Article

Effects of Plant-Emitted Monoterpenes on Anxiety: A Propensity-Matched Observational Cohort Study

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Abstract: Immersive experiences in green areas and particularly in forests have long been known to produce beneficial effects for human health. However, the exact determinants and mechanisms leading to healthy outcomes remain to be elucidated. The purpose of this study was to investigate whether inhaling plant-emitted biogenic volatile compounds, namely monoterpenes (MTs), can produce specific effects on anxiety. Data from 505 subjects participating in 39 structured forest therapy sessions in different Italian sites, were collected. Monoterpenes air concentration was measured at each site. STAI State questionnaires were administered before and after the sessions as a measure of anxiety. A propensity score matching analysis was then performed, considering an above-average exposure to inhalable air MTs as the treatment: the estimated effect was -1.28 STAI-S points (95% C.I. -2.51 to -0.06, $p = 0.04$), indicating that the average effect of exposure to high MT air concentrations during forest therapy sessions is to decrease anxiety.

Keywords: Anxiety; Biogenic volatile organic compounds; Forest therapy; Monoterpenes; α-Pinene; Propensity matching.

1. Introduction

The preventive and healing effects of green environments on psychophysical well-being have been documented extensively in the scientific literature, and numerous studies suggest that forest exposure is associated with a wide range of benefits, covering both psychological [1–5], and physiological aspects [6–9]. In particular, a few experimental studies have demonstrated the effects of forest immersion in stress mitigation and induction of physiological relaxation, especially with regard to anxiety levels [10–14], a topic analyzed in recent literature reviews [5,15,16].

The beneficial effects of visiting a forest derive from the combined stimulation of all senses by specific features of the natural environment [7], whose impact and quantitative assessment have not yet been fully elucidated. Biogenic organic volatile compounds (BVOCs) emitted by plants and soil in the forest atmosphere, and in particular Monoterpenes (MTs), as key constituents of BVOCs, have often been suggested as one of the

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determinants of the interaction between forest ecosystems and human health [17–20], especially those related to long-lasting effects on the immune system after forest exposure [18,21–25].

All plant organs from flowers to roots can generate and release MTs [26], with leaves generally responsible for the highest emission rates [27]. MT emission rates and chemical profiles vary widely among different species of forest trees [28,29], and they depend on physiological (plant age and developmental stage), and physicochemical factors, i.e., related to leaf structure (presence or absence of storage structures, stomatal openness) [30]. The individual perception of benefits induced by plant-emitted MTs stimulated research efforts to expedite the modeling and prediction of forest emissions [31,32], along with preliminary studies aimed at predicting nose-level concentrations of BVOCs [33]. Important efforts have also been made to design forest therapy programs and trails based on potential emissions of BVOCs released by dominant plant species [34].

Recently, several studies aimed at assessing and quantifying BVOCs in forest sites have been published [20,31,32,35,36], owing to the need of gaining further insights into forest characteristics potentially related to specific effects on health outcomes [34,37–42].

Overall, the evidence about MTs activity on human health mostly derives from *in vitro* or animal or indoor/laboratory experiments, usually with a small number of study participants [43], and very few studies taking into consideration MTs concentration along with the measurement of health outcomes [23,44]. Only recently, a few studies investigated the most important psychophysiological short-term effects of individual exposure to plant-emitted MTs, but such studies were limited to specific and/or numerically scarce target groups, for example, maladjusted soldiers [45], or to specific plant species, for example *Cinnamomum burmannii*, growing in partially controlled environments [46]. In general, even though the results of these studies are encouraging, quantitative experiments in natural environments are still lacking.

There is also a dearth of studies investigating any specific health effects of terpenes inhaled during forest exposure and their mechanism of action from a pharmacological point of view [47]. Only two studies measured the serum concentration of monoterpenes after forest exposure, reporting a six-fold increase of α-pinene levels after walking in a conifer forest for one hour [48], and a higher absorption of α-pinene after walking in an evergreen broadleaf forest for two hours, but only in participants with lower baseline blood concentrations of monoterpenes [35]. Although relevant, the impact of latter two studies was affected by small sample sizes. Moreover, some preclinical evidence exists from animal studies of possible anxiolytic activity of α-pinene [7,49–51].

Anxiety is a highly prevalent non-psychotic mental disorder, and one of the most increasing psychiatric disorders of the last decades. Anxiety disorders have a worldwide prevalence of up to 15% in the general population, and are more common in women [52,53]. The global burden of hypertension is extensively impacted from anxiety, both as a risk factor and as an exacerbating factor [54]. Anxiety is also a known risk factor for major adverse outcomes in heart diseases, especially in heart failure, chronic and acute coronary syndromes, and cardiomyopathies [55–58]. Finding new sustainable strategies to decrease the burden of anxiety is an important goal for researchers and would have a significant impact on health policies.

The aim of this study was to assess whether exposure to inhalable MTs during 3-hour-long forest therapy sessions can produce specific effects on anxiety levels. This study follows a previous pilot study, where the total concentration of BVOCs was preliminarily suggested as a contributing factor associated with short-term improvements in mood states, in particular with anxiety reduction of subjects involved in forest therapy sessions organized in remote green areas [14]. To the authors' best knowledge, this is the first attempt, grounded on a large number of participants, to clarify the extent of the effect on anxiety levels of MTs inhaled in a natural setting.

2. Materials and Methods

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2.1. Design, Participants, and Outcome Measures

Experiments were conducted between September 2021 and October 2022 in 39 sites, including 27 remote mountain forest sites, 3 hill forest sites, 1 remote costal pinewood forest site, 7 urban parks in Italy, and 1 remote mountain forest site in Carinthia, Austria, located at altitudes between 5 m a.s.l. and 1,800 m a.s.l. Figure 1 shows the location of these sites. During each session, one or more groups of participants, each made up of about 15-20 people, were guided along identified paths by trained operators, for a total duration of 3.0±0.5 hours.

Exclusion criteria were minor age (< 18 years), any acute illnesses requiring medications other than those usually taken on a daily basis for chronic diseases, and any major physical or mental health problems incompatible with outdoor walking for a few hours. Participants were allowed to attend only one session, to prevent any possible carry-over effect.

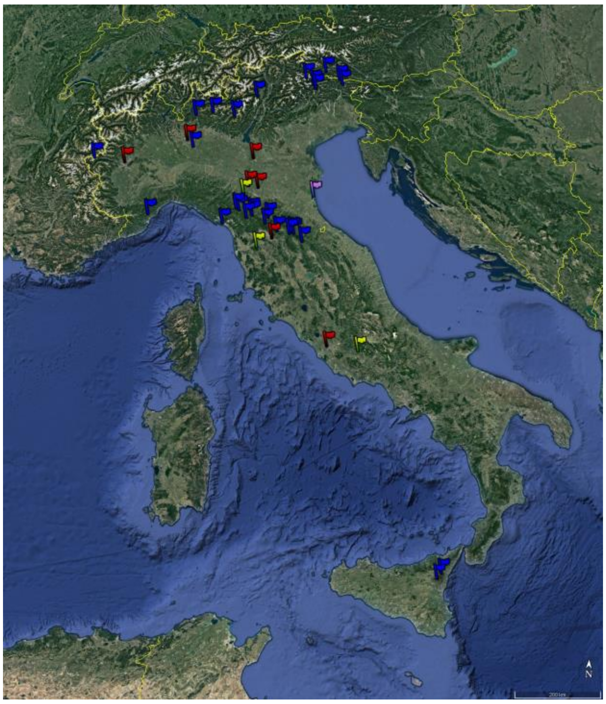

Figure 1. Location of the experimental sites. Blue: remote mountain; yellow: hill; violet: remote coastal pinewood; red: urban park.

Only participants who gave their informed consent were included in the data collection. All participants were treated in accordance with the ethical guidelines for research provided by the Declaration of Helsinki and its revisions, and the study was approved by the local ethics committee (CNR Ethics Committee n. 0069654/2021).

Overall, 655 participants were recruited, and 150 of them were excluded from the final analysis due to missing data in one or more key variables (i.e. demographic data, psychometric tests). The final sample consisted of 505 participants, with a higher share of females (65%). The distribution of participants in different age groups was as follows: 18-29 years (10%), 30-44 years (19%), 45-54 years (19%), 55-69 years (42%), and over 70 (10%).

Participants autonomously reached each forest therapy site. Upon arrival, participants were required to fill in the informed consent form. Then, every participant was presented with an anonymous questionnaire (with a unique ID code), which was aimed at collecting information about gender, age, place of residence, job, height, weight, chronic diseases, allergies, medications used, smoking habit, sport activities, experience in meditation practices. Moreover, the participants were asked to rate their subjective satisfaction in the relationships with friends and relatives with a 10-point Likert scale. Then, they were asked to fill in some psychometric questionnaires, namely the State-Trait Anxiety Inventory (STAI) and the Profile of Mood States (POMS) questionnaires.

The STAI questionnaire is widely used to measure state and trait non-disorder-specific anxiety, in both healthy and clinical populations [59]. It consists of a 40-item self-report measure of anxiety, using a 4-point Likert-type scale for each item. It has two scales: State anxiety, i.e., how one feels at a given moment (STAI-S), and Trait anxiety, i.e., how one generally feels (STAI-T), both consisting of 20 items. Total scores were calculated by summing up the items in each respective form of anxiety (ranging from 20 to 80), where higher scores reflect greater levels of anxiety. The POMS questionnaire consist of 40 items, each measured with a 5-point Likert scale, related to domains such as tension, depression, anger, vigor, fatigue, confusion, and self-esteem [60]. Although both the STAI-S and the POMS questionnaires are widely used to explore different domains, the STAI-S was chosen as the outcome measure of state anxiety, i.e. the outcome measure of this study. Unlike the POMS, the STAI-S questionnaire is often used with clinical and subclinical study populations, and it can detect subtle changes in anxiety levels, even when there are large differences in baseline scores, which occurs in groups differing for their vulnerability to anxiety, as is the case with highly heterogeneous participants to forest therapy sessions [61].

The STAI-S questionnaire was administered immediately before and after each forest therapy session in order to intercept any short-term effects (changes from baseline) elicited during the sessions. The STAI-T questionnaire was administered only at baseline, to better characterize the participants on the basis of their trait anxiety, which is the stable long-term tendency to anxiety. For a more comprehensive evaluation, the POMS questionnaires were also collected at baseline in order to characterize the participants in terms of other baseline mood states, i.e. non-anxiety-related POMS domains.

2.2. Forest Therapy Sessions

A professional psychologist or psychotherapist took part in each of the forest therapy sessions. The conduction protocol consisted of simple instructions to the participants to focus one's attention on the external environment. The protocol was chosen to give reproducible instructions for all the psychologists or psychotherapists involved, in order to minimize any interferences related to the therapists' personal styles and to achieve the most homogeneous experimental conditions possible.

The overall duration of each session was approximately 3 hours, including short, slow walks, interspersed with five stops, and a final walk, before filling out the STAI-S questionnaires after the forest therapy experience. Each session was performed in the morning, starting around 10:00 a.m. and ending around 01:00 p.m., local time (corresponding for all sessions to GMT+2). Forest therapy sessions were performed only if the weather was at least fair, without rain, and with comfortable temperatures within the range of 12 to 26°C, collected by hand-held thermometers at the BVOCs sampling sites. The standardization of the method of conducting all forest therapy sessions, along with the same timing, allowed the control of possible biases.

2.3. Measurement of Volatile Organic Compounds

The total and individual concentrations of different biogenic volatile organic compounds (BVOCs) in each forest site were measured through 1-hour air samplings performed at 9.00 a.m., 11.30 a.m., and 2.00 p.m. Air was passed to the adsorption traps by using portable pumps (Pocket389Pump, SKC Inc., PA, USA), that provided a constant flow of air of 200 ml/min, yielding sampling volumes of 12 l per hour.

Traps were made of inert metal tubes (8 cm × 0.3 cm i.d.) filled with Tenax TA and Carbograph 1TD (350 mg; 35/60 and 40/60 mesh, respectively), provided by Markes International, Ltd. (Llantrisant, UK), and they were stored at -20°C until the analysis. BVOCs, released from traps using a thermal-desorption unity series 2, (Markes International, Sacramento, CA, USA), were injected into a 60 m capillary column (HP-1, 0.25 mm I.D.) internally coated with a 0.25 μm film of polymethylsiloxane, provided by J&W Scientific

USA, Agilent Technologies (Palo Alto, CA, USA). BVOCs separation was performed on a 7890A gas chromatograph and eluted compounds were detected with a 5975C mass spectrometer (GC–MS, Agilent Technologies, Wilmington, USA).

Retention times and mass spectra were used for BVOCs identification, by comparison with the NIST 11 library, using the Agilent MassHunter Qualitative Analysis software. The compounds quantification with the Agilent MassHunter Quantitative Analysis software was achieved by comparing the responses of the samples with those generated by injecting dynamically-diluted volumes of a certified gas standard mixture from Apel-Riemer Environmental Inc., (Broomfield, CO, USA) [62–64].

Calibration curves were obtained with standard compounds (Sigma - Aldrich Chemical Co. St Louis, MO). The concentration of each compound was calculated in μgm^{-3} as the mean and standard error of the three replicates.

Among BVOCs, the air concentrations of MTs α-pinene, camphene, o-cymene, and sabinene were examined. For the purposes of the study, the MTs concentration data collected at 11.30 a.m. at each site were considered, because that timing was the most representative of the sessions. The total air concentration of MTs was selected as the principal exposure factor.

2.4. Data Analysis

Microsoft Excel was used to organize the data collected. The software used for statistical analyses was "R" [65], using RStudio ver. 2022.07.2 [66], and the packages "matchit" [67], "marginaleffects" and "stats" [65]. The threshold for statistical significance of the overall effect size was set at $p<0.05$.

3. Results

3.1. Propensity Score Matching

The total MTs air concentrations at any studied sites were analyzed, and the average concentration value, equal to 0.20 μgm^{-3} (range 0.01 to 0.99 μgm^{-3}), was established as a cut-off value between low- and high- MT exposure. The choice of the average concentration allowed the partitioning of the whole sample of participants to forest therapy sessions in two subsamples of comparable numerosity. High MT exposure was considered as the treatment of interest.

The propensity score matching [68–72] was performed to estimate the average marginal effect of the exposure to high MT air concentrations during forest therapy sessions on change-from-baseline STAI-S scores reported by the study participants and accounting for confounding by the following covariates (baseline characteristics): age, sex, height, weight, presence of hypertension, diabetes, dyslipidemia, asthma, allergies, session in an urban site, children, smoke habit, sport activities, experience in meditation practices, satisfaction from their relationship with friends and relatives, baseline STAI-T score, baseline POMS depression, vigor, fatigue, confusion, anger and self-esteem domains scores. Missing data in one or more of the aforementioned key covariates implied the exclusion of the participant from the final analysis.

Due to the short-term nature of state anxiety, which was the main outcome measure of this study, baseline characteristics were chosen in order to better match participants not only by physiological covariates, but also by psychological covariates, obviously including the baseline long-term tendency to anxiety (STAI-T score).

We first attempted a 1:1 nearest neighbor propensity score matching without replacement with a propensity score estimated using logistic regression of the intervention on the covariates. This matching specification yielded an acceptable, yet not fully satisfactory, balance (standardized mean differences for the covariates <0.15), so we instead tried with an optimal full matching on the propensity score, which yielded a satisfactory balance. The propensity score was estimated using a logistic regression of the intervention on the covariates. Baseline covariates were considered balanced across the high and low MT

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exposure groups in the matched sample if the absolute difference of the standardized difference was ≤0.1.

Figure 2 shows the balance of covariates before and after propensity matching for exposure to high total monoterpenes air concentration. After matching, all standardized mean differences for the covariates were below 0.1, indicating adequate balance. Since full matching uses all units allocated to the intervention and control, no units were discarded.

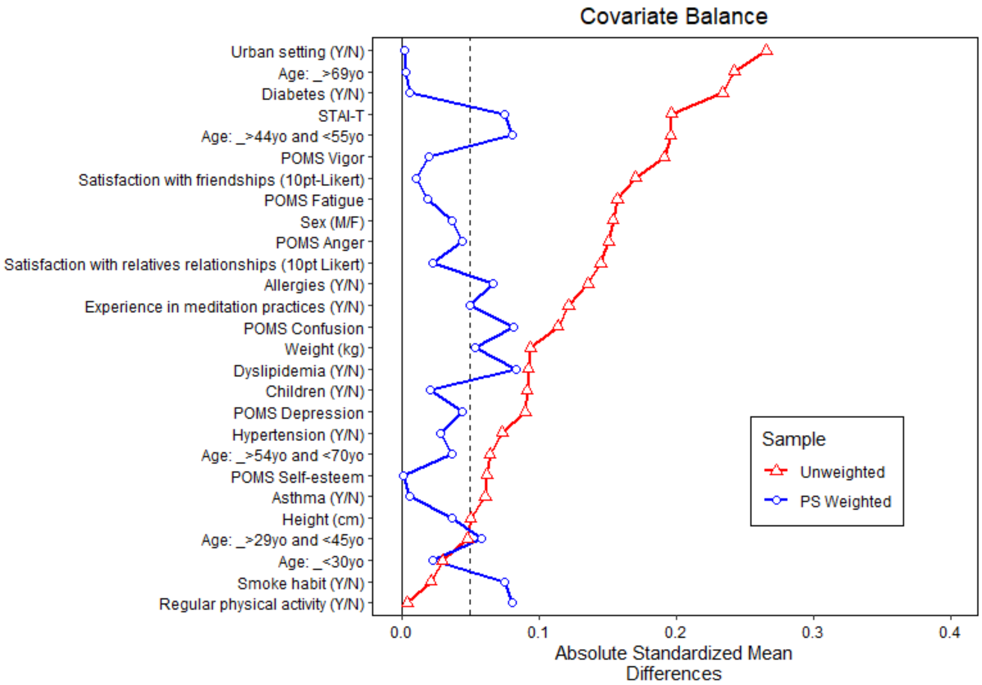

Figure 2. Balance of covariates before and after propensity matching for exposure to high total monoterpenes air concentration.

The efficacy endpoint, which was a significant short-term reduction in STAI-S scores after a forest therapy session, was compared across the propensity-matched groups fitting a linear regression model with the change-from-baseline (post minus pre) STAI-S score as the outcome of interest and the intervention, covariates, and their interactions as predictors. This analysis also included the full matching weights, in order to estimate the intervention effect and its confidence interval. A g-computation was then performed in the matched sample to estimate the average effect of the intervention in those who received it (ATT). A cluster-robust variance was used to estimate its confidence interval with matching stratum membership as the clustering variable.

An additional analysis was performed, using the propensity score matching to estimate the average effect of the exposure to above-the-average α-pinene air concentrations (0.10 μgm^{-3}) on change-from-baseline anxiety scores reported by the study participants, accounting for confounding by the aforementioned covariates. The same procedure and matching methods as described above were applied with similar results, with the only difference being that the propensity score was estimated using a probit regression of the intervention on the covariates, which in this case yielded better balance than a logistic regression (covariates standardized mean difference <0.1 vs <0.15).

3.2. Anxiolytic Effect of the Exposure to Monoterpenes and α-pinene

Based on the propensity score matching analysis described in Section 3.1, the estimated effect on anxiety levels, following the exposure to above-average total concentration of MTs, was -1.28 STAI-S points (95% C.I. -2.51 to -0.06, p = 0.04), indicating that the average effect of the exposure to high MTs air concentrations during forest therapy sessions is to decrease anxiety.

In the second analysis focused on α-pinene, the estimated effect was -1.31 STAI-S points (95% C.I. -2.15 to -0.12, p = 0.03), indicating that the average effect of the exposure to high α-pinene air concentrations in forests is to decrease anxiety, confirming the previous result and suggesting a pivotal role of α-pinene in the anxiety-reducing effect.

4. Discussion

This study showed for the first time a specific effect of the total concentration of inhaled MTs and, in particular, of α-pinene, on anxiety levels of participants to forest therapy sessions. Although this was not a randomized controlled trial, the propensity matching methodology, applied to a quite large cohort of participants to several, identically structured forest therapy sessions, allowed an accurate balance for all participants' variables, including their psychological baseline and involvement in experiences either in urban parks or in remote forests. This way, the participants could be partitioned into an intervention group, exposed to MT concentrations above a cut-off level derived from the available data, and a control group, exposed to lower concentrations.

The quite comparable effect sizes, equal to -1.28 STAI-S points (p=0.04) for high total MTs concentration, and -1.31 STAI-S points (p=0.03) for high α-pinene concentration, were about 28% of the average decrease in STAI-S observed across the entire sample (-4.6 points), which provided an estimate of the specific contribution of inhaled MTs or α-pinene to any decrease in STAI-S anxiety levels following a few hours in forest environments.

Inhalation of natural essential oils (EOs), of which MTs (including α-pinene) are important constituents, has long been the subject of clinical studies that showed significant effects on depression and anxiety disorders in humans, as well as of preclinical examinations, which confirmed significant effects on depression and anxiety-like symptoms in animal models [73,74].

On the one hand, the role of the cardiorespiratory system, responsible for the systemic absorption of volatiles and the subsequent delivery to organs including the central nervous system, and, on the other hand, the direct stimulation of the brain through olfactory organs, were hypothesized as fundamental for explaining the therapeutic efficacy of inhaled EO molecules on mood disturbances. Olfactory stimuli can directly affect mood, through direct anatomical and functional links of the olfactory system with the limbic system. However, more precise mechanisms of action can be revealed only by experiments with isolated EO constituents, including individual MTs such as α-pinene [73].

The results of this study allow the identification of a ubiquitous class of components, MTs and in particular α-pinene of the atmosphere of green areas, especially forests, as specific therapeutic agents with regard to anxiety levels. This finding substantiates the emerging concept of the environment as a "healing environment", a source of valuable elements actively promoting human health and well-being, and not only as a resource to be protected and preserved from pollution [75].

The elucidation and quantification of a specific healing mechanism of forest environments can offer a powerful tool to direct the widespread efforts to plan and optimize forest therapy trails, often relying on the prediction of MT concentration based on plant species and meteorological variables [31,32,34,76].

Studies documenting the effects of forest immersion experiences on anxiety and stress, such as those analyzed in recent comprehensive reviews [77,78], can be partially reinterpreted in the light of this study findings, and future experiments should take these results into adequate account.

Finally, this growing body of evidence indicates that green environments have an active and specific effect in reducing the burden of anxiety in the general population, thus impacting on not only mental but also indirectly cardiovascular health, with a great potential in terms of saving public health expenditure. Therefore, advocacy for the encouragement of protecting and creating green areas should be addressed.

The main limitations of this study were the lack of a randomized controlled trial design and a significant drop-out rate from the final analysis due to suboptimal control over the participants' compliance with filling out the self-report questionnaires. Another minor limitation consisted of the gender imbalance, with the dominance of female participants over males. These limitations should be considered for future studies on the topic.

5. Conclusions

The results of this study show for the first time that inhalation of plant-emitted MTs, and in particular α-pinene, produces a specific anxiolytic effect.

Our findings highlight the importance of focusing on the environment as a source of health-promoting factors, with the necessity to promote appropriate policies and raise awareness about environmental issues. Further perspectives are to investigate the possible effects of inhalable forest MTs on physiological outcome measures.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data are available by contacting the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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