the mice were relatively relaxed, with overall numbers of fecal grains that were only 81.7% to 97.6% that of the controls. Overall, VOCs from *J. chinensis* had beneficial effects on mice. Therefore, more *J. chinensis* should be planted in urban green spaces. However, VOCs from *P. chinensis* could cause adverse effects on mice. Therefore, it is suggested to minimize their planting in city or repairing their branches to keep away from the smelling range of humans.

Key words: VOCs; Mice; Open-field test; Spontaneous behavior; Habitat forest

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INTRODUCTION

Along with the rapid development of social economies and urbanization, environmental pollution has become an aggravating factor. The construction of urban residential forests has gained increasing attention. In the past, the construction of human settlements pursued visual beauty only in the traditional sense. The past studies show that human habitat forests play an important role in healthcare, as green plants release volatile organic compounds (VOCs) into the surrounding environment in the normal course of their growth (Farmer 2001; Miller *et al.* 2001; Li *et al.* 2002; Lu *et al.* 2002; Phillips *et al.* 2003; Li *et al.* 2014). These substances have certain effects on the physiology and psychology of the human body (Li *et al.* 2015), some of which can eliminate fatigue,

relieve stress, and improve spirits (Yatagai and Hong 1997; Kumie 2000). Some volatile benzene compounds can cause people to go into a trance and produce unsteadiness, numbness, and other phenomena (Ishiyama 2000; Sawada *et al.* 2000; Kumie 2000; Yatagai 2000). Therefore, one of the most highly researched questions within urban human habitat forest construction is how to take full advantage of the medical and health care effects of different tree species and to create an environment that is more conducive to human health.

When animals are in a new environment, they exhibit dramatic emotional changes (Bubna and Jahn 1994) and adapt to this environment after persistent exploration. The open-field test, a classical behavioral test, can be used to evaluate the excitability, adaptability, inquiry, tension, memory, and other behaviors of animals toward new environments. This testing method is good for evaluating animal emotional levels and their behavior in open environments (Crawley 1985). It also can be used to test the animal's central nervous system. Mice serve as an ideal animal model of human growth and development, biological processes, gene functions, and human disease because of the high similarities of their genome sequence, biochemical metabolism, and physiological characteristics to humans, with the added advantages of small individual differences, short reproductive cycle, strong reproductive capacity, *etc.* (Liu 2011).

Juniperus chinensis has the characteristics of strong germination, decay resistance, and resistance to pruning. The branches and leaves can also be used as medicine, promoting blood circulation and diuresis (Zhang and Ming 2001). *Pistacia chinensis* has the characteristics of strong wind resistance, long service life, seed oil, and leaves that turn bright red or orange in autumn. These two species are common and widespread in the northern area of China. Although some related studies (Li *et al.* 2014) have been conducted before, it is still not fully clear what specific effects their leaf and branch volatile matters have on human physiology and living environments when planted in large numbers. To greatly enhance the construction of forest-quality human habitats, timely research is needed, which plays a major role in guiding this research. Previously, *P. chinensis* and cypress were used in rat open-field tests (open-field test; Walsh and Cummins 1976). Here, the two species were still used as a case study. The effects of volatiles from the leaves of *P. chinensis* and cypress on mice were investigated in terms of excitement, exploring capacity, learning, memory capacity, and the state of anxiety to provide a scientific basis for the rational search for useful green species.

EXPERIMENTAL

Materials

Animals

Four-week-old Kunming mice, weighing approximately 17 to 20 g, were obtained from China Biologic Products, Inc. (Taian, China).

Plants

P. chinensis and *J. chinensis* were harvested from the campus of Shandong Agricultural University (Taian, China)

The *P. chinensis* was about 11.2 m in height and 24.5 cm in diameter at breast height (DBH), and the *J. chinensis* was about 4.8 m in height and 8.3 cm in DBH. The leaves facing the sun were selected and picked in the middle of June.

Custom-made processing box

Custom-made processing boxes were composed of seven no-cap glass containers with a diameter of 350 mm and were covered with a glass containing two 1 mm-diameter air holes (Fig. 1), which were designed to prevent the spread of volatile compounds and to ensure normal breathing for the mice. At the bottom of each box was a wire mesh with a small aperture on the partition plate, which was located below the mice, designed to prevent the direct contact between mice and plant branches and leaves, and to separate fecal grains from the container, which was in contact with the plants.

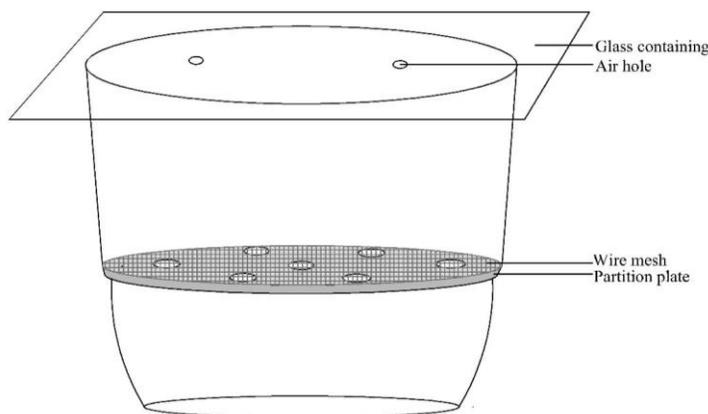


Fig. 1. Custom-made processing box

Experimental instrument

Mouse movement was observed using the Open-field Test Video Analysis System (ZH-FT, HuaiBeiZhengHua Biological Instrument Equipment Co., Ltd., Anhui, China).

Methods

Plant treatment

Every morning before 10 am, fresh branches and leaves were picked with a pair of scissors, cut into approximately 1-cm pieces and mixed evenly, with the aim of evenly distributing the volatiles. Different amounts (200, 400, and 600 g) were weighed using a balance and were added to different boxes. The effects of different VOC concentrations from branches and leaves on the locomotor activity in mice were observed. Plant volatiles were composed of a mixture of various substances from nature. This method was used to attempt to reflect the natural state of these volatiles as similar as possible.

Animal treatment

The mice were randomly divided into 7 groups with 8 mice in each group. The groups treated with 200, 400, and 600 g of *P. chinensis* were named *P200*, *P400*, and *P600*, respectively, and groups treated with the same amounts of *J. chinensis* were named *J200*, *J400*, and *J600*, respectively. Control mice that were exposed to no plants were termed CK.

Spontaneous motor activity

The experiment was conducted after the mice were allowed to grow accustomed to the lab environment for three days. Mice were placed into the custom-made processing boxes after recording their state at 9:50 am, with each box containing 8 mice. All the procedures conducted in this study were in accordance with ethical standards. Every plant corresponded to the same number of mice with a specific number. Mice were then removed and analyzed in the ZH-FT Open-field Test Video Analysis System (HuaiBeiZhengHua Biological Instrument Equipment Co., Ltd., Anhui, China) after they were processed in the box for 4 h from 10 am to 2 pm. Each batch of mice was observed for 5 min daily for 6 days a week.

SPME-GC-MS analysis of volatile compounds

A solid-phase microextraction (SPME) method together with Shimadzu GC-MS-QP 2010 (RTX-5MS capillary column) was used to extract the VOCs from the sampled tree leaves (Li *et al.*, 2014). In each analysis, 10 g of fresh branches and leaves (Here, the branches used were green twigs, which could carry on photosynthesis, and therefore they were grouped together with the leaves. They are called the functional leaves of the tree.) were weighed, cut into 0.5 cm pieces, and placed into a 100 mL extraction flask sealed with aluminum foil. The extraction was conducted at 40 °C using tips of 50/30 µm DVB/CAR/PDMS (divinyl benzene/ carbon molecular sieve/ polydimethylsiloxane). The tips were then inserted into the injection port of a GC-MS and desorbed at 250 °C for 3 min. The GC oven was programmed for 2 min at 35 °C, followed by a temperature increase at the rate of 6 °C ·min⁻¹ to 100 °C, and then by an increase of 8 °C ·min⁻¹ to 140 °C, and finally by an increase of 12 °C ·min⁻¹ to 250 °C, where it was maintained for 3 min. The flow rate of He, carrier gas, was 1.0 mL ·min⁻¹. The MS of the eluting compounds were generated at 70 eV and recorded each second (35 to 450 m ·z⁻¹). The ion source was electron impact (EI), which has a temperature of 200 °C and interface temperature of 230 °C. The volatiles were identified by screening the NIST 08 and NIST 08S libraries for comparable mass spectra and *via* comparison with authentic reference compounds. The relative percentages of the compounds were calculated using the areas of peak normalization method.

Data analysis

SPSS (SPSS, Chicago, IL, USA) and Microsoft® Excel 2003 for Windows software were used to perform one-way analyses of variance (ANOVA). A least significant difference (LSD) test was used for statistical evaluations, and the differences between the different treatments were examined for significance at the 5% level.

RESULTS AND DISCUSSION

Main Components of the Volatiles from *P. chinensis* and *J. chinensis*

As shown in Table 1, *P. chinensis* and *J. chinensis* both produced eight main volatile compounds, which comprised 82.83% and 74.67% of the total VOCs (Li *et al.* 2014), respectively. Olefinic was the most abundant component in *P. chinensis*, with beta-ocimene and beta-trans-ocimene contents of 31.37% and 20.97%, respectively.

These VOCs are used for anti-insect defenses (Arimura *et al.* 2004; Navia-Gine *et al.* 2009) and to inhibit fungal growth (Kishimoto *et al.* 2006; Toome *et al.* 2010); they can also be used for disinfection and deworming. Bornyl acetate was the most abundant compound in *J. chinensis*, followed by D-limonene, which comprised 27.52% and 24.19% of the total VOCs, respectively. The pharmacological actions of bornyl acetate are anti-diarrheal, analgesic, and anti-inflammatory; it also inhibits smooth muscle spasms (Wu *et al.* 2006). D-limonene has a stabilizing effect (Wang *et al.* 2005). Therefore, VOCs from the branches and leaves of *J. chinensis* have a high medical value.

To a certain extent, the peak area shows how much volatile matter is present; the total peak areas of *P. chinensis* were 1.92-fold higher than those of *J. chinensis*, from the same amounts of braches and leaves. The VOCs of the species both consist of the same volatiles, including D-limonene and caryophyllene; the peak areas of *P. chinensis* were 10.9 times greater than that of *J. chinensis* for caryophyllene and 1.04 times greater for D-limonene. Overall, there were differences in the VOCs and the pharmacological actions between *P. chinensis* and *J. chinensis*.

Table 1. Eight Types of Highly Represented Volatiles from *P. chinensis* and *J. chinensis* (Mean \pm SD)

Species	Relative content %	Peak area	Content of volatiles	Category
<i>P. chinensis</i>	31.372 \pm 7.171	42.8 \pm 7.59	beta-Ocimene	Alkene
	20.969 \pm 0.259	29.0 \pm 1.92	beta-trans-Ocimene	Alkene
	12.089 \pm 5.266	17.1 \pm 8.18	D-Limonene	Alkene
	9.552 \pm 0.642	13.2 \pm 0.18	Caryophyllene	Alkene
	3.330 \pm 0.371	4.63 \pm 0.77	2-Pinene	Alkene
	2.411 \pm 0.216	3.32 \pm 0.12	(4E,6Z)-2,6-Dimethyl-2,4,6-octatriene	Alkene
	1.862 \pm 0.484	2.61 \pm 0.80	7-Methyl-3-methylene-1,6-octadiene	Alkene
	1.246 \pm 0.059	1.73 \pm 0.17	Acetic acid cis-3-hexenylester	Ester
<i>J. chinensis</i>	27.515 \pm 1.790	18.6 \pm 1.70	Bornyl acetate	Ester
	24.188 \pm 0.517	16.4 \pm 2.22	D-Limonene	Alkene
	11.270 \pm 0.569	7.61 \pm 0.81	7-Methyl-3-methylene-1,6-octadiene	Alkene
	4.022 \pm 0.111	2.75 \pm 0.50	2,2-Dimethyl-3-methylenebicyclo[2.2.1]heptane	Alkane
	2.894 \pm 0.386	1.93 \pm 0.04	2,6-Dimethyl-2,7-octadien-6-ol	Alcohol
	1.750 \pm 0.209	1.21 \pm 0.33	Caryophyllene	Alkene
	1.552 \pm 0.431	1.11 \pm 0.46	3-(1-Methyl-2-propenyl)-1,5-cyclooctadiene	Alkene
	1.474 \pm 0.184	0.98 \pm 0.03	Cis-3-Hexene-1-ol	Alcohol

Changes in the Total Distance Covered by Mice in an Open Field under Different VOC Conditions

The total distance covered reflects the amount of animal movement, with a large amount of exercise indicating that the central nervous system is excited; conversely, a small amount of exercise indicates that the animal is suffering from a certain degree of depression (Wang *et al.* 2013). Buchbauer *et al.* (1991) measured sedation with lavender essential oil and found that mouse movement was reduced after inhaling this substance in a manner that was negatively correlated with the time and dose of inhalation. As can be seen from Table 2, during the first three days, the total distance covered by the mice first increased and then decreased with increasing amounts of *P. chinensis* VOCs, with the peak located between *P400* and *P600*; on the last three days, the total distance increased. There was a significant difference between the three treatments in the first two days ($P < 0.05$), while the subsequent difference was not significant ($P > 0.05$). *P200* was 1.02, 1.06, and 1.01 times greater than CK. In *P600*, the total distance covered by the mice was shorter than CK at the same time on the first three days, with a different result on the last three days ($P < 0.05$), which suggested that the animals' sense of smell was shocked by the high levels of *P. chinensis* VOCs, making the mice depressive. However, the mice appeared to adapt to the volatiles with increasing time. The total distances covered by the mice increased in *P200*, *P400*, and *P600* from the fourth day, when the mice moved from exploration to adaption, similar to the findings of Wang *et al.* (2011). In a study on the effect of a "forest bathing" treatment in *Phyllostachys edulis* forests on the spontaneous behavior of mice (Wang *et al.* 2015), it was found that the fifth day was a turning point, at which the stage of exploration transferred to the stage of adaptation. It was also found that the exploration stage was much longer than that in this study. This might be because the mice required more time to adapt to the forest park of this wild field environment than in a relatively quiet indoor environment. But overall, after a forest bath, the physical activities of the mice were increased and the excitabilities of the mice were enhanced. A large body of research shows that the effects of plant volatiles on animals are related to the excitation of the olfactory pathway and the excitability of brain regions such as the hypothalamus, hippocampus, and amygdala; the effects are also related to the plasticity of the nervous system (Tang *et al.* 2006; Lin and Ding 2007; Yang *et al.* 2007). After the experimental mice smelled the branches and leaves of *P. chinensis* volatiles, the central nervous system relaxed, leading to enhanced excitability and an increase in the amount of exercise.

With increasing amounts of *J. chinensis* volatiles, the total distance traveled by the mice decreased gradually, with values lower than CK. For the same treatment, there was no difference between *J200* and CK at the same time point ($P > 0.05$), whereas for *J600*, a significant difference was observed ($P < 0.01$, and $P < 0.05$ on day six). At *J600*, the total distance was reduced by 18.47%, 23.18%, 13.79%, 11.67%, 12.26%, and 10.81% from the first to the sixth day, respectively. These data suggested that *J. chinensis* reduces mouse excitability and that a higher concentration enhances this effect. These data also demonstrated that mice adapted to the presence of *J. chinensis* volatiles. In a report (Zhuo *et al.* 2015), low doses volatiles from leaves of *Sabina chinensis* promoted the excitement of mice, while high doses resulted in some depression of mice. Since *Sabina chinensis* is a variation species of *Sabina chinensis*, the following question is raised: is there a large difference between components of the volatiles from these two species? This question needs to be answered in further research.

After exposure to either *P. chinensis* or *J. chinensis*, on the second day, the total distance of the mice traveled increased sharply (by 4.8 to 5.2 times, respectively). Further research is needed on this abnormal behavior. In conclusion, mouse total distance decreased as the number of treatment days increased, which was in agreement with the observed characteristics of the total mouse distance traveled. Spontaneous activity was strong at first and then became weak, indicating that rodents first explore their new environment before adapting to it (Wang *et al.* 2003). Overall, *P. chinensis* improved the excitability of the mouse nerve center, whereas *J. chinensis* yielded the opposite effects.

Table 2. Effects of Different Treatments with *P. chinensis* and *J. chinensis* on Total Distance Traveled by Mice (Mean \pm SD)

Total distances (mm)	<i>P. chinensis</i>			CK	<i>J. chinensis</i>		
	P600	P400	P200		J200	J400	J600
Day 1	7197 $\pm 274^{dB}$	9246 $\pm 61^{aB}$	8753 $\pm 33^{bB}$	8141 $\pm 44^{cB}$	7761 $\pm 47^{cB}$	7192 $\pm 120^{dB}$	6337 $\pm 216^{eB}$
Day 2	35061 $\pm 1479^{dA}$	46577 $\pm 565^{aA}$	42839 $\pm 724^{bBA}$	40190 $\pm 467^{cA}$	39803 $\pm 120^{cA}$	35755 $\pm 756^{dA}$	30875 $\pm 1122^{eB}$
Day 3	8235 $\pm 63^{abB}$	8532 $\pm 192^{aBC}$	8368 $\pm 239^{abBC}$	8022 $\pm 98^{bcBC}$	7709 $\pm 47^{cdB}$	7350 $\pm 71^{dB}$	6916 $\pm 48^{eB}$
Day 4	8370 $\pm 170^{aB}$	8173 $\pm 271^{aC}$	7713 $\pm 119^{bCD}$	7441 $\pm 55^{bCD}$	7308 $\pm 67^{bC}$	7238 $\pm 46^{bB}$	6573 $\pm 150^{cB}$
Day 5	7917 $\pm 148^{aB}$	7561 $\pm 142^{bC}$	7305 $\pm 22^{cD}$	7250 $\pm 58^{cCD}$	7130 $\pm 22^{cdC}$	6921 $\pm 44^{dB}$	6361 $\pm 19^{eB}$
Day 6	8879 $\pm 360^{aB}$	8337 $\pm 262^{aBC}$	7614 $\pm 64^{bD}$	7162 $\pm 44^{bD}$	7056 $\pm 33^{bcD}$	6550 $\pm 128^{cdB}$	6388 $\pm 113^{dA}$

Note: The different lowercase letters within the same line indicate significant differences with treatments. The different uppercase letters within the same column indicate significant differences based on treatment time ($P < 0.05$).

Changes in Center Distance Traveled by Mice/Total Distance Traveled by Mice (C/T) in the Open Field under Different VOC Conditions

The open field was divided into a central activity zone and a marginal activity zone by Paulus and Geyer (1993). The central activity zone was not the zone rodents preferred, and activity was frequent here only in the exploratory stage (Treit and Fundytus 1989; Paulus *et al.* 1999). Table 3 shows the order of central grid game distances against total sport distances, which was $P600 < P400 < P200 < CK < J200 < J400 < J600$ when mice were exposed to volatiles from branches and leaves of *P. chinensis* and *J. chinensis*. The C/T was higher (1.02- to 1.33-fold) than that of the control group after the mice were exposed to volatiles from branches and leaves of *J. chinensis*, and the C/T rose with increasing volatile concentration with a difference that was significant ($P < 0.05$). In contrast, the C/T was lower than that of the control group after the mice were exposed to volatiles from the branches and leaves of *P. chinensis* (80.90% to 97.39% of the control group) and decreased with increasing volatile concentrations, with significant differences in the C/T observed only on the first two days ($P < 0.05$). These results indicated that volatiles from *Sabina chinensis* ‘Kaizuca’ were beneficial by increasing the explorative capacity of mice, whereas those from *P. chinensis* were not. Wang *et al.* (2011) found that *Platycladus orientalis* could also increase mouse explorative capacities, and Zhuo *et al.* (2015) found that a low concentration of *Sabina virginiana* could significantly

increase the total sport distance in the central activity zone, though more experiments are needed to identify whether volatiles from *J. chinensis*, *Platycladus orientalis*, and *Sabina virginiana* contained the same components given that they were all classified as part the same taxonomic family. In the same treatment, volatiles from the branches and leaves of both *P. chinensis* and *J. chinensis* presented the same C/T trend as treatment time increased (d1 < d2 < d3 < d4 < d5 < d6). No significant differences were observed on the first two days ($P > 0.05$), whereas significant differences were observed from the 3rd to the 6th days ($P < 0.05$). Mice had increased activity fluency in the central grid ($P < 0.05$) and demonstrated enhanced adaptability and explorative capacity as the treatment days progressed. This result was similar to that obtained in another study (Wang *et al.* 2015). Both of the results indicated that after exposure to certain volatiles, the mice could increase their exploring abilities, but this required an adaptation process.

Table 3. Effects of Different Treatments with *P. chinensis* and *J. chinensis* on the C/T (Mean \pm SD)

C/T (%)	<i>P. chinensis</i>			CK	<i>J. chinensis</i>		
	P600	P400	P200		J200	J400	J600
Day 1	2.88 $\pm 0.039^{fF}$	3.28 $\pm 0.009^{eE}$	3.40 $\pm 0.027^{dE}$	3.56 $\pm 0.010^{cE}$	3.63 $\pm 0.052^{cE}$	3.83 $\pm 0.030^{bE}$	4.12 $\pm 0.015^{aE}$
Day 2	3.28 $\pm 0.022^{fE}$	3.39 $\pm 0.026^{eDE}$	3.55 $\pm 0.010^{dDE}$	3.64 $\pm 0.006^{cDE}$	3.71 $\pm 0.045^{cE}$	4.01 $\pm 0.020^{bE}$	4.24 $\pm 0.006^{aE}$
Day 3	3.40 $\pm 0.010^{fD}$	3.53 $\pm 0.035^{eFD}$	3.69 $\pm 0.032^{deD}$	3.77 $\pm 0.070^{cdD}$	3.96 $\pm 0.010^{cD}$	4.40 $\pm 0.123^{bD}$	4.98 $\pm 0.098^{aC}$
Day 4	3.60 $\pm 0.038^{fC}$	3.70 $\pm 0.073^{eFC}$	3.88 $\pm 0.088^{deC}$	4.03 $\pm 0.038^{dC}$	4.31 $\pm 0.105^{cC}$	4.81 $\pm 0.050^{bC}$	5.04 $\pm 0.074^{aC}$
Day 5	4.12 $\pm 0.030^{fB}$	4.26 $\pm 0.058^{eFB}$	4.47 $\pm 0.076^{deB}$	4.51 $\pm 0.044^{dB}$	4.95 $\pm 0.092^{cB}$	5.26 $\pm 0.077^{bB}$	5.57 $\pm 0.087^{aB}$
Day 6	4.60 $\pm 0.053^{fA}$	4.75 $\pm 0.068^{eFA}$	4.85 $\pm 0.042^{deA}$	4.98 $\pm 0.061^{dA}$	5.45 $\pm 0.044^{cA}$	5.73 $\pm 0.029^{bA}$	6.61 $\pm 0.064^{aA}$

Note: The different lowercase letters within the same line indicate significant differences with treatments. The different uppercase letters within the same column indicate significant differences based on treatment time ($P < 0.05$).

Changes in Weight in the Open Field under Different VOC Conditions

Changes in mouse body weights reflect an impact on the appetite. Gaworski *et al.* (1992) evaluated the toxicity of citral inhalation and found that mice grew normally if the inhaled concentration was lower than 68 mg/L, but certain symptoms, including weight loss, hypopsia, dyspnea, and drooling, appeared if a higher inhaled concentration was present. As shown in the Table 4, the *P. chinensis* and control groups experienced the same changes in body weight over all 6 days. Body weight increased with the increase in processing number. However, body weight decreased with increases in the *P. chinensis* volatiles. For example, in the P200 treatment group, the increments in mouse body weight from the first to the sixth day were 66.67%, 64.86%, 69.23%, 75.00%, 78.57%, and 79.55% those of the control group. Additionally, in the P.3 treatment group, the increments in mouse body weight were -139.39%, -72.97%, -46.15%, -25.00%, -16.67%, and 29.55% those of the control group. The mouse body weight experienced a negative growth trend during the first five days, and with the increment of the processing number, the body weight variation in the treatment group was close to that of the control group on

the same day, suggesting that volatiles from *P. chinensis* branches and leaves could decrease appetite; the P600 treatment in particular led to anorexia. Additionally, Wang *et al.* (2012) reported that volatiles from camphor branches and leaves decrease appetite. Furthermore, volatiles from *P. chinensis* and camphor branches and leaves have very pungent odors, and most people have adverse reactions to these scents.

For the *J. chinensis* treatment group, with the increment of the processing number and the concentrations of volatiles from branches and leaves, the body weight variation did not change significantly, though the body weight variation in the treatment group was higher than that in the control group. Furthermore, the least variation in mouse body weight was observed in the J400 group that had been treated for 6 days; the body weight of that treatment group was 1.02 times that of the control variation. The group with maximal variation in mouse body weight was the J200 group after 1 day of treatment, with a body weight in the treatment group that was 1.58 times that of the control variation. These findings suggest that the volatiles from *J. chinensis* branches and leaves can improve mouse appetites slightly, similar to the results reported by Wang *et al.* (2015). On the whole, *P. chinensis* can reduce mouse body weight increments, whereas *J. chinensis* can increase mouse body weight.

Table 4. Effects of Different Treatments with *P. chinensis* and *J. chinensis* on Mouse Weights (Mean \pm SE)

Weight (g)	<i>P. chinensis</i>			CK	<i>J. chinensis</i>		
	P600	P400	P200		J200	J400	J600
Day 1	-0.46 $\pm 0.003^{dF}$	0.16 $\pm 0.007^{cC}$	0.22 $\pm 0.012^{cD}$	0.33 $\pm 0.012^{bcD}$	0.52 $\pm 0.099^{aA}$	0.45 $\pm 0.042^{abA}$	0.44 $\pm 0.094^{abA}$
Day 2	-0.27 $\pm 0.015^{dE}$	0.17 $\pm 0.009^{cC}$	0.24 $\pm 0.021^{bcD}$	0.37 $\pm 0.012^{abC}$	0.43 $\pm 0.092^{abA}$	0.47 $\pm 0.038^{aA}$	0.44 $\pm 0.040^{aA}$
Day 3	-0.18 $\pm 0.019^{dD}$	0.18 $\pm 0.010^{cBC}$	0.27 $\pm 0.015^{cCD}$	0.39 $\pm 0.013^{bC}$	0.41 $\pm 0.022^{bA}$	0.51 $\pm 0.044^{aA}$	0.47 $\pm 0.060^{bA}$
Day 4	-0.10 $\pm 0.003^{dC}$	0.21 $\pm 0.017^{cABC}$	0.30 $\pm 0.007^{bBC}$	0.40 $\pm 0.010^{aBC}$	0.42 $\pm 0.037^{aA}$	0.46 $\pm 0.041^{aA}$	0.43 $\pm 0.045^{aA}$
Day 5	-0.07 $\pm 0.012^{eB}$	0.22 $\pm 0.015^{dAB}$	0.33 $\pm 0.015^{cAB}$	0.42 $\pm 0.006^{bAB}$	0.51 $\pm 0.054^{aA}$	0.50 $\pm 0.032^{abA}$	0.43 $\pm 0.012^{bA}$
Day 6	0.13 $\pm 0.010^{eA}$	0.25 $\pm 0.020^{dA}$	0.35 $\pm 0.019^{cA}$	0.44 $\pm 0.007^{bA}$	0.53 $\pm 0.023^{aA}$	0.45 $\pm 0.035^{bA}$	0.53 $\pm 0.015^{aA}$

Note: The different lowercase letters within the same line indicate significant differences with treatments. The different uppercase letters within the same column indicate significant differences based on treatment time ($P < 0.05$). Body weight change: before and after change

Changes in Fecal Grains in the Open Field under Different VOC Conditions

Fecal grains reflect the degree of anxiety, with more fecal grains indicating a higher degree of curiosity and fewer meaning a lower degree of curiosity (Dong *et al.* 2012). Table 5 shows the total number of mouse fecal grains produced under the treatment conditions. This data shows that mice grew accustomed to the environment containing volatiles from the branches and leaves of two different plants as the treatment days progressed and, as a result, their degree of curiosity was reduced. The numbers of fecal grains in the group exposed to *P. chinensis* branch and leaf volatiles were clearly higher than those in the control group, with the number increasing in proportion to the volatile concentration, especially for mice exposed to P600 for 1 to 6 days, which were

2.22-, 2.16-, 2.10-, 2.08-, 2.02-, and 1.91-fold higher, respectively, than the control group, indicating that the mice were nervous in the environment containing *P. chinensis* volatiles and that this anxiety increased in proportion to the volatile concentration. However, mice did not show such anxiety in environments containing *J. chinensis* volatiles, as shown in Table 5. There was no relationship between fecal grains and volatile concentration, and fecal grain numbers in the treatment group were less than those in the control group. The reduction in *J600* was the highest, with the numbers of fecal grains of mice in condition *J600* being 79.27%, 80.52%, 83.10%, 87.30%, 88.33%, 92.98%, respectively, of those in the control group, indicating the mice were not nervous when exposed to *J. chinensis* volatiles and that they felt more comfortable as the concentration increased. Wang *et al.* (2011) found that volatiles from *Platycladus orientalis* arborvitae reduce the number of fecal grains, and Dong *et al.* (2012) found that the degree of stress decreased when mice were in an environment containing *Ligustrum lucidum*, which had the same effects as *J. chinensis* volatiles.

Table 5. Effects of Different Treatments with *P. chinensis* and *J. chinensis* on Mouse Fecal Grains

Number (pcs)	<i>P. chinensis</i>			CK	<i>J. chinensis</i>		
	<i>P600</i>	<i>P400</i>	<i>P200</i>		<i>J200</i>	<i>J400</i>	<i>J600</i>
Day 1	182	108	94	82	80	76	65
Day 2	166	98	88	77	72	70	62
Day 3	149	96	84	71	58	66	59
Day 4	131	96	80	63	58	60	55
Day 5	121	80	76	60	56	56	53
Day 6	109	76	77	57	49	52	53

Generally, *P. chinensis* volatiles may lead to the excitement of neural centers, resulting in reduced capacity to explore, loss of appetite, and increased anxiety. Further testing is needed to verify whether ocimene was the main contributor, given that its content reached 52.44%. In contrast to *P. chinensis* volatiles, *J. chinensis* volatiles may result in the enhancement of exploratory capacity and the elimination of stress. Its components include bornyl acetate, caryophyllene, limonene, and linalool, which have stabilizing effects. The roles that these compounds play in the aforementioned changes also require further study.

The results obtained from these experiments showed that high concentrations of *P. chinensis* volatiles could cause adverse effects on mice, such as mental strain, exploration ability declining, and inappetence. These effects may also be imposed on human if these trees were planted large-area in residential and recreation areas. However, Liu *et al.* (2012) proposed that *P. chinensis* performed functions of sterilization and carbon fixation and provided a good landscape effect as a colored-leaf tree. Therefore, this species could be planted as a landscape tree out of the smell range to achieve a fully positive effect. *J. chinensis* volatiles allowed mice to relax and improved their explorative

and memory capacities. Therefore, in addition to its ornamental value, *J. chinensis* should be planted in parks or residential areas to fully display its internal and external beauty.

CONCLUSIONS

1. VOCs from *P. chinensis* branches and leaves increased the total distance traveled by mice and also stimulated them. Concurrently, these volatiles increased the number of fecal grains produced by mice, which suggests increased anxiety in response to the presence of the volatile matter.
2. The exploratory capacity of mice was enhanced in the presence of *J. chinensis* volatiles, while *P. chinensis* volatiles had the opposite effect.
3. VOCs from the branches and leaves of *P. chinensis* had a negative influence on mouse appetites, especially when the treatment reached 600 g, which resulted in anorexia and negative growth in mice. *J. chinensis* VOCs had the opposite effect.

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