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Enhancement of essential oil yield in Ocimum americanum L. across ontogenetic phases by Bacillus subtilis MTCC 8141 and Trichoderma harzianum MTCC 936 and its termiticidal efficacy against Odontotermes parvidens

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Enhancement of essential oil yield in *Ocimum americanum* L. across ontogenetic phases by *Bacillus subtilis* MTCC 8141 and *Trichoderma harzianum* MTCC 936 and its termiticidal efficacy against *Odontotermes parvidens*

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Abstract

Essential oils from medicinal plants are increasingly recognized as sustainable sources of bioactive compounds for eco-friendly pest management. In the present study, we investigated the effects of soil inoculated with *Bacillus subtilis* (PGPR), *Trichoderma harzianum* (PGPF), and their consortium on growth-stage-dependent essential oil production, chemical composition, nutrient accumulation, and termiticidal activity of *Ocimum americanum* L. Plants were grown under four treatments: uninoculated control, *B.s* MTCC 8141, *T. h* MTCC 936, *O.a* + Mix (*B.s* MTCC 8141 + *T. h* MTCC 936) inoculation, and leaf essential oils were extracted at pre-reproductive, reproductive, and post-reproductive phases. Oil yield peaked at reproductive phase, highest in *B.s* MTCC 8141 treated plants (1.28 ± 0.10%) and mixed inoculated plants (1.27 ± 0.14%). GC-MS identified (+)-2-bornanone (camphor) as dominant (53.74% in *B. subtilis*, 50.38% in mix), followed by D-limonene and (3E)-3,7-dimethylocta-1,3,7-triene. Essential oil extracted from reproductive stage were tested against *O. parvidens* using contact toxicity, fumigant, and repellency assays. Essential oils extracted from O. *a* + *B.s* MTCC 8141 and *O.a* + Mix inoculated plants showed the strongest activity, with 100% mortality at 400 ppm in contact and fumigant assays, and complete repellency at 400 ppm. Our findings show microbial consortia as viable biostimulants for optimizing bioactive essential oil production and eco-friendly pest control.

Keywords- *Ocimum americanum*, microbial inoculation, essential oil, termiticidal activity

Introduction

The management of insect pests has traditionally depended on synthetic pesticides, which remain widely used in agriculture because of their rapid action and operational convenience. However, sustained reliance on these compounds has raised serious concerns regarding environmental contamination, residue accumulation in food products, disruption of non-target organisms, and risks to human health through occupational and dietary exposure (Zhou and Achal 2025). Repeated chemical control also accelerates the development of resistant pest populations and may disturb the ecological stability of agroecosystems (Al Naggar et al. 2025). These limitations have intensified interest in safer and more sustainable pest management tools. Among the most promising alternatives are essential oils derived from medicinal and aromatic plants, which are biodegradable, renewable, and rich in bioactive volatile compounds with diverse insecticidal properties (Gupta et al. 2023).

Essential oils are increasingly considered important components of sustainable pest management because their chemical complexity allows them to act through multiple modes of action (Gupta et al. 2023). Depending on their composition, they may induce direct toxicity, interfere with respiration or neurosensory signalling, suppress feeding, or alter insect behaviour through repellent and fumigant effects (Fouad et al. 2023). Their volatility also makes them suitable for managing pests that are difficult to control by contact alone (De Clerck et al. 2021). In contrast to many synthetic insecticides, which usually act on a narrow physiological target, essential oils often contain mixtures of monoterpenes, sesquiterpenes, and oxygenated derivatives that may act synergistically (Dassanayake et al. 2021). This broad-spectrum activity makes them attractive candidates for integrated pest management, particularly where reduced chemical input and safer residue profiles are priorities.

Termites are among the most destructive insect pests in tropical and subtropical regions. Their subterranean habit, concealed feeding behaviour, and organised colony structure make them difficult to detect and manage before substantial damage occurs (Tolossa 2022). In agricultural systems, termite activity can reduce plant vigour, compromise stand establishment, and diminish overall plant productivity (Abebe 2024). Conventional termite control has depended heavily on synthetic insecticides, but their limitations are increasingly evident in terms of persistence, environmental safety, and non-target effects. These constraints have made termites a strong target for botanical alternatives, particularly plant-derived volatiles that may function as contact toxicants, fumigants, and repellents (Oi 2022).

Within this broader context, species of the genus *Ocimum* are especially relevant because of their aromatic profile and wide range of biological properties. *Ocimum americanum* L. is a comparatively underutilized essential oil-bearing plant species that deserves greater attention in this regard. Its oil has been reported to contain biologically active constituents such as camphor, limonene, and other monoterpenoid compounds associated with

antimicrobial and insecticidal activity (Mahendran and Vimolmangkang 2023; Ragavendran et al. 2024). Although the species has been studied for several medicinal and pharmacological applications (Luanda et al. 2023), its potential as a source of pest-management agents remains insufficiently explored as compared to other *Ocimum* species. This is an important limitation because the utility of an essential oil depends not only on plant identity, but also on growth stage, environmental conditions, and rhizosphere interactions that shape its final yield and composition.

Plant ontogeny is a major factor influencing essential oil productivity. The quantity and chemical profile of volatile metabolites often vary across developmental phases as plants shift resources between vegetative growth and reproductive development (Paulus et al. 2019). In aromatic plants, reproductive-stage tissues frequently exhibit enhanced accumulation of secondary metabolites, although the pattern is species-specific (Hussien and Ali 2021). Such developmental variation has direct practical implications for harvest timing, oil quality, and biological efficacy (Aburigal and Mirghani 2020). Despite this, ontogenetic profiling has not been sufficiently integrated with microbial biostimulation studies in *O. americanum*. As a result, the developmental stage that provides the best balance between essential oil yield and insecticidal potential remains unclear.

In recent years, plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) have emerged as effective tools for improving plant performance and modulating secondary metabolism like essential oils (Yilmaz and Karik 2022). *Bacillus subtilis* is a well-established PGPR that promotes plant growth through phytohormone production, nutrient solubilization, siderophore release, and induction of systemic resistance (Adjei et al. 2025). Similarly, *Trichoderma harzianum* functions as a versatile PGPF that improves root development, nutrient acquisition, and overall plant vigour while also influencing metabolic processes (Chen et al. 2025; Geng et al. 2025). In aromatic crops, such beneficial microorganisms have been shown to affect biomass production and essential oil yield, suggesting that rhizosphere manipulation may be a practical means of optimising bioactive metabolite production (Tahami et al. 2017; Ebrahimi et al. 2026). However, the extent to which *B. subtilis*, *T. harzianum*, or their consortium can modulate essential oil production in *O. americanum* across developmental stages remains largely unexplored.

The use of microbial consortia is particularly attractive because bacterial and fungal partners may complement one another in the rhizosphere (Santoyo et al. 2024). Such interactions may be especially relevant in aromatic plants, where increased nutrient availability can translate into greater synthesis of volatile compounds (Singh et al. 2023). However, direct evidence linking microbial inoculation, ontogenetic variation, and essential oil

bioactivity in *O. americanum* is lacking. Even less information is available on whether these changes can be translated into improved termiticidal performance.

The present study was therefore designed to evaluate the influence of soil inoculation with *B. subtilis*, *T. harzianum*, and their consortium on growth-stage-dependent in *O. americanum* essential oil production, and termiticidal activity against *Odontotermes parvidens*. By integrating ontogenetic analysis with microbial biostimulation and termite bioassays, this study addresses an important gap in the literature and provides a basis for developing *O. americanum* as a source of eco-friendly pest management agents.

Materials and Methods

Plant material

O. americanum was selected as the experimental plant for the present study. Plant material was authenticated by Department of Life Sciences, Dibrugarh University, Assam, India. A voucher specimen (DU LSC 499) was deposited in the Herbarium of the Department of Life Sciences, Dibrugarh University (Fig. 1).

Selection of the bioinoculants

Two plant growth-promoting microorganisms, namely the bacterial strain *Bacillus subtilis* (*B.s*) MTCC 8141 and the fungal strain *Trichoderma harzianum* (*T. h*) MTCC 936, were used as microbial inoculants. Both strains were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Their selection was based on the plant growth-promoting properties documented in the MTCC catalogue.

This selection of *B.s* MTCC 8141 and *T. h* MTCC 936 was based on their well-documented plant growth-promoting attributes and on evidence that *Bacillus-Trichoderma* consortia frequently outperform single-strain inoculations in enhancing host biomass and secondary metabolite accumulation, including essential-oil constituents in aromatic plants (Tiwari et al. 2017; Salih et al. 2026). The complementary mechanisms exhibited by these taxa provide the rationale for employing this bacterial-fungal combination to evaluate consortium-mediated enhancement of essential-oil yield and composition in *O. americanum* across successive ontogenic stages.

Preparation of the bioinoculants

Inoculum preparations of *B.s* MTCC 8141 and *T. h* MTCC 936 were carried out according to the procedure of Gupta et al. (2018), with slight modifications to suit the experimental requirements of the present study (Fig. 2 S1).

For inoculum preparations of *B.s* MTCC 8141, stock cultures were maintained on Luria-Bertani (LB) agar and propagated in LB broth at 28 ± 2 °C for 72 h to obtain actively growing cells. Bacterial cultures were harvested by centrifugation at 6,000 × g for 5 min. The cell pellets were washed and resuspended in sterile 0.85% (w/v) NaCl. Cell density was adjusted to 3×10^8 CFU mL^{-1} by serial dilution and viable counting on LB agar (Gupta and Pandey 2015).

For inoculum preparations of *T. h* MTCC 936, cultures were maintained on potato dextrose agar (PDA) and grown in potato dextrose broth at 28 ± 2 °C for 7 days to ensure abundant sporulation. Spore suspensions were harvested and centrifuged at 6,000 × g for 5 min. The resulting pellets were resuspended in 0.1 M phosphate buffer (K_2HPO_4, pH 7.0) to obtain a final concentration of 1.8×10^4 CFU mL^{-1}, as determined by serial dilution and plating on PDA (Gupta et al. 2018).

An incubation temperature of 28 ± 2 °C was selected in accordance with established protocols for the co-cultivation of *Bacillus* and *Trichoderma* spp. This condition was further validated in preliminary laboratory trials, in which both *B.s* MTCC 8141 and *T. h* MTCC 936 exhibited robust growth, thereby confirming its suitability for preparing viable inoculant suspensions.

In vitro compatibility of *B.s* MTCC 8141 and *T. h* MTCC 936

Mutual compatibility of *B.s* MTCC 8141 and *T. h* MTCC 936 was evaluated using a dual-culture plate assay on a defined minimal medium. The basal medium comprised: (10 g L^{-1}) $NaNO_3$ (3 g L^{-1}), K_2HPO_4 (0.5 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.5 g L^{-1}), KCl (0.5 g L^{-1}), $FeSO_4 \cdot 7H_2O$ (0.01 g L^{-1}), agar (15 g L^{-1}). Media were sterilized by autoclaving at 121°C for 15 min and dispensed into sterile 90 mm petri dishes under aseptic conditions.

B.s MTCC 8141 adjusted to 10^8 CFU mL^{-1} and *T. h* MTCC 936 adjusted to 10^4 spores mL^{-1} were placed on opposite sides of each plate, maintaining a centre-to-centre distance of 40 mm. Inoculated plates were incubated at 28 ± 2°C for 7 days under stationary conditions. Following incubation, plates were visually examined for reciprocal growth patterns and interaction zones. Compatibility was inferred from the uninterrupted radial expansion of *B.s* MTCC 8141 colonies and the characteristic cottony mycelial growth of *T. h* MTCC 936 towards the plate centre, with no observable zone of inhibition or antagonistic interaction between the two organisms (Fifani et al. 2022).

Experimental design in field condition

The experimental framework was developed with some modifications as described by Gupta and Pandey (2015). A pot-to-field experiment was conducted in medicinal nursery of Dibrugarh University to evaluate the effect of microbial inoculation on essential-oil yield in *Ocimum americanum* L. across three ontogenic phases: pre-

reproductive, reproductive, and post-reproductive. Four treatments were established: (i) un-inoculated control (O.a + C), (ii) B.s MTCC 8141 inoculation (O.a + B.s), (iii) T.h MTCC 936 inoculation (O.a + T.h), and (iv) combined inoculation with both microbes (O.a + Mix).

Seeds of *O. americanum* were collected from multiple locations in Dibrugarh, Assam, India. As no formal seed repository exists for this wild species in the region, seeds were selected using a traditional two-step procedure: winnowing in bamboo baskets under natural airflow to retain dense, viable seeds, followed by flotation testing in distilled water, where sinking seeds were considered viable and floating seeds were discarded. Selected seeds were rinsed twice with distilled water and surface-sterilized with 2% NaOCl for 5 min.

For each treatment, 150 healthy seeds were bio-primed with the respective inoculant suspension (10 ml) in the presence of 2% carboxymethyl cellulose as an adhesive (Fig. 4 S1). Seeds were incubated on a rotary shaker at 25 °C and 110 rpm for 6 h to facilitate uniform microbial adhesion and penetration. Treated seeds were sown in individual pots under greenhouse conditions (25-30 °C, 70-80% relative humidity, 12-h photoperiod). Each pot received 10 mL of the corresponding inoculum at sowing. Seedlings were grown for 3-4 weeks until the 8-10 leaf stage, after which uniform seedlings (selected on the basis of height and leaf number) were transplanted to field plots.

The field experiment was laid out in a randomized block design comprising four independent plots (300 × 4 sq. ft. each), with 60 plants per plot and per plot receiving one treatment. The four plots were assigned as follows: Plot 1 served as the un-inoculated control (O.a + C); Plot 2 was inoculated with *B. subtilis* MTCC 8141 (O.a + B.s); Plot 3 was inoculated with *T. harzianum* MTCC 936 inoculation (O.a + T.h); and Plot 4 received the combined inoculation of *B. subtilis* and *T. harzianum* in a 1:1 ratio (O.a + Mix). A distance of 50 m was maintained between four plots to minimize cross-contamination. Prior to transplanting, each plot was drenched with 10 mL of the respective inoculum suspension. In the combined treatment (O.a + Mix), *B. subtilis* and *T. harzianum* were applied together in a 1:1 proportion. A second application of bioinoculants (10 mL per plant) was made after the pre-reproductive phase to maintain microbial activity in the rhizosphere (Fig. 5 S1).

Assessment of plant growth parameters

Growth performance of *O. americanum* was evaluated across three distinct phenological phases under field conditions in all four experimental plots. At each growth phase, representative plants were harvested from all plots to determine fresh and dry biomass. Fresh weight (g/plant) was recorded immediately after excision whole plant. Samples were then oven-dried at 60-70°C until constant weight was achieved, and dry weight (g/plant) was

recorded. Concurrently, soil pH and temperature were monitored in all four plots throughout the three growth phases (Gupta and Pandey 2015).

Essential-oil extraction and yield determination

Essential oil was extracted from freshly harvested leaves of untreated and microbial-inoculated *O. americanum* plants at the pre-reproductive, reproductive, and post-reproductive phases (Fig. 6 S1). For each treatment and ontogenic phase, five independent plants were taken, with each replicate consisting of 100 g of freshly collected leaves obtained from an individual plant. The samples were subjected to hydrodistillation in a Clevenger-type apparatus for 4 hours following the method of Gupta and Pandey (2015). Thus, five independent extraction replicates were obtained for each treatment at each ontogenic phase ($n = 5$ per treatment per phase). These replicate values were used for the statistical analysis of essential-oil yield.

After distillation, the oil was collected and its volume was recorded. Percentage (%) of essential oil concentration was calculated as follows:

$$\text{\% of Essential oil} = \frac{\text{Essential oil obtained (ml)}}{\text{Weight of loaded mass (g)}} \text{ X } 100$$

GC-MS analysis

Essential-oil samples from untreated control and microbial-inoculated *Ocimum americanum* plants were analysed by gas chromatography-mass spectrometry (GC-MS) at the CSIR-North East Institute of Science and Technology (CSIR-NEIST), Jorhat, Assam, India. Chromatographic separation was performed using a PerkinElmer gas chromatographic system coupled to a mass spectrometer and fitted with a fused-silica capillary column. Helium was used as the carrier gas at a flow rate of 0.4 mL min^{-1}. Essential-oil samples were introduced in split mode, using split ratios ranging from 1:10 to 1:100. The injector and transfer-line temperatures were maintained at 250 and 280 °C, respectively. The oven temperature was initially held at 50 °C for 2 min and then increased at 5 °C min^{-1} to 220 °C. The oven was subsequently maintained at 300 °C for 10 min. The chemical constituents of the essential oil were identified by comparing their retention indices and mass-spectral fragmentation patterns with reference spectra available in the CSIR–NEIST spectral library and with published literature. The relative abundance of each constituent was calculated from the integrated peak area and expressed as a percentage of the total chromatographic peak area (Gupta and Pandey 2015).

Collection and identification of the termites

The termites from the soil mound were collected in boxes from Dibrugarh University campus and immediately brought to the Department of Life Sciences (Fig. 7 S1). Further, the termites were transferred to the rearing tray and termiticidal activity was performed using essential oil of *O. americanum* (Chantarapitak et al. 2025).

The collected termites (both workers and soldiers) were preserved in 80% alcohol and morphological identification were done in Zoological Survey of India (ZSI), Kolkata, India.

Termiticidal bioassay

Essential oil obtained from *Ocimum americanum* was evaluated for termiticidal activity against *Odontotermes parvidens*. Test solutions were prepared at five concentrations: 25, 50, 100, 200, and 400 ppm. A 2% (v/v) dimethyl sulfoxide (DMSO) solution served as the solvent control to distinguish the effect of the essential oil from any effect attributable to the solvent. Two commercial termiticides - Agenda (Chlorpyriphos 20% EC) and Tricel (Fipronil 2.92 % EC), were used as positive controls for comparative assessment of the bioactivity of the essential oil (Fig. 7 S1). The termiticidal potential of the oil was assessed through three complementary bioassays: contact-toxicity, fumigant and repellency assays. Each treatment concentration, including the negative and positive controls, was evaluated in five independent replicates (Chantarapitak et al. 2025).

Contact-toxicity assay

Filter paper disks were placed in petri dishes lined with 1 g of moistened sand as food source. Ten worker termites were released per dish. Different concentrations (25, 50, 100, 200, and 400 ppm) of essential oil were pipetted in the petri dish. Mortality was recorded at 15 minutes, 30 minutes, and 1hour post-exposure; termites were considered dead if no movement occurred upon probing. LC50 values and LC90 were calculated via probit analysis using SPSS v.26 (Chantarapitak et al. 2025). The mortality % was calculated by the following formula.

$$\% \text{ Mortality} = \frac{\text{Number of dead termites}}{\text{Total number of termites}} \times 100$$

Fumigant assay

Petri dish lids were covered with a thin layer of absorbent cotton plug. Different concentrations (25, 50, 100, 200, and 400 ppm) of *O. americanum* essential oil and the controls corresponding to test concentrations were directly pipetted onto the cotton plug and allowed to volatilize for 5 min. Ten worker termites were placed at the bottom of the petri dish with moist sand (1 g), ensuring no direct contact with the treated lid. Mortality was assessed at 15 minutes, 30 minutes, and 1hour post-exposure. LC50 and LC90 were calculated via probit analysis using SPSS v.26 was determined by probit analysis (Perrine et al. 2022). The mortality % was calculated by the following formula.

$$\% \text{ Mortality} = \frac{\text{Number of dead termites}}{\text{Total number of termites}} \times 100$$

Repellency assay

Filter papers were cut in half; one half received 1 mL oil dilution, the other solvent control, both air-dried for 5 min. Treated and control halves were placed adjacent in Petri dishes with ten worker termites released at the midline junction. Termite distribution was recorded after 10 minutes (Bakaruddin et al. 2016).

Repellency index (%) = [(Nc - Nt)/ (Nc + Nt)] × 100,

Nc = termite numbers on control

Nt = termite numbers treated halves

Statistical analysis

The SPSS software (V21, SPSS Inc., Chicago, IL) was used to do the analysis of variance (ANOVA) technique, one way ANOVA and post hoc analysis. Microsoft excel was used to compute the regression equations, R^2 values, and standard errors of means. LC50 values (concentration lethal to 50% of termites) and LC90 (concentration lethal to 90% of termites) were calculated via probit analysis using SPSS v.26.

Results

In vitro compatibility of *B.s* MTCC 8141 and *T. h* MTCC 936

Following seven days of incubation at 28 ± 2 ºC, no visible zone of inhibition was detected between *B. s* MTCC 8141 and *T. h* MTCC 936 on minimal medium. Both microorganisms exhibited uninhibited growth. *T. h* MTCC 936 developed the characteristic cottony mycelial growth, whereas *B. s* MTCC 8141 formed distinct colonies in close proximity to the fungal mycelium. Under the experimental conditions, neither microorganism showed detectable antagonism toward the other, indicating compatibility between the two strains (Fig. 3 S1).

Assessment of plant growth parameters

The pH of the growing medium remained within the range of 6.0-6.5 throughout all three phenological phases. The pre-reproductive phase occurred from March to May at 60 DAT, with temperatures ranging from 26 to 28 °C. The reproductive phase extended from June to September at 180 DAT, during which the temperature ranged from 26 to 32 °C. The post-reproductive phase occurred from October to February at 270 DAT, with temperatures ranging from 12 to 22 °C. During the pre-reproductive phase, the combined application of *Bacillus subtilis* MTCC

8141 and *Trichoderma harzianum* MTCC 936 produced the highest fresh and dry weights, recording 31.13 ± 1.56 and 28.00 ±1.40 g respectively. In comparison, the control plants recorded 23.84±1.19 g fresh weight and 22.50 ± 1.15 g dry weight, corresponding to increases of 30.6% and 24.4%, respectively, under the combined treatment. During the reproductive phase, the mixed microbial treatment again resulted in the greatest biomass accumulation. Fresh and dry weights reached 60.01 ± 3.60 and 54.25 ± 2.75 g respectively, compared with 43.36 2.17 and 38.40 1.92 g in the control. Thus, combined inoculation increased fresh weight by 38.4% and dry weight by 41.3% relative to the untreated control. During the post-reproductive phase, the combined treatment produced the highest fresh weight, 122.90 ± 6.15 g whereas *B. subtilis* MTCC 8141 applied individually resulted in the highest dry weight - 99.35 ± 4.97 g (Table 1)

Table 1 Effect of *B.s* MTCC 8141and *T. h* MTCC 936 on plant growth parameters in three growth phases of *O. americanum*

Treatments	Parameters	
	Fresh weight (g)	**Dry weight (g)**
Pre-reproductive phase		
Control	23.84 ± 1.19	22.50 ± 1.12
***O.a* + *B.s* MTCC 8141**	29.82 ± 1.49	26.00 ± 1.30
***O.a* + *T. h* MTCC 936**	25.83 ± 1.29	24.53 ± 1.61
***O.a* + Mix (*B.s* MTCC 8141 + *T. h* MTCC 936)**	31.13 ± 1.56	28.00 ± 1.40
Reproductive phase		
Control	43.36 ± 2.17	38.40 ± 1.92
***O.a* + *B.s* MTCC 8141**	48.48 ± 2.43	45.68 ± 2.28
***O.a* + *T. h* MTCC 936**	48.30 ±	44.52 ±

	2.28	2.23
***O.a* + Mix (*B.s* MTCC 8141 + *T. h* MTCC 936)**	60.01 ± 3.60	54.25 ± 2.71
Post-reproductive phase		
Control	48.67 ± 2.14	45.30 ± 1.58
***O.a* + *B.s* MTCC 8141**	106.40 ± 5.08	99.36 ± 4.97
***O.a* + *T. h* MTCC 936**	58.48 ± 2.92	54.45 ± 2.01
***O.a* + Mix (*B.s* MTCC 8141 + *T. h* MTCC 936)**	122.90 ± 6.15	85.52 ± 4.28

Essential-oil extraction and yield determination

Essential-oil yield from *O. americanum* leaves was significantly enhanced by microbial inoculation across all growth phases as shown in Fig. 1. In the pre-reproductive phase, oil concentration increased from 0.20 ± 0.04% in uninoculated control plants to 0.33 ± 0.03% in plants receiving the mixed inoculum of *B.s* MTCC 8141 and *T. h* MTCC 936, with a significant treatment effect ($F_{3,16} = 7.20$, $p = 0.003$). In both, plants inoculated with mixed inoculum and *B.s* MTCC 8141 differed significantly from the control, whereas plants inoculated with *T. h* MTCC 936 alone did not. During the reproductive phase, essential-oil yield peaked (0.90 -1.28%), with significant treatment effects ($F_{3,16} = 6.19$, $p = 0.005$) and higher oil in plants inoculated with the mixed consortium (ptukey = 0.010) and *B.s* MTCC 8141 (ptukey = 0.008) relative to uninoculated controls. In the post-reproductive phase, plants inoculated with mixed inoculum significantly exceeded the control (0.84 ± 0.11%; $F_{3,16} = 10.5$, $p < 0.001$; ptukey < 0.001), and the mixed treated plants also yielded more oil than *T. h* MTCC 936 alone (ptukey = 0.003), while other pairwise differences among inoculated treatments were non-significant. Residuals were normally distributed in all analyses (Shapiro–Wilk $p > 0.27$) (Supplementary file S3).

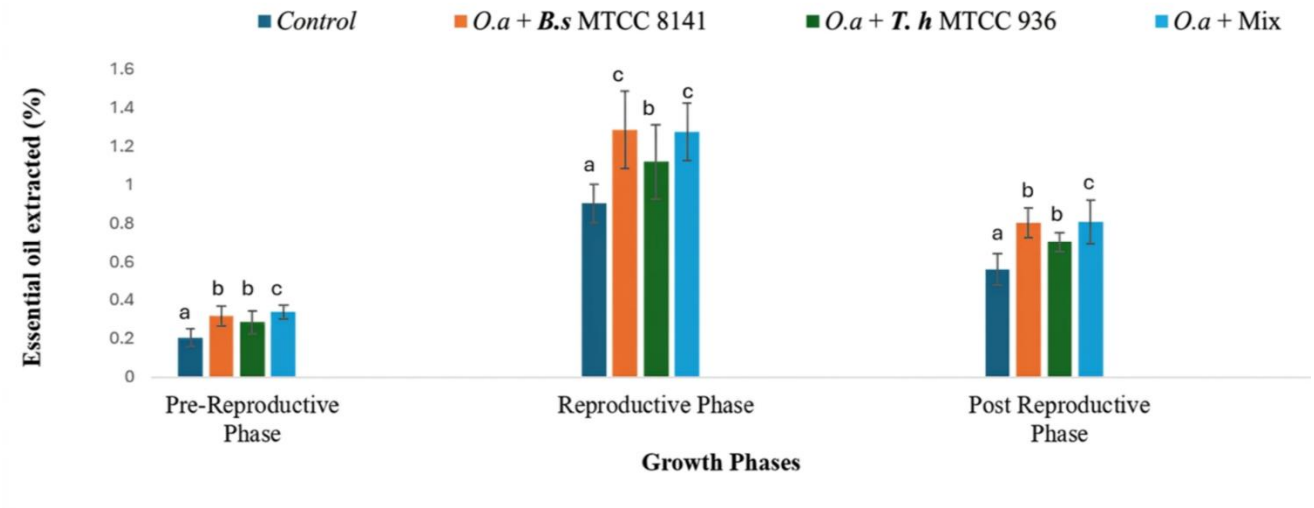

Fig. 1 Enhancement of essential oil (%) by *B. s* MTCC 8141 and *T. h* MTCC 936 in different growth phases of *O. americanum* leaves.

Identification and characterization of extracted essential oil from *O. americanum* by GC-MS analysis

Essential oil extracted from the reproductive phase exhibited the highest yield among all growth stages (Table 1 S1). Therefore, GC-MS analysis was performed exclusively on reproductive-phase oil. GC-MS analysis of essential oil from the reproductive phase (Fig. 1-4 S2) of *O. americanum* identified 15-17 constituents per treatment (Table 1-4 S1), dominated by monoterpenes and smaller contributions from sesquiterpenes. In uninoculated controls plants, (+)-2-bornanone was the major compound (46.93%), followed by D-limonene (10.33%) and (3E)-3,7-dimethylocta-1,3,7-triene (9.27%). Plants inoculated with *B.s* MTCC 8141 increased the compound (+)-2-bornanone to 53.74% while maintaining D-limonene (10.4%). Plants inoculated with *T. h* MTCC 936 produced a similar profile to the control (46.97% of (+)-2-bornanone, 10.48% of D-limonene), and the plants inoculated with both *B.s* MTCC 8141 + *T. h* MTCC 936 (mixed inoculum) showed an intermediate pattern (50.38% of (+)-2-bornanone, 10.24% D-limonene). Sesquiterpenes such as caryophyllene and α-selinene varied modestly among treatments. Overall, bio inoculation, especially plants inoculated with *B.s* MTCC 8141, enhanced the relative abundance of the key oxygenated monoterpene (+)-2-bornanone, indicating that these microbial inoculants can modulate both yield and chemical composition of *O. americanum* essential oil in field condition.

Termiticidal bioassay

The termiticidal activity of reproductive-phase essential oils extracted from *O. americanum* was evaluated against *Odontotermes parvidens* Holmgren & Holmgren, 1917 belonging to the family Termitidae and subfamily Macrotermitinae (Fig. 8 S1). Three biological assays were performed - contact-toxicity, fumigant, and repellency assays. These assays were performed to determine whether the microbial treatments that enhanced essential-oil production also influenced the biological activity of the extracted oils. Mortality was assessed at different oil concentrations and exposure periods in the contact and fumigant assays, whereas repellency was evaluated 10 min after exposure (Fig. 9 S1). The results of these bioassays are presented separately below.

Contact-toxicity assay

Contact toxicity of *O. americanum* essential oils against *O. parvidens* increased with exposure time and concentration, whereas the DMSO control caused no mortality throughout the assay (Table 2). As the mortality data did not meet the normality assumption at 15 min, 30 min, or 1 h (Shapiro–Wilk, $p \leq 0.010$), treatment and concentration effects were evaluated using Kruskal-Wallis tests followed by Dwass-Steel-Critchlow-Fligner pairwise comparisons (Supplementary file S3).

Mortality differed significantly among samples after 15 min ($\chi^2 = 132$, $df = 6$, $p < 0.001$) and 30 min ($\chi^2 = 113$, $df = 6$, $p < 0.001$). After 15 min, mortality ranged from 36-52% in essential oil extracted from untreated plants, 48-80% in essential oil extracted from *B.s* MTCC 8141inoculated plants, 36-56% in essential oil extracted from *T. h* MTCC 936 inoculated plants, and 50-76% in consortium derived essential oil. After 30 min, mortality increased to 56-80%, 70-100%, 60-78%, and 72-100%, respectively. The oil extracted from the plants inoculated with mixed inoculum and *B.s* MTCC 8141 essential oils showed significantly higher activity than several other samples and caused complete mortality at 400 ppm after 30 min. Agenda and Tricel caused 12-42% and 18-48% mortality after 15 min, respectively, while the corresponding values after 30 min were 42-74% and 36-80%.

After 1 h, mortality ranged from 72-94% with essential oil from untreated plants, 88-100% with essential oil extracted from *B.s* MTCC 8141 inoculated plants, 80-96% with essential oil derived from *T. h* MTCC 936 and 88-100% from consortium inoculated plants. Complete mortality was observed at 200 and 400 ppm with the essential oils extracted from the plants inoculated with *B.s* MTCC 8141 and mixed inoculum. Agenda and Tricel caused mortality of 72-86% and 74-100%, respectively. The concentration effect was significant at 15 min ($\chi^2 = 21.1$, $df = 4$, $p < 0.001$), 30 min ($\chi^2 = 32.0$, $df = 4$, $p < 0.001$), and 1 h ($\chi^2 = 16.3$, $df = 4$, $p = 0.003$). Therefore, under the concentrations tested in the experiment, essential oils obtained from *B.s* MTCC 8141 and mixed inoculated plants exhibited the greatest contact toxicity against *O. parvidens*.

Table 2 Effects of essential oil from *O. americanum* against *O. parvidens* Holmgren & Holmgren, after 15 minutes, 30 minutes and 1 hour of exposure.

Sample	Time	Concentration	% Mortality ± SD	LC_{50} (LL-UP)	LC_{90} (LL-UP)	χ^2	p value
Agenda	15 minutes	25	12 ± 4.47[a]	892.54 (389.12-11093.65)	41575.76 (564.15-4588.24)	4.71	1.00
		50	18 ± 4.47[a]				
		100	22 ± 4.47[a]				
		200	28 ± 8.37[a]				
		400	42 ± 8.37[a]				
	30 minutes	25	42 ± 4.47 [a]	59.91 (27.34-96.70)	2831.45 (862.57-8388.95)	3.07	1.00
		50	44 ± 5.48 [a]				
		100	54 ± 5.48 [a]				
		200	68 ± 4.47 [a]				
		400	74 ± 5.48 [a]				
	1 hour	25	72 ± 8.37[a]	5.96 (0.150-16.95)	249.59 (133.72-1513.62)	8.19	0.99
		50	76 ±5.48[a]				
		100	80 ± 7.07[a]				
		200	86 ± 5.48[a]				
		400	72 ± 8.37[a]				
Tricel	15 minutes	25	18 ± 4.47[ab]	429.74 (224.18-2587.90)	26077.75 (3628.77-18756.22)	4.02	1.00
		50	24 ± 5.48[ab]				
		100	36 ± 5.48[ab]				
		200	40 ± 7.07[ab]				
		400	48 ± 8.37[ab]				
	30 minutes	25	36 ± 5.48[ab]	53.89 (31.10-77.91)	937.24 (460.58-4167.76)	6.60	1.00
		50	46 ± 11.40[ab]				
		100	66 ± 5.48[ab]				
		200	72 ± 8.37[ab]				
		400	80 ± 7.07[ab]				

	1 hour	25	74 ±5.48[ab]	6.90 (1.13-14.77)	112.01 (76.08-207.90)	15.09	0.89
		50	84 ± 11.40[ab]				
		100	82 ± 8.37[ab]				
		200	96 ± 5.48[ab]				
		400	100± 0[ab]				
DMSO	15 minutes	25	0 ± 0	-	-	-	-
		50	0 ± 0				
		100	0 ± 0				
		200	0 ± 0				
		400	0 ± 0				
	30 minutes	25	0 ± 0	-	-	-	-
		50	0 ± 0				
		100	0 ± 0				
		200	0 ± 0				
		400	0 ± 0				
	1 hour	25	0 ± 0	-	-	-	-
		50	0 ± 0				
		100	0 ± 0				
		200	0 ± 0				
		400	0 ± 0				
O.a + control	15 minutes	25	36 ± 5.77[bc]	261.75 (103.81-66540.83)	499052.45 (760.29-35944.00)	4.54	1.00
		50	38 ± 5.00[bc]				
		100	42± 5.00[bc]				
		200	48 ± 8.16[bc]				
		400	52 ± 10.95[bc]				
	30 minutes	25	56 ±5.48[bc]	15.01 (0.14-38.26)	3485.79 (703.89-301651.22)	8.54	1.00
		50	60 ± 15.81[bc]				
		100	68 ± 8.37[bc]				
		200	70 ± 12.25[bc]				

		400	80 ± 7.07[bc]				
	1 hour	25	72 ± 8.37[ab]	5.51 (0.04-13.)	202.30 (106.69-1448.13)	6.13	1.00
		50	82 ± 4.47[ab]				
		100	86± 5.48[ab]				
		200	88± 4.47[ab]				
		400	94 ± 5.48[ab]				
O.a + *B.s* MTCC 8141	15 minutes	25	48 ± 4.47[d]	33.91 (8.11-60.35)	2241.06 (674.01-1101.56)	3.12	1.00
		50	54 ± 5.48[d]				
		100	62 ± 4.47[d]				
		200	68 ± 4.47[d]				
		400	80 ± 7.07[d]				
	30 minutes	25	70 ± 7.07[d]	11.67 (4.16-19.83)	134.76 (96.38-230.29)	13.79	0.93
		50	74 ± 8.94[d]				
		100	86 ± 11.40[d]				
		200	90 ± 7.07[d]				
		400	100 ± 0.00[d]				
	1 hour	25	88± 4.47[c]	3.49 (0.01-12.96)	30.79 (11.98-47.66)	13.07	0.95
		50	94± 8.94[c]				
		100	98 ± 4.47[c]				
		200	100 ± 0.00[c]				
		400	100 ± 0.00[c]				
	15 minutes	25	36 ± 11.40[c]	141.77 (59.51-983.10)	72804.00 (142386-6690.13)	8.83	0.99
		50	42 ± 10.95[c]				
		100	54 ± 5.48[c]				
		200	54 ± 5.48[c]				
		400	56± 13.42[c]				
	30 minutes	25	60 ± 12.25[bc]	11.44 (0.08-31.37)	2218.50 (540.37-38631.58)	6.65	1.00
		50	66 ± 8.94[bc]				
		100	70 ± 7.07[bc]				

O.a + *T. h* MTCC 936		200	76 ± 4.89[bc]				
		400	78± 8.37[bc]				
	1 hour	25	80 ± 12.25[bc]	5.48 (0.07-13.27)	128.98 (74.91-374.78)	13.38	0.94
		50	84 ± 8.94[bc]				
		100	86 ± 8.94[bc]				
		200	92 ± 4.00[bc]				
		400	96 ± 5.48[bc]				
O.a + Mix (*B.s* MTCC 8141 + *T. h* MTCC 936)	15 minutes	25	50 ±7.07 [d]	29.01 (3.24-57.33)	3715.93 (834.38-1860.85)	3.35	1.00
		50	54 ± 5.48 [d]				
		100	62 ± 4.47 [d]				
		200	70 ±7.07 [d]				
		400	76 ± 5.48 [d]				
	30 minutes	25	72 ±4.47 [d]	12.15 (3.33-21.60)	113.47 (78.48-212.46)	8.87	0.99
		50	76 ± 5.48 [d]				
		100	82 ± 4.47 [d]				
		200	96 ± 5.48 [d]				
		400	100 ±0.00 [d]				
	1 hour	25	88 ± 4.47 [c]	1.80 (0.01-7.65)	28.83 (9.87-44.49)	10.57	0.98
		50	96 ± 5.48 [c]				
		100	98 ± 4.47 [c]				
		200	100 ± 0.00 [c]				
		400	100 ± 0.00 [c]				

Results are expressed as means ± SD; distinct letters indicate significance among treatments ($p \leq 0.05$)

Fumigant Assay

Fumigant toxicity of the essential oils against *O. parvidens* increased with exposure time and concentration, whereas the DMSO control caused no mortality (Table 3). Because mortality data were non-normal at 15 min, 30 min, and 1 h (Shapiro-Wilk, $p \leq 0.002$), non-parametric analyses were used (Supplementary file S3).

Treatment significantly affected mortality after 15 min ($\chi^2 = 100$, $df = 6$, $p < 0.001$) and 30 min ($\chi^2 = 93.4$, $df = 6$, $p < 0.001$). At 15 min, mortality ranged from 24-48% in essential oil from untreated plants, 32-60% in essential oil extracted from *B.s* MTCC 8141 inoculated plants, 24-52% in essential oil extracted from *T. h* MTCC 936 inoculated plants, and 38-64% in consortium derived essential oil plants. After 30 min, the corresponding ranges were 44-82%, 42-90%, 44-64%, and 66-92%, respectively. The consortium extracted essential oil showed the highest fumigant activity at both exposure periods.

The 1-h analysis also showed a significant concentration effect ($\chi^2 = 17.5$, $df = 4$, $p = 0.002$). After 1 h, mortality reached 100% at 100-400 ppm with essential oil extracted from *B.s* MTCC 8141 inoculated plants and consortium-inoculated plants, whereas mortality reached 96% with essential oil extracted from *T. h* MTCC 936 inoculated plants and 94% with essential oil extracted from untreated plants.

The fumigant assay demonstrated that the volatile fraction of the essential oils was highly active against *O. parvidens*, particularly after prolonged exposure. Consortium-derived essential oil produced the highest early mortality, whereas essential oils extracted from *B.s* MTCC 8141 and mix inoculated plants achieved complete mortality at 100 ppm after 1 h.

Table 3 Effects of Essential oil from *O. americanum* against *O. parvidens* Holmgren & Holmgren, after 15 minutes, 30 minutes and 1 hour of exposure

Sample	Time	Concentration	% Mortality ± SD	LC_{50} (LL-UP)	LC_{90} (LL-UP)	χ^2	p value
Agenda	15 minutes	25	22 ± 8.37 [a]	674.34 (248.94-4226.74)	2606.03 (8306.88-80524.00)	8.87	0.99
		50	32 ± 8.37 [a]				
		100	34 ± 8.94 [a]				
		200	38 ± 13.04 [a]				
		400	46 ± 8.94 [a]				
	30 minutes	25	42 ± 4.47 [a]	59.91 (27.34-96.70)	2831.45 (862.57-8388.95)	3.07	1.00
		50	44 ± 5.48 [a]				
		100	54 ± 5.48 [a]				
		200	68 ± 4.47 [a]				
		400	74 ± 5.48 [a]				
	1 hour	25	64 ± 5.48 [a]	17.1 (6.54-27.65)	161.39 (110.01-320.39)	10.42	0.98
		50	70 ± 10.00 [a]				
		100	80 ± 7.07 [a]				
		200	90 ± 7.07 [a]				
		400	100 ± 0.00 [a]				

Tricel	15 minutes	25	18 ±4.47 [ab]	410.10 (230.63-1575.40)	14471.21 (2868.17-13178.74)	5.11	1.00
		50	24 ± 5.48 [ab]				
		100	26 ± 5.48 [ab]				
		200	34 ± 5.42 [ab]				
		400	52 ± 8.37 [ab]				
	30 minutes	25	40 ± 7.07 [ab]	64.94 (37.56-96.36)	1510.64 (629.74-11233.97)	7.39	0.99
		50	42 ± 8.37 [ab]				
		100	52 ± 8.37 [ab]				
		200	68 ± 4.47 [ab]				
		400	80 ± 10.00 [ab]				
	1 hour	25	74 ± 8.94 [ab]	11.20 (4.18-18.59)	96.33 (71.46-148.67)	13.90	0.93
		50	76 ±11.40 [ab]				
		100	88 ± 8.37 [ab]				
		200	96 ± 5.48 [ab]				
		400	100 ± 0.00 [ab]				
DMSO	15 minutes	25	0 ± 0	-	-	-	-
		50	0 ± 0				
		100	0 ± 0				
		200	0 ± 0				
		400	0 ± 0				
	30 minutes	25	0 ± 0	-	-	-	-
		50	0 ± 0				
		100	0 ± 0				
		200	0 ± 0				
		400	0 ± 0				
	1 hour	25	0 ± 0	-	-	-	-
		50	0 ± 0				
		100	0 ± 0				
		200	0 ± 0				
		400	0 ± 0				
O.a + control	15 minutes	25	24 ± 5.48 [bc]	421.37 (189.31-1476.47)	102863.40 (5677.85-16895.5)	2.33	1.00
		50	32 ± 4.47 [bc]				
		100	38 ± 4.47 [bc]				
		200	44 ± 5.48 [bc]				
		400	48 ± 4.47 [bc]				
	30 minutes	25	44 ± 5.48 [bc]	31.12 (11.39-50.85)	839.23 (385.58-5496.91)	3.56	1.00
		50	62 ±4.47 [bc]				
		100	66 ± 5.48 [bc]				
		200	76 ± 5.48 [bc]				
		400	82 ± 4.47 [bc]				
	1 hour	25	74 ± 5.48 [ab]	16.23 (1.27-26.88)	167.41 (93.51-744.82)	11.37	0.97
		50	80 ± 15.81 [ab]				
		100	86 ± 5.48 [ab]				
		200	92± 4.47 [ab]				
		400	94 ± 5.48 [ab]				
O.a + *B.s* MTCC 8141	15 minutes	25	32 ± 4.47 [d]	133.52 (67.76-388.95)	22052.48 (2529.11-4029.00)	2.49	1.00
		50	42 ± 5.48 [d]				
		100	48 ± 4.47 [d]				
		200	54 ± 5.48 [d]				
		400	60 ± 7.07 [d]				
	30 minutes	25	42 ± 4.47 [d]	31.67 (15.38-47.64)	444.09 (257.16-1316.42)	6.02	1.00
		50	64 ± 5.48 [d]				
		100	72 ± 8.37 [d]				
		200	78 ± 4.47 [d]				
		400	90 ± 7.07 [d]				
	1 hour	25	82 ± 4.47 [c]	5.81 (0.27-15.61)	39.15 (27.93-57.70)	9.30	0.99
		50	90 ±10.00 [c]				
		100	100 ± 0.00 [c]				

		200	100 ± 0.00 [c]				
		400	100 ± 0.00 [c]				
O.a + *T. h* MTCC 936	15 minutes	25	24 ± 5.48 [c]	311.38 (165.64-1868.43)	30710.14 (3555.14-12091.40)	3.58	1.00
		50	30 ± 7.07 [c]				
		100	38 ± 4.47 [c]				
		200	46 ± 5.48 [c]				
		400	52 ± 8.37 [c]				
	30 minutes	25	44 ± 5.48 [bc]	45.98 (0.15-108.29)	48679.66 (1451291-34083.80)	11.76	0.97
		50	48 ± 4.47 [bc]				
		100	52± 10.95 [bc]				
		200	58 ± 13.04 [bc]				
		400	64 ± 18.17 [bc]				
	1 hour	25	72 ± 8.37 [bc]	10.97 (2.06-18.15)	206.03 (118.92-785.95)	18.29	0.74
		50	78 ± 10.95 [bc]				
		100	82 ± 10.95 [bc]				
		200	88± 13.04 [bc]				
		400	96 ± 5.48 [bc]				
O.a + Mix (*B.s* MTCC 8141 + *T. h* MTCC 936)	15 minutes	25	38 ± 4.47 [d]	65.52 (17.64-127.89)	12684.08 (1713.94-19483.4)	5.10	1.00
		50	48 ± 8.37 [d]				
		100	58 ± 4.47 [d]				
		200	62 ± 8.37 [d]				
		400	64 ± 8.94 [d]				
	30 minutes	25	66 ± 5.48 [d]	7.50 (0.14-21.07)	533.45 (229.11-11434.4)	8.19	0.99
		50	72 ± 8.37 [d]				
		100	76 ± 5.48 [d]				
		200	80 ± 7.07 [d]				
		400	92 ± 8.37 [d]				
	1 hour	25	88 ±4.47 [c]	4.04 (0.04-18.01)	43.67 (25.76-63.13)	4.40	1.00
		50	90 ± 7.07 [c]				
		100	100 ± 0.00 [c]				
		200	100 ± 0.00 [c]				
		400	100 ± 0.00 [c]				

Results are expressed as means ± SD; distinct letters indicate significance among treatments ($p \leq 0.05$)

Repellent Assay

Repellency was assessed 10 min after exposure to the essential oils (Table 4). The repellency data were non-normally distributed (Shapiro–Wilk $W = 0.747, p < 0.001$); therefore, treatment effects were analysed using the Kruskal–Wallis test followed by Dwass–Steel–Critchlow–Fligner pairwise comparisons.

Repellency differed significantly among samples ($\chi^2 = 80.3$, $df = 6$, $p < 0.001$) and concentrations ($\chi^2 = 59.5$, $df = 4$, $p < 0.001$). DMSO showed no repellency at any concentration. Repellency of the essential oils generally increased with concentration, ranging from 66.78-91.10% in oil from untreated plants, 74.11-100% in essential oil extracted from *B.s* MTCC 8141 inoculated plants, 68.19-100% in essential oil extracted from *T. h* MTCC 936 inoculated plants, and 74.22-100% in consortium derived oil. At 400 ppm, all three microbe-derived essential oils produced 100% repellency, compared with 91.10% for essential oil from untreated plants and 97.78% for Tricel.

Pairwise comparisons indicated that the microbe-derived oils showed significantly greater repellency than Agenda at selected comparisons ($p \leq 0.016$), whereas differences among the three microbial treatments were not significant ($p \geq 0.562$). Repellency at 100, 200, and 400 ppm was significantly higher than at 25 ppm ($p \leq 0.004$). Overall, the essential oils showed strong short-term repellency against *O. parvidens*, with the highest response observed at 400 ppm following the 10-min exposure period.

Table 4 Effects of Essential oil from *O. americanum* against *O. parvidens* Holmgren & Holmgren, after 15 minutes, 30 minutes and 1 hour of exposure

Sample	Concentration	Repellent Rate (%) ± SD
Agenda	25	61 ± 5.48 [a]
	50	72 ± 6.71 [a]
	100	73 ± 4.47 [a]
	200	74 ± 2.24 [a]
	400	87.20 ± 3.75 [a]
Tricel	25	56.112 ± 2.30 [ab]
	50	74.5 ± 1.41 [ab]
	100	88.104 ± 1.74 [ab]
	200	88.104 ± 1.74 [ab]
	400	97.776± 4.97 [ab]
DMSO	25	0±0
	50	0±0
	100	0±0
	200	0±0
	400	0±0
O.a + control	25	66.78 ± 18.70 [ab]
	50	74.11 ± 2.31 [ab]
	100	74.11 ± 2.31 [ab]
	200	86.10 ± 6.21 [ab]
	400	91.100 ± 4.97 [ab]
O. a + *B. subtilis*	25	74.14 ± 2.32 [b]
	50	74.11± 2.31 [b]
	100	86.10± 6.21 [b]
	200	91.10± 4.97 [b]
	400	100.00± 0.00 [b]
O. a + *T. harzianum*	25	68.19 ± 10.08 [b]
	50	74.11 ± 2.31 [b]
	100	86.10 ± 6.21 [b]
	200	91.10 ± 4.97 [b]
	400	100.00 ± 0.00 [b]
O. a + Mix	25	74.22 ± 2.37 [b]
	50	74.11 ± 2.31 [b]
	100	86.10 ± 6.21 [b]
	200	91.10 ± 4.97 [b]
	400	100.00 ± 0.00 [b]

Results are expressed as means ± SD; distinct letters indicate significance among treatments ($p \leq 0.05$)

Discussion

The present study provides experimental evidence that plant developmental stage and microbial inoculation influence essential-oil production and biological activity in *O. americanum*. The reproductive phase was identified as the most productive stage for oil extraction, while inoculation with *B. s* MTCC 8141, alone or in combination with *T. h* MTCC 936, further enhanced oil production and improved the activity of the extracted oil against *O. parvidens*. Similar studies have shown that beneficial microorganisms can increase biomass and essential-oil accumulation by improving nutrient acquisition and regulating secondary metabolism (Çakmakçı et al. 2020; Lv et al. 2024). Thus, the present findings integrate phenology-based harvesting with microbial biostimulation and provide a scientifically supported basis for developing *O. americanum* essential oil as a biological resource for termite management.

The improved biomass under microbial treatments may reflect complementary functions of the bacterial and fungal inoculants. *Bacillus* species can promote growth through nutrient solubilisation, phytohormone production, siderophore synthesis, and modulation of plant defence, whereas *Trichoderma* can improve root development, nutrient acquisition, and plant physiological performance. (Lyngwi et al. 2016; Adjei et al. 2024; Jiménez Bremont et al. 2026; Santoyo et al. 2024). The absence of antagonism observed in vitro may have allowed both strains to establish simultaneously and contribute to plant growth. The present results are also consistent with studies showing that microbial inoculation can increase plant biomass together with essential-oil production and aroma quality in medicinal and aromatic plants (Gupta et al. 2016). However, because the consortium did not outperform *B. s* MTCC 8141in every parameter, its effect should be described as treatment-dependent and strain compatibility rather than universally synergistic.

Essential-oil yield was highest during the reproductive phase in all treatments. This result agrees with recent work in aromatic plants showing that flowering or reproductive development can be associated with maximum oil accumulation (Tonce et al. 2017; Kholiya et al. 2022). A similar pattern has also been reported for *O. gratissimum*, in which the full-flowering phase was considered the most suitable harvest stage for obtaining maximum oil yield (Kholiya et al. 2025). This may be because plants had accumulated sufficient biomass while maintaining active secondary metabolism (Kholiya et al. 2022). Developmental changes in essential-oil yield are well documented in aromatic plants, although the maximum-yielding stage differs according to species, genotype, environment, and the plant organ examined (Ben Youchret-Zallez et al. 2026). The identification of the reproductive phase as the most productive stage is therefore practically relevant for maximising oil recovery from this species (Giri 2019; Abd-ElGawad et al. 2023). Microbial inoculation further increased oil yield at each phase. During the reproductive

phase, plants inoculated with *B. s* MTCC 8141and the mix inoculum produced the highest oil content compared with the untreated plants. This response is consistent with reports that rhizobacteria can stimulate essential-oil accumulation in basil and other aromatic species by improving nutrient availability and altering plant signalling (Ebrahimi et al. 2026). Microbial inoculation may have enhanced essential-oil production by priming jasmonate, salicylic acid and ethylene mediated signalling networks involved in plant defence and terpenoid metabolism. Microbial elicitors and phytohormone related effects may stimulate glandular-trichome activity, terpene biosynthesis, root development, and nutrient acquisition, thereby contributing to the higher oil yield and increased relative abundance terpenoids in microbial treated plants (Cappellari et al. 2019; Hembram et al. 2026). However, because hormonal responses, trichome density, and terpene-biosynthetic gene expression were not measured, this mechanism remains a hypothesis requiring further validation.

GC-MS analysis revealed that the reproductive-phase essential oil contained 14-17 identified constituents, with 16 compounds detected in the essential oil extracted from untreated control, 14 in the essential oil extracted from *B. s* MTCC 8141 treated plants, 17 in the essential oil extracted from *T. h* MTCC 936 treated plants, and 15 in the essential oil extracted from consortium treatment. The compounds were dominated by monoterpenes and oxygenated monoterpenes, with smaller contributions from sesquiterpenes. (+)-2-Bornanone, corresponding to camphor was the major constituent in all treatments. Although the *B. s* MTCC 8141 derived essential oil contained the fewest identified constituents, it showed the highest relative abundance of (+)-2-bornanone (53.74%), followed by the consortium essential oil (50.38%). This indicates that chemical diversity and the relative dominance of a major constituent were not directly related. This pattern is broadly comparable with previous reports in which camphor and limonene were major constituents of *O. americanum* oil, although their proportions vary according to genotype, season, locality, and cultivation conditions (Mahendran and Vimolmangkang 2023; Paudel et al. 2025).

The essential oil extra ted from the reproductive phase exhibited substantial toxicity against *O. parvidens* in all three bioassays- contact- toxicity, fumigant and repellent assays. Mortality increased with exposure time and concentration, while the DMSO control produced no mortality. This confirms that the observed activity was associated with the essential oils rather than the solvent. Essential oil extracted from *B. s* MTCC 8141 and mix inoculated plants generally caused the greatest contact mortality, while essential oil extracted from mix inoculated plants showed particularly strong fumigant activity during the early exposure period. The fumigant response is important because essential oils can act through volatile compounds that enter the insect respiratory system without direct contact. The strong activity observed here may be related to the high proportion of camphor and

the presence of limonene, compounds previously associated with insecticidal, fumigant, and repellent effects (Rosette et al. 2020; Opiyo 2022). The essential oils also produced concentration-dependent repellency, reaching 100% at 400 ppm after 10 min in all microbial treatments. This response was distinct from mortality and indicates rapid behavioural avoidance of the treated area. Essential-oil repellency may reduce termite orientation and feeding before lethal exposure occurs, thereby providing a complementary mode of action to contact and fumigant toxicity (Bacci et al. 2015; Chantarapitak et al. 2025). Similar behavioural effects have been reported for camphor-rich essential oils against insect pests, including termites. The strong repellency observed at the highest concentration may be useful for reducing termite entry into treated areas (Fu et al. 2015; Santos et al. 2024). However, excessive repellency can also limit contact between termites and treated surfaces, potentially reducing delayed toxicity. Field performance will therefore depend on the balance between repellency, vapour action, residual persistence, and exposure to the treated material (Fouad et al. 2023; Chantarapitak et al. 2025).

Conclusion

The present study establishes a relationship between microbial biostimulation, phenological timing, essential-oil production, and termite control. Reproductive-stage harvesting maximised essential oil yield, while *B. s* MTCC 8141 and mix inoculants (*B.s* MTCC 8141 + *T. h* MTCC 936) produced essential oil with enhanced relative camphor abundance and strong contact, fumigant, and repellent activity against *O. parvidens*. Thus, microbial inoculation can be used not merely to increase *O. americanum* biomass, but to improve the production of essential oil with potential value in termite management. Future studies should validate these results under field conditions, quantify the absolute concentrations of key constituents, assess effects on non-target organisms, and determine whether the observed laboratory activity can be retained in a stable and practically applicable formulation.

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Authors' Contributions

Munmi Borkataky supervised the study. Nilakhi Kakoti conceptualised and conducted the research, performed data curation, statistical analysis, investigation, and visualisation, and prepared the original manuscript draft. Both the authors reviewed and approved the final version of the manuscript.

Ethics Approval

This is not applicable

Consent to participate

This is not applicable

Consent to publish

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Clinical Trial Number

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Declaration of Competing Interest

The authors declare no competing interest

Data availability

All data required are available in the manuscript

Supplementary Information

All supplementary materials are provided in SM1, SM2, SM3

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