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The Potential Benefits of Therapeutic Treatment Using Gaseous Terpenes at Ambient Low Levels

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Featured Application: residents who have less chance to contact with natural monoterpene emitted from plants.

Abstract: Biological volatile organic compounds (BVOCs) are emitted abundantly from the flora. Among BVOCs, monoterpenes (MTs) have been used for aromatherapy (e.g., forest bathing) to treat human physiological and psychological conditions. However, relatively little is known about the therapeutic effects of MTs at low part per billion by volume (ppbv) levels. The effects of artificial MTs on human subjects were assessed by both olfactory and therapeutic parameters (brain alpha waves and stress index). Gaseous standards of three monoterpenes (i.e., α-pinene, β-pinene, and *d*-limonene) prepared at low ppbv levels were used individually and as mixtures. Fifty-nine healthy and non-asthmatics volunteer university students were selected for the test. All human subjects inhaled low ppbv levels of monoterpene in the testing room. Brain alpha waves and stress index were investigated during the inhalation time. Questionnaires were also used after testing. It was found that the detection threshold of MTs was close to 5 ppbv. When the MT levels increased from 0 to 20 ppbv, the mean values of brain alpha waves derived from all participants increased from 9.8 to 15.1. In contrast, the stress index values declined from 46.2 to 34.7. The overall results suggest that MTs have great potential to positively affect the relaxation state of subjects in a manner similar to forest bathing in terms of short-term effect. They can thus be applied as potential therapeutic media for mental health care.

Keywords: monoterpene; psychology; physiology; brain wave; forest bathing

1. Introduction

In general, natural aromatherapy has been shown to be a potential and effective solution to treat harmful psychological conditions such as anxiety, depression, and anger. Aromatic compounds called phytoncides or wood essential oils of plant constituents have been identified as the cause of psychological effects [1–4]. Monoterpenes (MTs: $C_{10}H_{16}$) are a class of biological volatile organic

compounds (BVOCs) consisting of two isoprene units. They are the second most abundant BVOCs emitted from plants [5]. The emission and composition of MTs have been widely investigated (Table 1) [6–15]. Of these compounds, *α*-pinene, *β*-pinene, and *d*-limonene are predominant. Many studies have explored the effects of MTs on human health with respect to their composition and concentration level [1,3,4,16–21]. It was also documented that several MTs may exert adverse health effects on humans [21]. However, these adverse effects could only occur when MTs concentrations exceeded the threshold level of human sensory irritation or when they reacted with ozone to produce harmful by-products.

Table 1. Comparison of concentration levels (pptv) and composition ratios (%) of monoterpene compounds emitted from forests in different studies.

Site	*α*-Pinene	*β*-Pinene	*d*-Limonene	3-Carene	Myrcene	Camphene	etc.	ΣM. Terpene	Ref.
Castelporziano, Italy	97 (33%)	36 (12%)	162 (55%)	bdl	bdl	bdl	0	295	[8,9]
Chiaotou, Taiwan	100 (28%)	253 (72%)	bdl	bdl	bdl	bdl	0	353	[8]
Balbina, Amazonia, Brazil	1100 (44%)	500 (20%)	350 (14%)	bdl	50 (2%)	100 (4%)	400 (16%)	2500	[10]
Ilomantsi, Finland	100 (64%)	16 (10%) *	10 (6%)	22 (14%)	-	2 (1%)	6 (4%)	156	[7]
Austin Cary Forest, FL, USA	125 (59%)	86 (41%)	bdl	bdl	bdl	bdl	bdl	211	[11]
Blodgett Forest, CA, USA	104 (14%)	311 (43%)	76 (11%) **	210 (29%)	10 (1%)	6 (1%)	5 (1%)	722	[6]
Mainz, Germany	117 (28%)	98 (23%)	74 (18%)	49 (12%)	24 (6%)	bdl	59 (14%)	421	[14]
Djougou, Benin	300 (55%)	200 (36%)	50 (9%) **	bdl	bdl	bdl	0	550	[12]
Oshiba plareau, Japan	70 (41%)	26 (15%)	74 (44%) **	bdl	bdl	bdl	0	170	[13]
Jönköping, Sweden	9730 (50%)	320 (2%)	1300 (7%)	7000 (36%)	210 (1%)	700 (4%)	340 (2%)	19,600	[5]
Odae Chanamu forest, Korea	133 (45%)	55 (19%)	61 (21%)	bdl	bdl	44 (15%)	bdl	293	[15]
Seonam temple forest, Korea	32 (31%)	38 (37%) *	13 (13%)	bdl	-	7 (7%)	11 (11%)	107	[15]
Juknokwon forest, Korea	77 (27%)	103 (36%) *	74 (26%)	bdl	-	12 (4%)	24 (8%)	291	[15]

* *β*-pinene+myrcene, ** *d*-limonene + β-phellamdrene, bdl: below detection limit, "-": not detected, pptv: part per trillion by volume.

Forest environments are known to affect various human senses, including vision, olfaction, and auditory and tactile sensations [22]. To date, much effort has been applied to assess the effect of such environmental conditions on human wellbeing. Some of those studies focused on the complex mixtures of BVOCs released from materials such as wood chips or wood-extracted oil [23–28]. However, it is not easy to determine the relative significance of a particular component among many different BVOC components.

The effects of forest bathing (e.g., Shinrin-Yoku) on human psychology have been well documented [22]. However, the MT levels in a forest can vary widely from pptv to ppbv, due to the combined effects of many factors such as reactivity, temperature, wind speed, and emission rate of MTs from the flora. Moreover, the effects of forest bathing, such as those of phytoncides, are complex, since they include all elements associated with senses (i.e., vision, olfaction, audition and tactile sensations) [22]. Therefore, it is not easy for a human to consistently recognize the odor of MTs. Also, the presence of MTs may not have an effect on human psychology and physiology. Therefore, the results of these previous studies could be biased if they are used to assess the effects of forest bathing. Thus, we attempted to investigate the effect of MTs at fixed concentrations and exposure times on young human subjects in the laboratory to remove the visual and auditory effects present in a forest.

It was found that a relaxed physiological state was observed at relatively low concentrations of *α*-pinene (e.g., 0.3–3.0 part per million by volume-ppmv) [28]. Likewise, the inhalation of *d*-limonene (~0.3 ppmv) was reported to induce a comfortable and soothing state [28]. Further, the inhalation of *d*-limonene (2.5 ppmv) for 90 s was reported to provide physiological and psychological relaxation [29]. However, those tests were conducted using MTs prepared at artificially high concentrations (e.g., at sub-ppmv levels) rather than at those which are actually present in ambient air.

No research has been conducted to directly assess the effects of ambient-level MTs on human psychology using standard samples made of three major MTs (*α*- pinene, *β*- pinene, and *d*- limonene) at

low ppbv levels. Accordingly, we used a cohort of 59 subjects to investigate the human psychological response to inhaling the three major MTs at low ppbv levels by monitoring two parameters: alpha waves and stress index.

2. Materials and Methods

2.1. Apparatus and Materials

A standard gas generation system based on the permeation technique (Dynacalibrator 230, Valco Instruments Co. Inc., Houston, TX, USA) was used for the generation of MT standard gases for olfactory tests. A zero-air generator (M701, Teledyne Advanced Pollution Instrumentation Inc., San Diego, CA, USA) was used to supply zero air. The outlet air of the zero-air generator did not contain any quantifiable VOCs. In this work, three MTs (i.e., α-pinene, β-pinene, and d-limonene) were selected as the main target because of their general abundance (see Table 1). They were generated using a permeation tube system containing them at high concentration levels in (near) pure forms (>99%) (100-106-1805-U90, 100-103-1851-U90, and 100-022-1855-U90, Valco Instruments Co. Inc., TX, USA). The outlet concentrations of the MTs were maintained in the range of 2 to 20 ppbv. For the actual human olfactory test, the mixture gas was made to flow at 5 L/min into a funnel, which was placed close to the subject's nose. The test mixture of the MTs generated from the permeation system was also analyzed to determine their real concentrations and compositions using a gas chromatograph (6890, Agilent Technologies, Santa Clara, CA, USA) coupled with a mass-selective detector (5975, Agilent Technologies, USA) as reported elsewhere [30]. To pretreat the sample gas, an adsorption tube trap [30,31] or absorption needle trap [32] can be employed. In this study, MTs samples were collected by an adsorption tube trap made of 110 mg of Tenax TA (60/80 mesh, Sigma-Aldrich Inc., Louis, MO, USA) and 100 mg of Carbotrap (20/40 mesh, Supelco, USA). The analytical procedures for the quantitation of the selected targets have been reported elsewhere [30].

Temporal stability in the generation of MT gases for the olfactory test was assessed for up to 12 h. Once the generation system was equilibrated with regard to temperature, the target gases collected from the outlet of the generation system were measured hourly for 12 h. The temporal variability was assessed in terms of the relative standard deviation (RSD), and the values were stable (<2.5%).

2.2. Test Subjects

A total of 59 healthy and non-asthmatics volunteer subjects were recruited for the test. Among the volunteers, those who had problems with olfactometric capabilities were excluded. The age of the students ranged between 22 to 33 years. They were divided into two groups. The first group consisted of 29 subjects (23 males), who took part in the first experiment (see Section 2.3.1). The second group comprised 30 subjects (15 males), who participated in the second experiment (see Section 2.3.2). Informed consent was obtained from all individual participants included in the study. All participants were asked not to use perfume and strong smelly cosmetics at least 24 h prior to the experiment.

2.3. Experimental Procedure

Due to the variability of MT levels in ambient air (see Table 1), it is important to select proper concentration levels for human olfaction tests. Two types of olfactory experiments were designed and conducted. In our first experiment (Exp 1), measurements of target gases were carried out using individual standard gases of MT, wherein the detection threshold values of each MT were measured. In this study, the detection threshold was defined as the MT concentration at which 50% of participants could distinguish their presence from the background. This approach was adopted by following the odor threshold procedures from the US EPA (1990) [33]. A questionnaire was used to investigate the relative preference among the three MTs by each participant because of their various mixing ratios in ambient air (Table 1). Information regarding the relative preference was then used to determine a mixing ratio for the three target MTs in the next experiment (Exp 2). To this end, a mixture of the

three MTs (α-pinene, β-pinene, and *d*-limonene = 1:0.5:0.5) was generated. Hence, during Exp 2, each participant inhaled the mixture over 2.5 min to quantitatively assess the therapeutic potential of the MT gases.

2.3.1. Assessment of the Human Olfactory Response to the Odors of α- Pinene, β- Pinene, *d*-Limonene (Exp 1)

Exp 1 was conducted to determine the human olfactory response at given concentration levels of each MT. Each participant was led into a clean testing room (L × W × H = 7 × 7 × 3 (147) m^3) and inhaled a test MT mixture dispensed from a funnel close to the nose for 2.5 min (Figure 1). The exposure time was 2.5 min because it was sufficient to observe physiological changes [29].

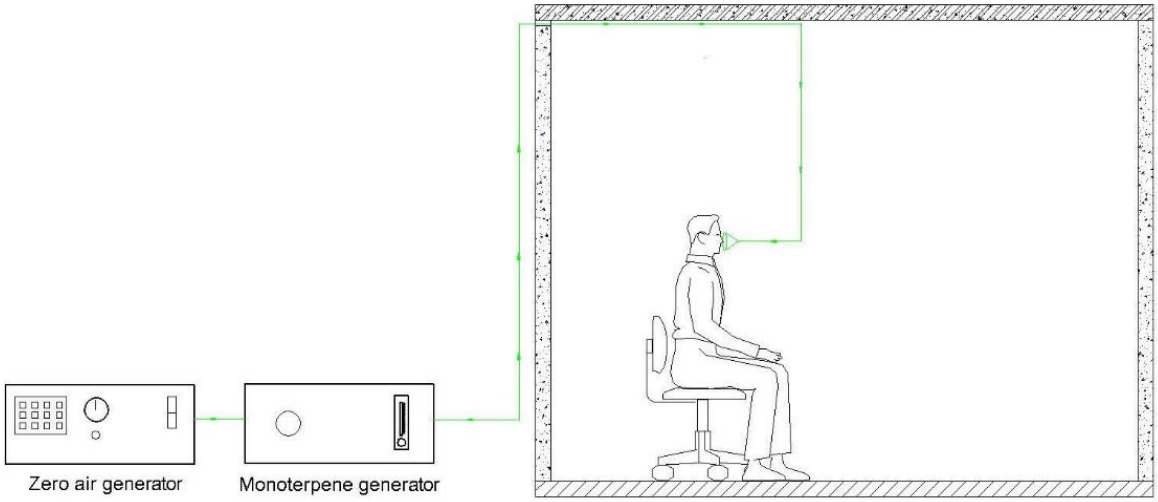

Figure 1. Experimental scheme for monoterpene (MT) exposure in Exps 1 and 2.

Sequentially, each participant inhaled each MT standard prepared at four different concentration levels (i.e., 2, 3, 5, and 7 ppbv). The different concentration were presented randomly. To this end, the permeation tubes for standard MT gases of α- pinene, β- pinene, and *d*- limonene were used at permeation rates of 201, 252, and 253 ng/min, respectively. For every single MT tested, each participant inhaled samples at a certain concentration level for 2.5 min. To avoid contamination of the room during the experiment [34], a 15 min break was taken by all participants before the inhalation of a mixture at a different concentration levels. The testing room was ventilated with fresh air during this time, at a flow rate of approximately 30 m^3/min. The participant seated in the room during break time. The inhalation testing room was maintained at a temperature of 25 ± 1 °C and a relative humidity of 50 ± 5%. Before and after each experiment where one kind of MT was tested, the room was ventilated with fresh air for one hour using fans. After inhaling the MTs at all concentration levels, the participants were asked to answer a questionnaire (see Table 2).

Table 2. Questionnaire for the assessment of human responses to the odors of α- pinene, β- pinene, *d*- limonene.

1. What is your gender? ① Male ② Female
2. Do you smoke? ① Yes ② No
3. How is your current physical condition? ① Excellent ② Fine ③ Good ④ Not good ⑤ Not very good
4. What do you think about sample 1 smell? ① Very refresh ② Refresh ③ Normally ④ Uncomfortable ⑤ Very uncomfortable
5. What do you think about sample 2 smell? ① Very refresh ② Refresh ③ Normally ④ Uncomfortable ⑤ Very uncomfortable
6. What do you think about sample 3 smell? ① Very refresh ② Refresh ③ Normally ④ Uncomfortable ⑤ Very uncomfortable
7. What level can you smell clearly? Sample 1: ① 2ppbv ② 3ppbv ③ 5ppbv ④ 7ppbv Sample 2: ① 2ppbv ② 3ppbv ③ 5ppbv ④ 7ppbv Sample 3: ① 2ppbv ② 3ppbv ③ 5ppbv ④ 7ppbv
8. Which sample do you think has the best odor? ① Sample 1 ② Sample 2 ③ Sample 3
9. Which sample has the worst odor among the three kinds of samples? ① Sample 1 ② Sample 2 ③ Sample 3
10. After the experiment, how is your physical condition? ① Excellent ② Fine ③ Good ④ Not good ⑤ Not very good
11. What do you think about your psychology state (stress, anger, sleepy) after smelling? ① Very stable ② Stable ③ Normally ④ Unstable ⑤ Very Unstable
12. Please write if you are suffering from diseases or symptoms. (Example: Nose allergies, Sinusitis, etc.)
13. Please write a comment about the experiments.

2.3.2. Effects of Mixed Monoterpenes on Human Psychology and Physiology (Exp 2)

The second group was recruited for Exp 2, which employed the same test chamber used in Exp 1. The participants in Exp 2 inhaled a mixture of MT gases instead of individual gases, as explained above. Here, a mixture of MTs consisting of α-pinene, β-pinene, and *d*-limonene was generated using permeation tubes as described above. As shown in Table 1, the ratio of β-pinene to α-pinene varied from 0.03 to 1.34, and that of *d*-limonene to α-pinene ranged from 0.00 to 1.67. Their average mixing ratios were about 1:0.7:0.4 (refer to Table 1). On the other hand, the results of the Exp 1 showed that people preferred *d*-limonene over α-pinene or β-pinene. Consequently, we set the mixing ratio of the 3 MTs to be α-pinene/β-pinene/*d*-limonene = 1:0.5:0.5. Although outdoor ambient MT concentrations are often variable from pptv to low ppbv levels (Table 1), people could clearly detect MT odors at the ppbv level (from Exp 1). It was found that the detection threshold of MT in this study was 5 ppbv (from Exp 1). However, 100% of participants could detect the odor at 7 ppbv. Moreover, MTs at ambient levels exert their effects on humans via olfactory mechanisms rather than via the blood-borne route through the lungs [22]. Hence, 7 ppbv was selected as the minimum concentration of MTs in this work to make sure that all participants could detect the MTs' odors. Furthermore, the maximum total concentration of MTs in the ambient air was about 20 ppbv (Table 1). Thus, the total concentration of the mixture was varied as follows: 0, 7, 15, and 20 ppbv (see Table 3). The total concentration was calculated on the basis of the total mass of all compounds in the mixture. The mass of each compound was derived from its calibration curve. Each participant inhaled different levels of the mixed MTs

for 2.5 min. For this test, a break was given at 15 min intervals between measurements at different concentration levels.

Table 3. Mixing ratios and concentrations of MTs for Exp 2.

No.	Total Concentration (ppbv)	Mixing Ratio		
		α-Pinene	*β*-Pinene	*d*-Limonene
1	0	0.0	0.0	0.0
2	7	1.0	0.5	0.5
3	15	1.0	0.5	0.5
4	20	1.0	0.5	0.5

During exposure to MTs, the participant's heart rate was continuously monitored using a heart rate variability (HRV) sensor (UbioMacpa, Bio-sense Creative Co. Ltd., Republic of Korea) to evaluate the subject's physiological condition. HRV (including blood pressure and pulse rate) has been used frequently to estimate changes in the autonomic nervous activity [22]. In the present research, the HRV values were interpreted on the basis of a stress index: good (HRV < 24), temporary stress (25 < HRV < 35), primary stress (35 < HRV < 45), accumulated stress (45 < HRV < 60), and chronic stress (HRV > 60). Moreover, the brain waves of the participants were also measured using a brain wavemeter (EEG, Neuroharmony Co., Republic of Korea). The relaxed status of each subject was presented as the alpha value of the brain wave (i.e., alpha wave). Note that the alpha waves are unitless and observed in the frequency range of 8 to 12 Hz in association with physical relaxation [35]. The measured data for alpha waves were downloaded through the software system of the brain wavemeter.

After inhaling the MT standard test mixtures, questionnaires were administered to assess the psychological conditions, which were related to the stress index and alpha wave (Table 4).

Table 4. Questionnaire for the evaluation for the effects of mixed MTs on human psychology.

1. What is your gender? ① Male ② Female
2. Do you smoke? ① Yes ② No
3. Currently, do you have rhinitis symptoms? ① Yes ② No
4. How is your psychological stability condition? ① Very comfortable ② Comfortable ③ Normal ④ Uncomfortable ⑤ Very uncomfortable
5. After the experiment, how is your psychological stability condition? ① Very comfortable ② Comfortable ③ Normal ④ Uncomfortable ⑤ Very uncomfortable
6. Please write if you are suffering from diseases or symptoms. (Ex: Nose allergies, Sinusitis, etc.)
7. Please write a comment about the experiments.

Analysis of variance (ANOVA) and STATGRAPHICS Centurion XV software ver. 15.2.05 (Statpoint Technologies Inc., Warrenton, VA USA) were used to analyze the stress index and alpha waves. The stress index and alpha wave values ($p \leq 0.05$) among participants were evaluated statistically using Fisher's least significant difference (LSD) procedure. All experimental materials and procedures involved in these experiments have been reviewed and approved by the Konkuk University Institutional Review Board (reference number: 7001355-201507-HR-065, email: irb@konkuk.ac.kr).

3. Results

3.1. Effect of Individual α-Pinene, β-Pinene, and d-Limonene on Humans

As seen in Tables 5 and A1 , 50% of the participants could detect the odors of the MTs at 5 ppbv. Therefore, the human olfactory MT detection threshold was set to 5 ppbv. On the other hand, it was found that 100% of participants could detect the odors of the MTs at 7 ppbv.

Table 5. Participants' ability to detect MTs' odor (%), as obtained from Exp 1.

Concentration (ppbv)	Ratio of Participants % (Ratio of Gender %)								
	α-Pinene			*β*-Pinene			*d*-Limonene		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
2	0.00 (0.00)	3.45 (16.7)	3.45	0.00 (0.00)	0.00 (0.00)	0.00	0.00 (0.00)	0.00 (0.00)	0.00
3	17.2 (21.7)	3.45 (16.7)	20.7	10.3 (13.0)	3.45 (16.7)	13.8	13.8 (17.4)	3.45 (16.7)	17.2
5	44.8 (56.5)	20.7 (100)	65.5	31.0 (39.1)	20.7 (100)	51.7	48.3 (60.9)	20.7 (100)	69.0
7	79.3 (100)	20.7 (100)	100	79.3 (100)	20.7 (100)	100	79.3 (100)	20.7 (100)	100

In this research, it was observed that *d*-limonene was the preferred odor (62% of participants), followed by *β*-pinene (24% of participants). In contrast, *α*-pinene was the least favorite odor in this study. Most of the participants preferred *d*-limonene as it is preferably used in many commercial and popular products (as orange, lime, lemon, etc.).

3.2. The Effects of Mixtures of Monoterpenes on Human Psychology and Physiology

Variations in alpha waves and stress index values observed in Exp 2 are shown in Figures 2 and 3, respectively.

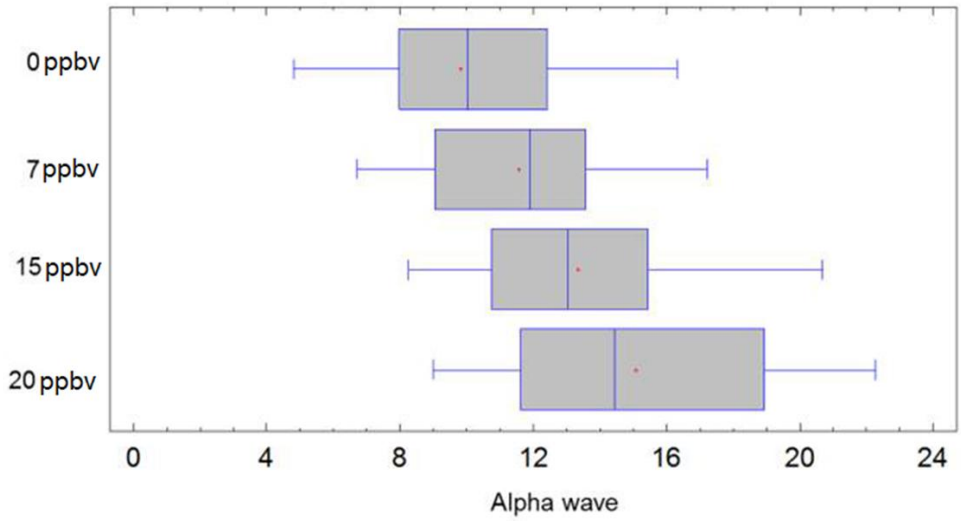

Figure 2. The alpha wave values of 30 participants (15 male and 15 female) at different monoterpene concentrations, as obtained from Exp 2.

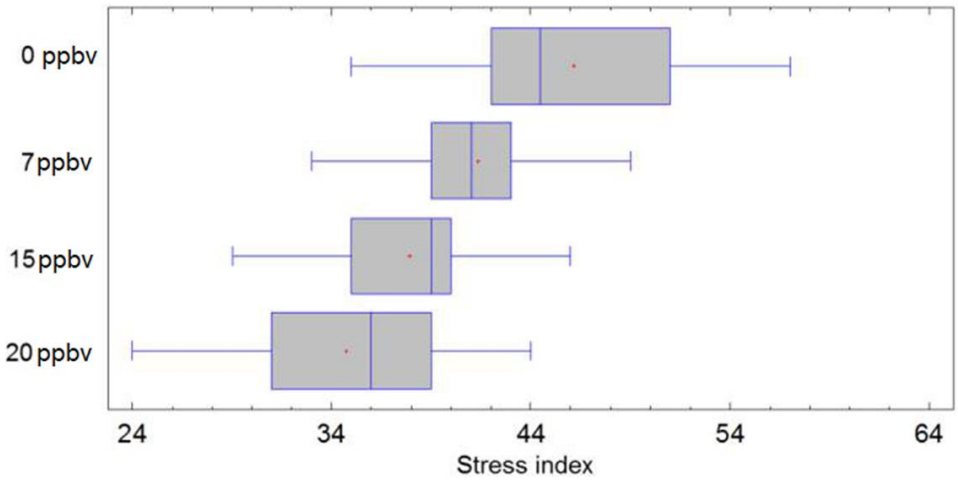

Figure 3. The stress index values from 30 participants (15 male and 15 female) at different monoterpene concentrations, as obtained from Exp 2.

As shown in Figure 2, the increase in MT concentration caused an increase in alpha waves, indicating that the participants felt relaxed in the presence of MTs, but this response was dose-dependent. All participants showed the most physiological benefit at 20 ppbv MT (mean of the alpha waves = 15.1 ± 4.0, refer to Table A2) compared to background conditions (mean of alpha wave = 9.8 ± 3.0, refer to Table A2). This pattern was confirmed by the LSD results. The mean values of alpha waves changed significantly (p value = 0) with increasing concentration of MTs. The increasing alpha patterns were similar for both gender groups (Table 6). Although female olfaction was more sensitive than that of males (obtained from Exp 1), there was no statistically significant (p-values > 0.05) difference in mean alpha wave values between males and females.

Table 6. Mean values of alpha wave and stress indexes of male and female participants obtained from Exp 2.

Parameter	Gender	n	Background	7 ppbv	15 ppbv	20 ppbv
Alpha wave	Male	3	9.3 (± 3.2)	11.3 (± 3.1)	13.1 (± 3.3)	15.4 (± 4.2)
	Female	3	10.4 (± 2.7)	11.8 (± 3.0)	13.5 (± 3.6)	14.7 (± 3.8)
	p-value [1]		0.319	0.696	0.746	0.626
Stress index	Male	3	45.8 (± 5.5)	41.5 (± 3.7)	38.9 (± 4.4)	35.3 (± 4.8)
	Female	3	46.5 (± 7.0)	41.3 (± 6.3)	37.0 (± 4.4)	34.1 (± 5.6)
	p-value [1]		0.752	0.916	0.257	0.533

[1] p-values were obtained from t-tests (95% confidence) to compare the means of male and female participants. Values in the parentheses are standard errors.

As illustrated in Figure 3, the increase of MT concentrations resulted in the decrease of the stress index values. In addition, the mean background stress index of both male and female participants was 46.2 ± 6.2 (refer to Table A2) and was higher at 20 ppbv MT (34.7 ± 5.2, refer to Table A2). This indicates that MT exposure helps reduce human stress. Since the concentrations of MTs were at ppbv levels, their effects must occur via the olfactory processing pathway, not via the blood-borne route through the lungs [28]. The alpha wave data showed that there was also no significant difference (p-values > 0.25) in mean stress index between males and females (Table 6).

Figure 4 shows variations in alpha waves and stress index with respect to the concentrations of MTs within 2.5 min. The alpha waves of males (slope: 0.292) increased more rapidly than those of females (slope: 0.214). This indicates that the alpha waves of male were more sensitive to inhaling MTs than those of females. On the other hand, the stress index of females (slope: −0.611) decreased more rapidly than that of males (slope: −0.497). This indicates that stress in females was reduced

more readily by inhaling MTs than in males. Furthermore, when comparing the variations in alpha waves and stress index between males and females as a function of MT concentration, the alpha waves (r^2 = 0.999) and stress index (r^2 = 0.995) of females were slightly more consistent than those of males (alpha wave: r^2 = 0.982; stress index: r^2 = 0.981). This indicates that MT affected female psychology more than male psychology, because the olfactory system of females is more sensitive than that of males [36].

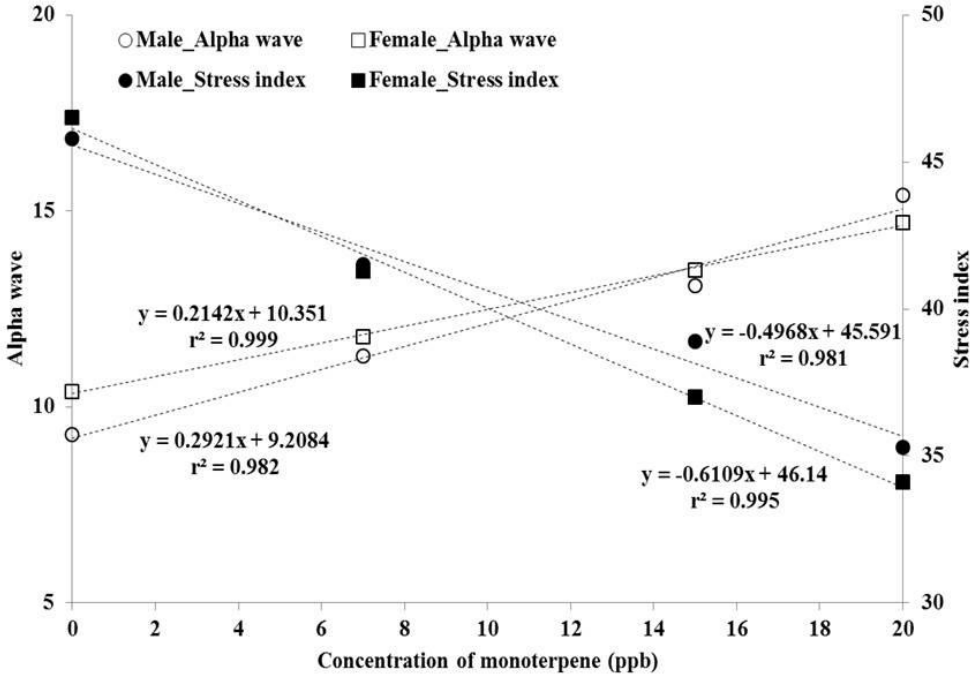

Figure 4. Variations of the mean values of alpha waves and stress index of 30 participants with respect to the concentration of MTs, as obtained from Exp 2.

The results obtained from the questionnaires also showed similar psychological patterns (refer to Table A3). Only 44% of the participants felt comfortable before inhaling the MTs. In contrast, after inhaling the MTs, 87% of the participants felt comfortable. The distribution of comfortable female and male participants increased from 40% and 47% to 80% and 93%, respectively.

4. Discussion

In terms of MT detection threshold, our results are similar to those of previous studies. Cain et al. (2007) reported that the detection threshold of limonene was 2 to 4 ppbv [37]. In contrast, the P100 threshold of α-pinene was about 15 ppbv (90 μg/m^3) [38]. Furthermore, a larger fraction of female subjects was able to detect the odor of 5 ppbv MTs compared to male subjects (Table 5). This indicates that the female olfactory response is more subtle than the male response. It was suggested that women have more neurons and glial cells than men [36]. Moreover, the odor perception of women is more effective than that men [39].

The concentration of MTs in our experiments was adjusted on the basis of their natural levels in forests. Moreover, the concentrations of MTs can vary depending on many factors such as season, climate, and forest composition [22]. Therefore, the concentration of MTs must be chosen on the basis of their detection limits when studying MTs effects on human psychology. The results of this study showed that all participants could clearly sense the MTs at a concentration of 7 ppbv, indicating that MTs might not affect human psychology when they are present under the detectable smell level (2 ppbv). In a study, it was concluded that the strong odor of α-pinene (~16.7 ppmv) was uncomfortable for the subjects [28]. However, this concentration level was much higher (e.g., in ppmv level) than that

in ambient level (e.g., in ppbv level). In this study, the objective was exposure to the low (ppbv) levels of MTs. Thus, it is not easy to directly compare those results to ours.

It was found that the inhalation of low ppbv levels of MTs was helpful to improve the psychology of all subjects in this study. These results are consistent with those reported previously regarding the inhalation of mixed MTs [23–26,28]. Participants who smelled Japanese cedar wood reported enhanced comfort and a relaxation after 40–60 s [26]. The smell of Hiba oil also mitigated depression and anxiety [25], while enhancing contingent negative variation [24]. Moreover, after inhaling Taiwan cypress, the performance of the participants was improved with respect to certain tasks [27]. Besides, their maximum blood pressure also decreased [27]. It was also reported that the inhalation of 0.3–3 ppmv of α-pinene and 0.3 ppmv of d-limonene from air greatly improved the comfort of subjects [28]. In addition, Cedrol was shown to decrease heart rate and systolic blood pressure [23].

Accordingly, the inhalation of single MTs revealed a good effect on human psychology. However, high concentrations of MTs could bring about adverse effects on psychology. In addition, mixtures of MTs also helped improve the subjects' comfort at low ppmv levels of MTs. On the basis of this study, it is suggested that the exposure to ppbv levels of artificial MTs should help improve human psychology and physiology. In the combination with an image display device (such as projector, television, and visual-reality device), the use of artificial monoterpenes would generate similar positive effects as a forest bathing, without visiting a real forest. It would offer diverse therapeutic opportunities for those who cannot access the forest environment with ease.

5. Conclusions

In conclusion, the individual and integrated effects of MTs on human physiology and psychology were investigated in a laboratory setting. Monoterpenes (α-pinene, β-pinene, and *d*-limonene) at ambient forest concentration levels were artificially generated and tested. It was observed that the human olfactory detection thresholds of α-pinene, β-pinene, and *d*-limonene were close to 5 ppbv. In terms of physiological and psychological effects, we found that the alpha waves of male participants were more sensitive to MTs than those of females. However, the female participants went into a state of relaxation faster than the males after inhaling MTs. Decreased stress index and increased brain alpha waves were observed after inhaling MTs, indicating that MTs relaxed the subjects physiologically and psychologically. These results suggest that using MTs can help improve human psychological wellbeing in a short-term after inhaling. This will help people who are unable to travel to a natural environment. This can also help people relax within a short period of time and with low cost. This is also a potential therapeutic method to treat psychiatric patients. Hence, more researches should be conducted to explore the potential therapeutic benefits of airborne terpenes at low ppbv levels. To evaluate the changes in stress and other physiological and psychological conditions in relation to terpenes in more details, we are proposing to use wearable devices such as those for electrocardiogram (ECG), photoplethysmogram (PPG), galvanic skin response (GSR) recording, etc. in the future works.

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Appendix A

Table A1. Summary of MT olfaction tests at four different concentrations of each MT standard gas (Exp 1).

Raw Counting Data					
No.	**Concentration (ppbv)**	2	3	5	7
	Compounds	Number of participant			
1	*α*-pinene	1	6	19	29
2	*β*-pinene	0	4	15	29
3	*d*-limonene	0	5	20	29
Ratio of Participant Could Smell MT Odor					
No.	**Concentration (ppbv)**	2	3	5	7
	Compounds	Ratio of participant (%)			
1	*α*-pinene	3.45	20.7	65.5	100
2	*β*-pinene	0.00	13.8	51.7	100
3	*d*-limonene	0.00	17.2	69.0	100
The Test of Preference between Three Tested MTs					
No.	Compound	Male	Female	Total	Percentage (%)
1	*α*-pinene	4	0	4	13.8
2	*β*-pinene	6	1	7	24.1
3	*d*-limonene	13	5	18	62.1

Table A2. Summary of alpha waves and stress index data in the psychological test (Exp 2).

Order	**Code**		**Age**	**Alpha Wave Intensity vs MT (ppbv)**				**Stress Index vs MT (ppbv)**			
				0	**7**	**15**	**20**	**0**	**7**	**15**	**20**
1	F1	F1	21	12.70	13.57	14.05	15.90	40	39	36	32
2	F2	F2	21	12.55	12.58	13.40	16.39	57	51	35	24
3	F3	F3	21	12.93	14.34	18.53	20.12	44	42	40	40
4	F4	F4	21	10.40	13.02	13.91	15.87	51	47	42	36
5	F5	F5	22	9.95	15.20	15.86	16.25	39	37	33	28
6	F6	F6	22	10.72	13.41	19.25	20.55	57	56	44	41
7	F7	F7	21	10.17	10.22	10.74	11.61	52	43	40	40
8	F8	F8	21	8.09	10.07	10.74	11.24	50	38	39	39
9	F9	F9	21	6.07	6.74	10.61	10.88	42	41	40	39
10	F10	F10	26	10.42	10.39	11.87	13.31	43	33	34	32
11	F11	F11	21	8.08	9.05	9.13	9.38	35	32	26	25
12	F12	F12	23	10.09	10.03	11.27	11.99	47	37	35	29
13	F13	F13	30	11.03	13.49	13.95	14.96	44	40	39	35
14	F14	F14	23	5.85	7.20	9.20	10.93	41	40	38	38
15	F15	F15	34	16.33	17.20	20.70	21.18	56	43	34	34
16	M1	M1	24	8.06	11.17	12.76	13.34	48	48	46	44
17	M2	M2	25	8.52	12.58	13.32	13.94	43	41	40	37
18	M3	M3	25	4.82	6.69	9.60	10.03	54	43	41	37
19	M4	M4	24	9.70	14.76	15.46	17.34	48	44	41	40
20	M5	M5	23	7.50	12.61	13.84	15.30	42	40	39	35
21	M6	M6	24	7.99	7.95	11.92	18.93	43	41	40	40
22	M7	M7	24	12.84	13.94	16.90	19.98	43	39	38	37
23	M8	M8	23	9.31	11.13	11.93	13.94	48	42	39	37
24	M9	M9	23	12.41	14.92	18.06	19.72	37	36	35	31
25	M10	M10	24	13.17	13.29	14.89	21.31	42	41	38	36
26	M11	M11	23	10.92	11.12	11.85	11.94	45	40	29	24
27	M12	M12	25	6.15	8.30	9.19	11.21	45	39	38	31
28	M13	M13	23	5.84	7.56	9.91	13.14	39	36	32	31
29	M14	M14	24	5.61	7.68	8.26	9.00	56	49	45	36
30	M15	M15	26	15.97	16.28	19.13	22.26	54	43	42	34

Note: F is female, M is male.

Table A3. Summary of participants' answers to the questionnaire for the psychological test (Exp 2).

No.	Code	Before inhaling MT					After Inhaling MT				
		Very Stable	Stable	Normal	Unstable	Very Unstable	Very Stable	Stable	Normal	Unstable	Very Unstable
1	F1			x				x			
2	F2			x			x				
3	F3		x				x				
4	F4			x					x		
5	F5			x					x		
6	F6			x				x			
7	F7		x					x			
8	F8			x				x			
9	F9			x				x			
10	F10			x					x		
11	F11		x				x				
12	F12	x					x				
13	F13			x			x				
14	F14		x				x				
15	F15		x				x				
16	M1			x				x			
17	M2	x					x				
18	M3					x		x			
19	M4	x					x				
20	M5		x					x			
21	M6				x			x			
22	M7			x			x				
23	M8			x				x			
24	M9	x					x				
25	M10			x				x			
26	M11			x			x				
27	M12			x				x			
28	M13		x						x		
29	M14		x					x			
30	M15		x				x				

Note: F is female, M is male, x is selected.

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