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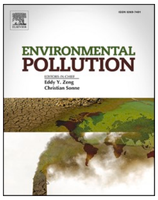

Ecotoxicological assessment of basil essential oil on soil nematode communities☆

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ABSTRACT

Intensive agriculture has over-exploited natural resources and altered the functioning of soil systems, mainly because of the abuse of synthetic products to increase yields. To improve sustainability, the European Commission has adopted the New Green Deal program, which aims to reduce the use of synthetic products by 50% and restore biodiversity by limiting human impact. Essential oils (EOs) can be sustainable alternatives to synthetic pesticides for crops and agricultural products protection against pests and diseases and, therefore, are attracting increasing interest from the scientific community. However, while most studies have focused on *in vitro* effects on target organisms (*i.e.,* soil borne-phytopathogens, insects, phytoparasitic nematodes), the impact of EOs on natural soil communities remains unexplored. This study employed a field experiment using the Before-After-Control-Impact (BACI) approach to investigate the potential effects of basil EO on nematode communities. Chickpea seeds were coated with a high concentration (25% v/v) of *Ocimum basilicum* EO in different formulations, with the basil EO and chitosan combination standing out for its potential applicability as a seed-coating treatment for future crop use. The results demonstrated that the basil EO and chitosan seed coating had no significant negative impact on nematode taxonomic diversity or functional indices. Furthermore, the taxonomic community structure—recognized as the most sensitive detection metric—revealed that the observed effects were primarily influenced by temporal changes, likely reflecting the natural progression of the crop system rather than the EO treatment itself. These findings are particularly promising, as they suggest that EO-based coatings can be compatible with maintaining soil health.

1. Introduction

Intensive agriculture has over-exploited natural resources and altered ecosystems functioning with many deleterious effects, not only on environmental health but also on humans. This was mainly due to the massive and indiscriminate use of synthetic products applying to increase crop yields. The European Union (EU) has, therefore, adopted several policies to protect and improve the quality of waters and soils in its countries. According to the European Green Deal (EDG), EU intends to prevent the contamination of air, water and soils. Among the most important initiatives of the EGD, there is the "Farm to Fork" strategy, that aims to transform agriculture into a more sustainable system by reducing the use and risk of chemical pesticides by 50%, cutting nutrient losses by 50%, lowering the use of fertiliser by 20%, and increasing the share of agricultural land under organic farming to at least 25% (Torre et al., 2021; Ferraz et al., 2022). Hence, synthetic chemicals must be replaced with more sustainable formulates, such as those based on biocidal compounds produced by many plants. Among them, essential oils (EOs) extracted from aromatic plants have been acknowledged for a strong bioactivity on crop pests.

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An EO is a hydrophobic liquid containing volatile chemical compounds; it can be found in many organs of the plant itself (*e.g.*, glandular hairs, leaves, flowers, roots, fruits, stems, bark). EOs are secondary metabolites that play a significant role in the interaction of the plant with its environment and are involved in plant protection against biotic stress with significant documented fungicidal, antimicrobial, nematocidal, insecticidal, and insect repellent activities (Torre et al., 2021; Catani et al., 2022, 2023; da Silva et al., 2022; Farina and Conti, 2024). Since EOs are not soluble in water and degrade rapidly, even in soil, they are not expected to pose risks to the environment (Benelli et al., 2020; Pavela et al., 2020; Pintong et al., 2020). In recent years, an increasing attention to scientific research on the properties of EOs has been observed, due to their potential for crop protection, representing a relevant, green alternative, to standard pesticides used in the control of pests and crops' diseases. However, despite the increasing interest on the topic, the number of commercial EOs employed in this sector is very low. One of the reasons behind this gap is that most published data on the biological efficacy of EOs are tested *in vitro* or in pot screenings, and the investigations are mostly focused to manage target pests. Conversely, only a few studies are focused on the effects of EOs on non-target organisms, which is a crucial step in demonstrating their eco-friendly role and developing authorizations as botanical pesticides (Ferraz et al., 2022). This phenomenon is partly due to the common perception that plant components, being natural, are also safe. However, EOs may be potentially toxic also to non-target organisms (Martins et al., 2013; Bilen, 2014; Zàrybnický et al., 2018; Falkowski et al., 2020); therefore, a critical assessment of their possible impact on the overall fauna across terrestrial trophic and aquatic chains must be undertaken (Pavela and Benelli, 2016).

Ferraz et al. (2022) reviewed the literature on the (eco)toxicological effects of plant extracts and EOs. Most studies are focused on the aquatic environment (77%), especially aquatic invertebrates (45%), while only 23% of the studies focused on the potential toxicity of plant-derived products to terrestrial ecosystems. It is interesting to note that some EOs showed no toxic effects on non-target organisms, or they were only observed at high concentrations, whereas others were toxic at concentrations below the limits set by international regulations and, in some cases, even at very low concentrations. Furthermore, the standard tests for compound approval address only a fraction of the possible effects of a chemical on the environment; only acute toxicity effects of individual chemicals and on a few species are evaluated, while cumulative effects or effects on the food chain and ecosystems are lacking, thus underlining the importance of investigations in the field and at the community organization level (Hägerbäumer et al., 2015; Ferraz et al., 2022; Schratzberger et al., 2023).

For instance, it has been noticed that some EOs can lead to a change (decrease) in soil pH level, causing changes in bacterial populations and soil respiration (Bilen, 2014). Martins et al. (2013) found that *Eucalyptus* EO can have direct toxic effects on non-target soil fauna and indirect effects on source quality and availability across the trophic chains. Although these products are generally considered innocuous and safer than their synthetic counterparts, their low impact on non-target organisms should be further investigated and, especially, demonstrated through field studies.

Invertebrates contribute to ecosystem functioning and play a significant role in maintaining good soil health (Martins et al., 2013). Nematodes, with their basic body plan, occur with high abundances in all terrestrial habitats and climatic regions (Wilson and Kakouli-Duarte, 2009; Semprucci and Balsamo, 2012; van den Hoogen et al., 2020; Cocozza di Montanara et al., 2022). They occupy different trophic levels and provide important services by connecting different ecosystems' components and participate in sedimentary, trophic, and ecological processes. They stimulate nutrient cycling, especially nitrogen, and regulate decomposition processes by grazing on microbes (Jiang et al., 2017). Moreover, nematodes promote soil biodiversity by helping to maintain a healthy environment (Neher, 2001; Hodda et al., 2009). The generation time and short life cycle of nematodes, as well as the simple methods for their sampling and extraction, make them ideal environmental bioindicators (Wilson and Kakouli-Duarte, 2009; Balsamo et al., 2012). Besides, the permeability of their body walls makes them highly vulnerable to the possible addition of chemicals in the surrounding environment (Bongers, 1990; Ferris et al., 2001; Van Den Hoogen et al., 2019). A suitable method to assess soil health is to observe changes in the nematode community structure. Indeed, community structure, as a level of organization, can provide information of greater ecological relevance thanks to the disappearance/appearance of nematode species with different behavioural styles and life-cycle characteristics (Schratzberger et al., 2023). For this level of organization, small-scale microcosms or field studies are the only options (Hägerbäumer et al., 2015), and field experiments are recommended to avoid a decrease in taxa related to soil manipulation rather than to the addition of chemicals (Čerevková et al., 2017).

EOs derived from the aromatic plant of basil (*Ocimum basilicum* L., Lamiaceae family) have high potential in many fields of application. A recent bibliometric analysis revealed that the EO obtained from *O. basilicum* is one of the most frequently cited alongside *Thymus vulgaris*, *Lavandula* spp., *Origanum* spp., *Mentha* spp., *Citrus* spp., and *Cymbopogon* spp. (Catani et al., 2022). Many studies have reported its relevant antimicrobial, fungicidal, nematicidal, insecticidal and insect repellent properties (*e.g.*, Oxenham et al., 2005, Hussain et al., 2008, Zhang et al., 2009; Basantia, 2017, Pierattini et al., 2019; Bedini et al., 2024; Djebbi et al., 2024). Thus, it is regarded as one of the most promising EOs for future applications in crops and agriculture products protection.

Fusarium is one of the broadly spread fungi affecting crops and causes important yield losses depending on cultivar susceptibility and climatic conditions (*e.g.*, Jiménez-Díaz et al., 2015; Jendoubi et al., 2017). The most represented constituents of *O. basilicum* EO (*i.e.*, 1,8-cineole, linalool, methyl chavicol, and eugenol, according to the chemotype-Lawrence, 1985) have a well-known inhibitory effect against *Fusarium* (Reuveni et al., 1984; Fandohan et al., 2004; Dambolena et al., 2010; Basantia, 2017; Harčárová et al., 2021; Torre et al., 2021) including *F. oxysporum ciceris* (FOC). The FOC disease attacks chickpeas crops (Jiménez-Díaz et al., 2015; Jendoubi et al., 2017), and coating the chickpeas seed with *O. basilicum* EO seems to reduce *Fusarium* attacks (Kocić-Tanackov et al., 2011). In this regard, chitosan (CHI) is a natural, non-toxic, and edible substance (a copolymer derived by deacetylation of chitin, Badawy and El-Aswad, 2012) that is having a wide application in combination with EOs because it limits the high EOs volatility and produces a coating on seeds (*e.g.*, Ascrizzi et al., 2021; Farina et al., 2022; Sravani et al., 2023; Abenaim and Conti, 2023; Djebbi et al., 2024).

Like many EOs, *O. basilicum* constituents are supposed to have little or no adverse effects on the environment and non-target organisms, but data on the actual impact of this substance on non-target organisms are currently lacking (https://echa.europa.eu/it/information-on-chemicals). Thus, to detect possible disturbing effects on soil health status, a field experiment was conducted using chickpea seeds (*Cicer arietinum* L., Fabaceae family) tanned with high concentrations of *O. basilicum* EO (25% v/v), and seeds coated with and without CHI. In particular, the seed treatment that we were most interested in understanding if it has an impact on non-target fauna for applications in the agricultural field is the one in which the basil EO has been combined with CHI (Ascrizzi et al., 2021; Abenaim and Conti, 2023).

Since the study of the post-impact alone is not always powerful enough to detect the effects of an environmental disturbance, the experiment was performed according to a BACI (Before-After-Control-Impact) strategy. This method permits to distinguish the biological community changes related to differences in time elapsed or space *vs.* the changes caused by an environmental disturbance (Seger et al., 2021). The BACI approach, in fact, allows a comparison of the community parameters between "before" and "after" an induced perturbation; likewise, the “controls” document the community status in samples fully sheltered from the perturbation, while “impacts” represent the community under a potential stress.

This study aims to investigate the effects of one of the most promising EOs on the diversity, as well as on the taxonomic and functional structure of nematodes’ assemblages, since they are known as excellent bioindicators of soil quality.

As mentioned above, we applied a BACI approach by carrying sampling before sowing (baseline condition) and after 7, 14, 28, and 56 days after sowing, to detect the possible effects of the treatments on the structure and composition of the nematode community. For that, control soil plots with untreated seeds, soil plots with seeds coated with EO 25% + CHI, plots with seeds coated only with CHI and plots with seeds coated only with EO 25% were defined.

2. Material and methods

2.1. Essential oil (EO) origin and analysis

O. basilicum EO was purchased from Sigma Aldrich (CAS number: 8015-73-4). It was diluted (5%) in HPLC-grade *n*-hexane and then injected into a GC-MS apparatus. Gas chromatography – electron impact mass spectrometry (GC/EIMS) analyses were performed with an Agilent 7890B gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an Agilent HP-5 MS (Agilent Technologies Inc., Santa Clara, CA, USA) capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and an Agilent 5977B single quadrupole mass detector (Agilent Technologies Inc., Santa Clara, CA, USA). Analytical conditions were as follows: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; injection of 1 μL; split ratio 1:25. The acquisition parameters were as follows: full scan; scan range: 30–300 *m/z*; scan time: 1.0 s. The identification of the constituents was based on the comparison of their retention times with those of the authentic samples (when available), comparing their linear retention indices relative to the series of *n*-hydrocarbons (C6-C25). Computer matching was also used against a commercial (National Institute of Standards and Technology, 2014) and a laboratory-developed mass spectra library built up from pure substances and components of commercial EOs of known composition and MS literature data (Adams, 2007).

Quantitative comparisons of relative peaks areas (%) were performed between the same chemicals in the different samples.

2.2. Seeds treatment

Chickpeas seeds (*Cicer arietinum* L.) tanned in *O. basilicum* EO at concentration of 25% v/v with or without CHI were used for the experiment.

According to Peng and Li (2014), commercial chitosan (CHI) extracted from crab shells (CAS number: 9012-76-4) was dissolved in a solution consisting of deionized water and glacial acetic acid (1% v/v) to obtain a 2% (w/v) CHI solution. The solution was stirred for 4 h at 25 °C by a magnetic stirrer. In addition, tween 80 (1% v/v) and the *O. basilicum* EO at the dose of 250 mL of EO L^{-1} of solution were added to obtain the CHI-EO solution (EO 25% + CHI). The CHI-EO solution was stirred for 4 min by the magnetic stirrer at room temperature. The EO 25% solution was obtained by mixing deionized water with 1% (v/v) tween 80 and EO at the dose of 250 mL of EO L^{-1} of solution.

For coating, chickpea seeds were immersed for 1 min in the individual solutions of CHI, EO 25% + CHI, or EO 25%. The excess liquid was drained by placing the seeds on a metal grid under the flow hood for 1 h. Finally, the coated seeds were air-dried for 24 h under laboratory conditions (24 °C and 65% RH).

2.3. Field site and soil sampling design

The field trial was conducted in Urbania (43°69′92″N; 12°48′80″E), a town located in central Italy, close to the Apennines (Fig. 1). The climatic condition in the region is characterized by hot summers, with an average daily temperature of 28 °C and longer winters. The soil is mainly characterized by clay (95%) with a low fraction of sand (4%). Soil humidity (% H_2O) was, on average, 15.3 ± 0.9, soil pH 7.93 ± 0.06, conductibility 467.33 ± 4 μS/cm, and available phosphorus lower than 3 μg/g. Sowing took place in April 2022. The trial was set up on a randomized plot design with four rows separated by buffer strips, while each treatment was carried out in triplicate, resulting in 16 plots overall (4 × 4 m per plot). The treatments comprised different variations: seeds coated with EO 25% + CHI, only with CHI, with EO 25%, or untreated seeds (Ctrl). Since EO seed coating could have effects on the natural soil fauna as well as on the target organisms of the treatment, soil samples were collected to assess possible changes in the nematode community. According to the BACI approach, soil sampling was scheduled before sowing and after 7, 14, 28, and 56 days from sowing for each treatment (*i.e.,* CHI, EO 25%, EO 25% + CHI) and control samples (*i.e.*, Ctrl, soil plots with untreated seeds), starting in April 2022 and ending in July 2022. Five samples per plot were taken randomly at a soil depth of 20 cm, mixed, and homogenized by hand. The litter above each sample area was removed before the cores were collected. Soil samples were placed in plastic bags, labelled, and kept in a portable refrigerator at 4 °C until arrival at the laboratory (Landi et al., 2022).

2.4. Nematode assemblage analysis

Once in the laboratory, nematodes were isolated from 100 g of mixed and homogenized fresh soil samples using the Baermann multi-funnel method extraction (after 48 h) and then fixed in a formalin solution (4%) (Viaene et al., 2021). All the nematodes present in each sample were counted under a stereomicroscope (Leica G26, zoom 50 ×). Nematodes were then mounted on permanent slides according to Seinhorst (1959, 1962), and the first 100 individuals encountered were identified at family or genus level under a light optical microscope (Optiphoto-2 Nikon) using taxonomical keys from Bongers (1988), Andrássy (2005, 2007, 2009), Manzanilla-Lopez and Marban-Mendoza (2012), and Perry and Moens (2013).

Nematodes were assigned to trophic groups – bacterivores, fungivores, herbivores, omnivores, and predators (Yeates et al., 1993; Okada and Harada, 2007) – and to colonizer-persister (c-p) guilds (Bongers and Bongers, 1998). Data on nematodes were used to calculate the following functional indices: Maturity (MI) and Plant Parasitic (PPI) indices according to Bongers (1990) and food web indicators (BI, basal index; EI, enrichment index; SI, structure index; CI, channel index) according to Ferris et al. (2001).

In detail, the maturity index (MI, Bongers, 1990) was calculated as the weighted average of the individual colonizer-persister (c-p) values: MI = Σv (i) f (i), where v is the c-p value of genus i and f (i) is the frequency of that genus. This index is based on the gradual discrimination among *r*-strategist nematodes (colonizers, *i.e.*, c-p 1 and c-p 2), intermediate colonizers (*i.e.*, c-p 3) and *k*-strategist genera (persisters, *i.e.*, c-p 4 and c-p 5). The maturity index for free-living nematode taxa (MI) may be viewed as a measure of disturbance, with smaller values being indicative of a more disturbed environment and larger values characteristic of a less disturbed environment. The MI decreases with the increasing of the microbial activity and pollution-induced stress. The PPI is comparable to the MI but computes only for the plant-feeding nematodes (*e.g.*, feeding = 1), with the rationale that their abundance is determined by the vigour of their host plants which, in turn, is determined by system enrichment (Bongers et al., 1997; Ferris and Bongers, 2009). Low values of PPI indicate a dominance of ectoparasites in the plant-parasitic nematode community, while higher values indicate a predominance of (semi-) endoparasites (Du Preez et al., 2022).

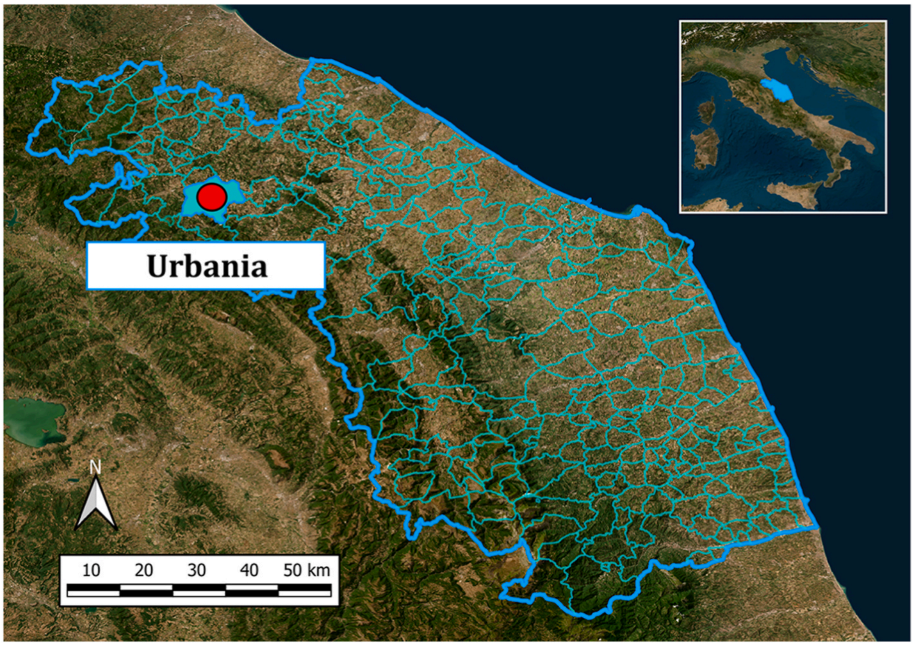

Fig. 1. Location of the experiment carried out from April to June 2022. Map generated using QGIS version 3.28.

The EI (a measure of opportunistic bacterivore and fungivore nematodes), CI (indicator of predominant decomposition pathways), SI (indicator of food web state affected by stress or disturbance) and BI (indicator of food web structure and complexity) are calculated based on feeding habits and life strategies of the nematodes (Ferris et al., 2001). These indices allow us to quantify the soil food-web state defining the enrichment and structure trajectories. In particular, functional guilds of soil nematodes characterized by trophic groups and by life strategies were used to obtain a diagram of soil food web condition (basal, structured, enriched) and the four quadrants have been interpretated as follows: annual crop agriculture with frequent disturbance and exogenous inputs ($SI < 50$ and $EI > 50$, Quadrat A); perennial crop or more sustainable agriculture with exogenous input but minimal physical disturbance ($SI > 50$, $EI > 50$, Quadrant B); natural forests and grasslands, undisturbed with recycling of endogenous resources ($SI > 50$, $EI < 50$, Quadrant C); resource deprived systems in stressed environments ($SI < 50$, $EI < 50$, Quadrant D) (Ferris et al., 2001).

The Shannon (H′) and Pielou (J, both log 2) indices were further calculated on the collected data, to provide a measure of the alpha diversity of the nematode community (Shannon and Weaver, 1949; Pielou, 1966).

2.5. Statistical data analysis

Biotic data, consisting of the abundance of the nematode fauna, were used to construct a taxa-by-treatments and period matrix.

The multivariate, non-metric multi-dimensional scaling (nMDS) analysis, based on Bray-Curtis (dis)similarity measures (square-root transformed values), was used to visualize differences in the structure of nematode assemblages between treatments and control (*i.e.*, CHI, EO 25% + CHI, EO 25%, Ctrl). The resulting MDS plots were overlaid with bubble plots representing the time elapsed from the beginning of the experiment: before sowing and after 7, 14, 28, and 56 days. Significant differences in the two above-cited factors were further checked using ANOSIM (Analysis of Similarities). The nematode taxa contributing most to (dis)similarities among treatments and periods were identified using the Similarity Percentages-species contributions (SIMPER) test performed on the transformed Bray-Curtis similarity matrix (cut-off 50%).

These multivariate analyses were performed using Primer software (version 5) (Clarke and Gorley, 2006).

The significance of the differences in the biotic univariate measures, *i.e.*, total nematode abundance, H′, J, MI, PPI, EI, CI, SI, and BI were checked using an Analysis of Variance (ANOVA). Data that did not meet the normality and homoscedasticity assumptions of ANOVA (Kolmogorov-Smirnov and Levene tests) were log $(1 + x)$ transformed. The Tukey's test was applied for the multiple comparisons when significant differences ($p < 0.05$) were detected (SPSS software, v.17).

3. Results

The complete composition of the tested *O. basilicum* EO is reported in Table 1. Its analysis evidenced a methyl chavicol chemotype, as this phenylpropanoid accounted for up to 78.5% of the total composition. The only two other detected compounds showing relative abundances over 2% were *trans*-α-bergamotene and 1,8-cineole, accounting for 4.6% and 4.3%, respectively.

Forty-four different genera belonging to 7 plant parasitic and 14 free-living nematode families were identified in soil samples collected in the present experiment. Tylenchidae (25%), Dorylaimidae (20%), Cephalobidae (10%), and Hoplolaimidae (10%) were the most abundant families. Fungivore and bacterivore nematodes dominated the community (39% and 33%, respectively), followed by omnivores/predators and herbivores (both 14%).

The effects of the different treatments and periods on nematode community structure are visualized by non-parametric multidimensional scaling (nMDS, Bray-Curtis similarity, square root transformation, Fig. 2). The MDS analysis overlaid with bubble plots representing the time elapsed since the start of the experiment clearly highlights a separation of the samples collected after 56 days. ANOSIM revealed that the period had a greater effect than treatments on the taxonomic structure of the nematode community (ANOSIM, $R = 0.27$; $p = 0.001$ for period and $R = 0.16$; $p = 0.002$ for treatment). Moreover, pairwise ANOSIM tests confirmed that the most distinct community structure was observed in samples collected 56 days after sowing ($R = 0.40–0.48$; $p = 0.001$). The slight but significant differences between treatments were primarily attributable to the comparisons between EO 25% and CHI ($R = 0.35$; $p = 0.001$) and EO 25% and the Ctrl ($R = 0.25$; $p = 0.004$).

The percentage contribution to the average dissimilarity between the treatments and sampling periods, measured by the SIMPER analysis, is

Table 1
Complete composition of the essential oil of *Ocimum basilicum*.

Compounds	l.r.i.[a]	Relative abundance (%)[b]
α-pinene	941	0.3
sabinene	976	0.2
β-pinene	982	0.5
myrcene	993	0.3
limonene	1032	0.2
1,8-cineole	1034	4.3
(*E*)-β-ocimene	1052	1.4
fenchone	1087	0.2
linalool	1101	1.3
fenchol	1113	0.2
camphor	1143	0.7
menthone	1154	0.1
borneol	1165	0.2
menthol	1173	0.5
α-terpineol	1189	0.5
methyl chavicol	1197	78.5
endo-fenchyl acetate	1223	0.4
bornyl acetate	1287	0.3
β-elemene	1392	0.4
methyl eugenol	1403	0.9
β-caryophyllene	1420	0.3
trans-α-bergamotene	1438	4.6
α-bulnesene	1505	0.1
trans-γ-cadinene	1513	1.0
δ-cadinene	1524	0.2
4-methoxycinnamaldehyde	1570	0.7
spathulenol	1576	0.1
1.10-*di*-*epi*-cubenol	1614	0.2
epi-α-cadinol	1640	1.4
Monoterpene hydrocarbons		2.8
Oxygenated monoterpenes		8.7
Sesquiterpene hydrocarbons		6.4
Oxygenated sesquiterpenes		1.6
Diterpene hydrocarbons		–
Phenylpropanoids		80.1
Non-terpene derivatives		–
Total identified (%)		99.7

[a] Linear retention index on a HP-5 MS capillary column.
[b] Detection threshold ≥0.1%.

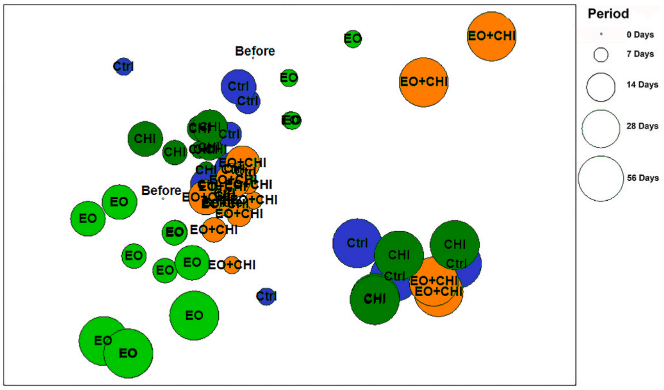

Fig. 2. Non-metric multi-dimensional scaling (nMDS) performed on the nematode community (square root transformed data) from each variant condition *i. e.,* baseline condition (before = white), control soil plots with untreated seeds (Ctrl = blue), plots with seeds coated only with chitosan (CHI = dark green), plots with seeds coated only with *O. basilicum* EO at concentration of 25% v/v (EO = light green), soil plots with seeds coated with basil essential oil and chitosan (EO + CHI = orange). Bubble sizes represent the time elapsed since the start of the experiment: sampling before sowing and at 7, 14, 28, 56 days from sowing. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

reported for the top discriminating taxa accounting for up to 50% of the cumulative percentage (Table S1). The highest dissimilarity was observed between EO 25% *vs.* CHI (average dissimilarity: 65%), followed by the average dissimilarity found between EO 25% *vs.* Ctrl (64%) and EO 25% *vs.* EO 25% + CHI (63%). In all these pair-wise comparisons, EO 25% samples were distinguished by the highest abundance of members belonging to the Cephalobidae, Aphelenchidae, and Tylenchidae families (Table S1). Regardless of the differences between treatments and between EO 25% and Ctrl, an overall discrepancy in the nematode community composition has been observed between the time periods, with samples collected after 56 days from the sowing showing the largest dissimilarity (average dissimilarity: 70–75%), due to the presence of members from the Diplogasteridae family and dauers (Table S2).

The total average abundance of nematodes (expressed as ind./100 g of mixed soil sample) are reported in Fig. 3. The analysis of variance (ANOVA) documented significant differences only between periods (ANOVA, F = 8.002; df = 67; $p < 0.01$) underlining higher abundances after 56 days (Tukey's test $p < 0.05$) (Fig. 3).

In Fig. 4, the trends in alpha diversity are presented by dividing the different seed-coating treatments across the four sampling intervals, for illustrative purposes only, as the statistical analyses were conducted on the entire dataset. The ANOVA did not reveal significant differences in alpha taxonomic diversity between treatments ($p > 0.05$), but a marked difference was observed between sampling periods (ANOVA, df = 67, H': F = 37.9, $p < 0.001$; J: F = 22.1, $p < 0.001$). Tukey's test highlighted that the lower alpha diversity was attributable to the sampling conducted 56 days after sowing and especially in relation to EO 25% (Tukey's test, $p < 0.001$). The k-dominance curves, providing a visual representation of nematode biodiversity trends, confirmed a marked dominance in the nematode community in the final sampling, especially due to Diplogasteridae (Fig. 5).

As with alpha diversity, MI and PPI trends are reported by dividing the different seed-coating treatments across the four sampling times. The PPI values remained consistent across both factors (ANOVA, $p > 0.05$), while a significant decrease in MI values was observed between sampling dates (ANOVA, df = 67, F = 3.1, $p < 0.05$), particularly between 7 and 56 days (Tukey's test, $p < 0.05$) (Fig. 6).

The EI, SI, BI, and CI values calculated for the faunal components are summarized in Table 2. Among these indices, only EI showed significant differences between treatments (ANOVA, df = 67, F = 5.15, $p < 0.05$). This difference was primarily due to the lower EI values for EO 25% compared to EO 25% + CHI and the Ctrl (Tukey's test, $p < 0.05$) (Table 2). Conversely, EI, CI, and BI displayed significant variations across the temporal intervals (ANOVA, EI: F = 11.9, $p < 0.001$; CI: F = 6.21, $p < 0.001$; BI: F = 10.2, $p < 0.001$). Tukey's test revealed that the highest EI values occurred 56 days after sowing ($p < 0.001$), while CI and BI values were significantly lower at this final sampling point ($p < 0.01$) (Table 2).

Plots of enrichment vs. structure indices are graphically displayed in Fig. 7 and S1. Fig. 7 assembles the four panels according to periods, while Fig. S1 focuses on seed treatments. The intersection of EI and SI primarily positioned the samples in quadrants "B" and "C," with only a few samples (e.g., baseline conditions, CHI 7 days, and EO after 14 days) falling in quadrant "D." As indicated by ANOVA, the primary driving factor appears to be the sampling period, as nearly all samples from the final interval clustered within quadrant B (Fig. 7D).

4. Discussion

Despite the considerable efforts of many international research groups and an ever-increasing amount of scientific literature on the possible applications of EOs in agriculture and agricultural products (Catani et al., 2022), few EO-based products are appearing in the market (Mohan et al., 2011). This limitation may be a consequence of regulatory barriers for commercialization (*i.e.*, cost of toxicological and environmental evaluations) or the fact that the effectiveness of EOs toward pests and diseases is not as apparent nor obvious as that observed with the use of currently available products (Mohan et al., 2011). Due to their high

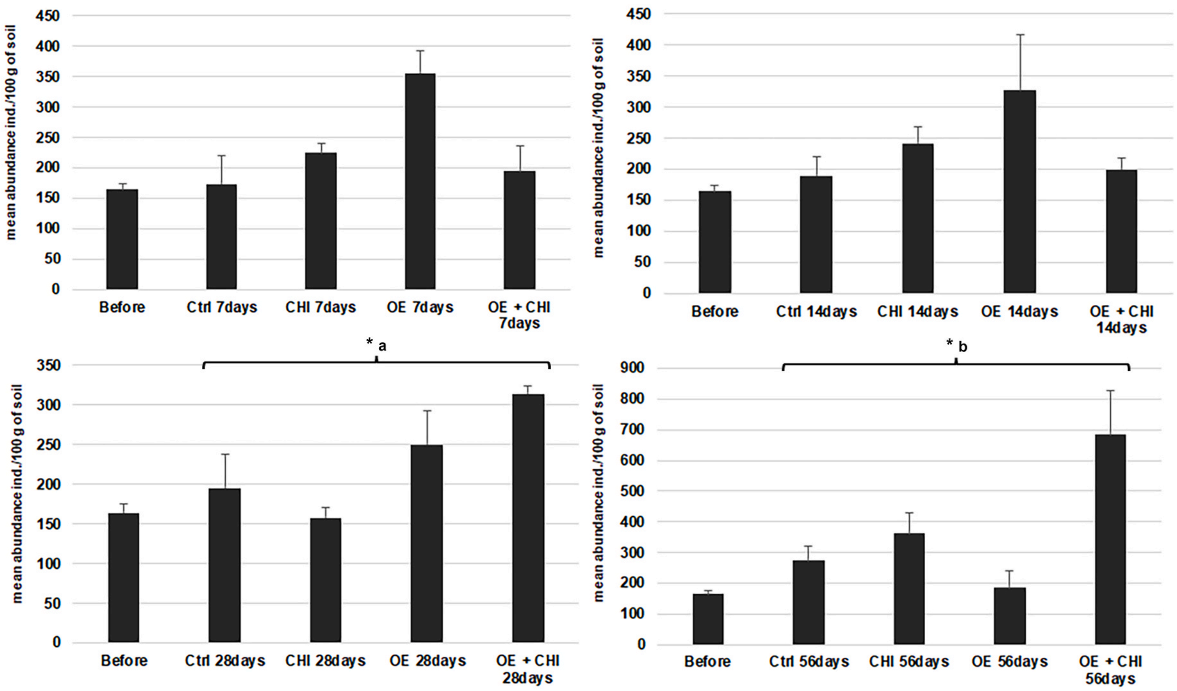

Fig. 3. Values of nematode mean abundance (±SD) from baseline condition (before) and each variant condition *i.e.,* control soil plots with untreated seeds (Ctrl), plots with seeds coated only with chitosan (CHI), plots with seeds coated only with basil essential oil (EO), soil plots with seeds coated with basil essential oil and chitosan (EO + CHI): (A) after 7, (B) 14, (C) 28, (D) 56 days after sowing. Three asterisks (*) followed by different letters represented a significance difference of $p < 0.05$ in the pairwise comparisons according to the Tukey's test.

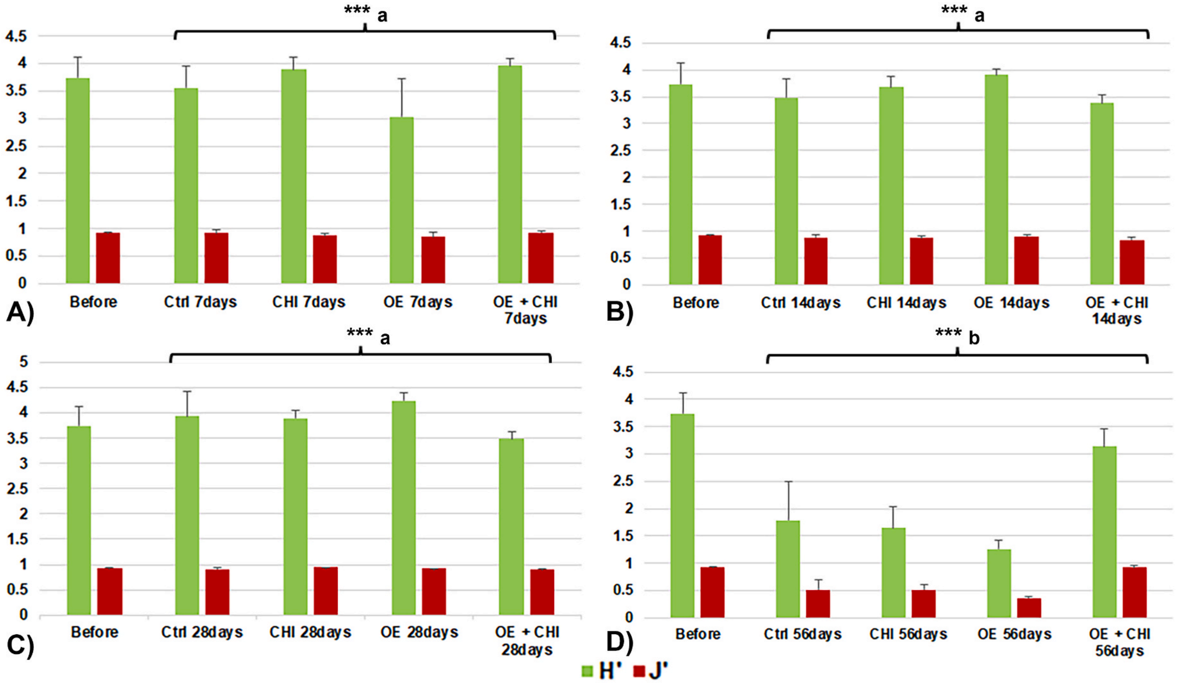

Fig. 5. k-dominance curves of nematode assemblage from each variant condition, *i.e.,* baseline condition (before), control soil plots with untreated seeds (Ctrl), plots with seeds coated only with chitosan (CHI), plots with seeds coated only with basil essential oil (EO 25%), soil plots with seeds coated with basil essential oil and chitosan (EO 25% + CHI) (A); before sowing (baseline condition) and after 7, 14, 28, 56 days after sowing period (B).

volatility, it's assumed that EOs represent a much lower risk to the environment compared to synthetic products. It is therefore expected that non-target organisms can be less affected because of the minimal residual activity, making EO-based pesticides potentially compatible with integrated pest management programs. However, the potential effects that the application of botanical-based products have on the ecosystems' health or on non-target animals are mostly unknown (Ferraz et al., 2022), which is why this survey was carried out in the field.

Among the treatments applied in this study, the combination of basil

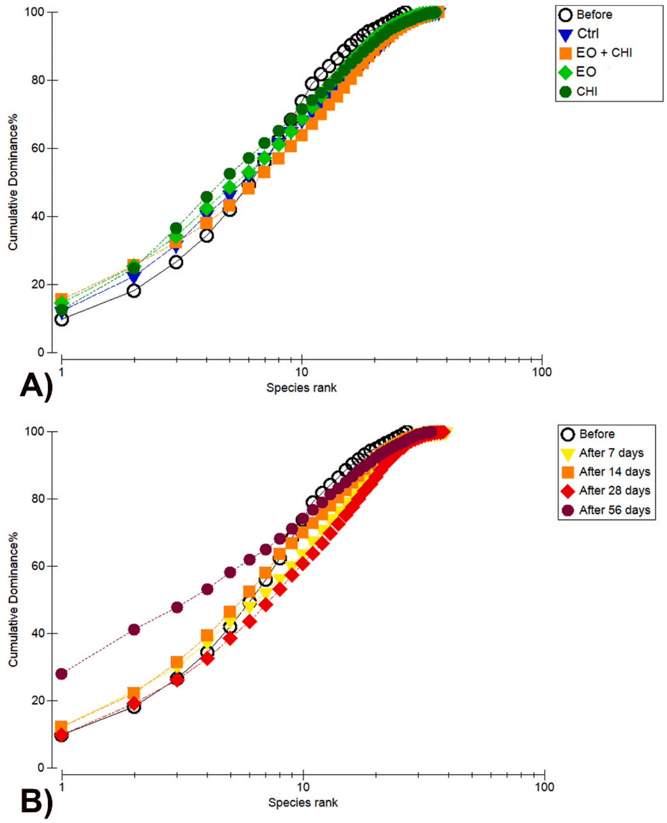

Fig. 4. Values of nematode Shannon-diversity (H′) and Pielou-evenness (J) indices (±SD) from baseline condition (before) and each variant condition *i.e.,* control soil plots with untreated seeds (Ctrl), plots with seeds coated only with chitosan (CHI), plots with seeds coated only with basil essential oil (EO), soil plots with seeds coated with basil essential oil and chitosan (EO + CHI): (A) after 7, (B) 14, (C) 28, (D) 56 days after sowing. Three asterisks (***) followed by different letters represented a significance difference of $p < 0.001$ in the pairwise comparisons according to the Tukey's test.

EO and CHI (EO 25% + CHI) was of particular interest to the authors due to its potential applicability as a seed-coating treatment for future agricultural use. In this respect, multivariate analysis showed no significant differences in the nematode community structure in soils containing seeds coated with EO 25% + CHI compared to other treatments. A slight variation in community structure was observed only with seeds treated solely with EO 25%, primarily due to an increased abundance of certain basal species (e.g., Cephalobidae, Aphelenchidae) and plant-parasitic nematodes (Tylenchidae). Nonetheless, the results of the multivariate analysis support the hypothesis that basil EO seed treatments, on their own, do not significantly alter the nematode community structure, as statistical analyses revealed that time was the primary influencing factor. Indeed, although the nematode taxonomic structure was significantly influenced by both factors, the faunal composition showed distinct changes only between sampling periods (Fig. 2). This variation is primarily attributable to successional changes associated with the development of the chickpea crop rather than the specific treatments applied (Bongers and Bongers, 1998; Ferris and Matute, 2003). Notably, the samples collected after 56 days exhibited a similar level of dissimilarity regardless of the treatment type (Fig. 2). This difference was largely due to the dominance of dauer larvae and members of the Diplogasteridae family, which is consistent with a surge in microbial activity (Bongers and Bongers, 1998; Ferris et al., 2001; Ferris and Bongers, 2009). Both Diplogasteridae and Rhabditidae nematodes are bacterial-feeding, enrichment-opportunists, characterized by short generation times (c-p 1). They produce numerous small eggs, leading to rapid populational growth (Abolafia, 2006; Steel et al., 2013). When food resources become scarce, they form dauer larvae (*i.e.,* non-feeding, resting L3-stages of c-p 1 nematode taxa), until conditions improve. The trends observed in k-dominance curves and alpha diversity further underscore the marked predominance of these few nematode components during the final sampling period (Figs. 3 and 4).

These findings highlight the importance of temporal dynamics and successional changes in shaping nematode communities, suggesting that the observed variations are driven more by shifts in nematode biological cycles than by applied treatments.

Among the functional indices, PPI values suggest a predominance of small and medium-sized ectoparasites (mainly Tylenchidae) among PPNs in the study area (Ferris and Bongers, 2009; Du Preez et al., 2022), and its overall trends did not reveal clear repulsion or attraction of PPNs in any between treatments or periods (Fig. 6). Overall, MI generally showed intermediate values across all treatments and time periods, consistent with a certain degree of soil food-web maturity (Du Preez et al., 2022), except at 56 days, where a slight successional setback was observed. This setback aligns with the findings of the multivariate analysis, which highlighted an increase in dauer larvae during the last time interval tested. Therefore, we can conclude that this aspect of the nematode assemblage also did not reveal any disturbance effect caused by the seed coating with EO and CHI.

As general observations, the BI values indicate that the system under study cannot be classified as a depleted or damaged soil food-web system, as the BI values remained below 50 (Du Preez et al., 2022). The CI, which provides an indirect measure of the dominant decomposition pathways in the soil (Ferris et al., 2001; Berkelmans et al., 2003; Du Preez et al., 2022), suggests that fungal decomposition predominated in the experimental samples. However, in the samples collected after 56 days, CI values dropped below 50, indicating a shift towards bacterial dominance. This observation aligns with the findings from the community structure analysis and MI trends.

Moreover, the enrichment *versus* structure indices diagram (EI *vs*. SI), representing the structure and enrichment conditions of the soil trophic chain, further supports these findings (Fig. 7 and S1). Most samples clustered in quadrants "B" and "C," with only a minor presence in quadrant "D." The most notable differences were related to the significant increase in EI values observed after 56 days, as previously discussed in detail.

Regarding EI, SI, BI, and CI indices, ANOVA consistently demonstrated that the time elapsed since sowing is the main discriminating factor — likely reflecting the progression of crop succession — since time accounted for the most pronounced and significant differences. The only notable differences between treatments were the statistically significant pairwise distinctions observed between EO 25% and EO 25% + CHI, as well as between EO 25% and the Ctrl. As highlighted in the multivariate analysis, these differences are minor and do not pose relevant risks to the safety of non-target organisms, as they remain minimal and unrelated to the specific effects of basil EO and CHI seed coatings.

Nonetheless, it cannot be excluded that a broader and more prolonged release of EOs into the environment and soil — even at lower concentrations than those tested in this study — could result in certain EO compounds being readily absorbed by the nematode cuticle, potentially causing sublethal effects. Therefore, further research is crucial to explore alternative application methods and formulations. Additional studies should consider different release systems and environmental conditions to ensure the safe and effective use of *O. basilicum* EO in agriculture before its widespread and routine application. Indeed, EO coatings in CHI represent only one of the many strategies available to mitigate the high volatility of EOs and enhance their application in crop protection. Depending on the target organism, EOs can be applied in various ways, including single applications, weekly irrigation, sprays, or through specialized formulations such as micro- or nano-capsules and emulsions, which can prolong their biocidal effects.

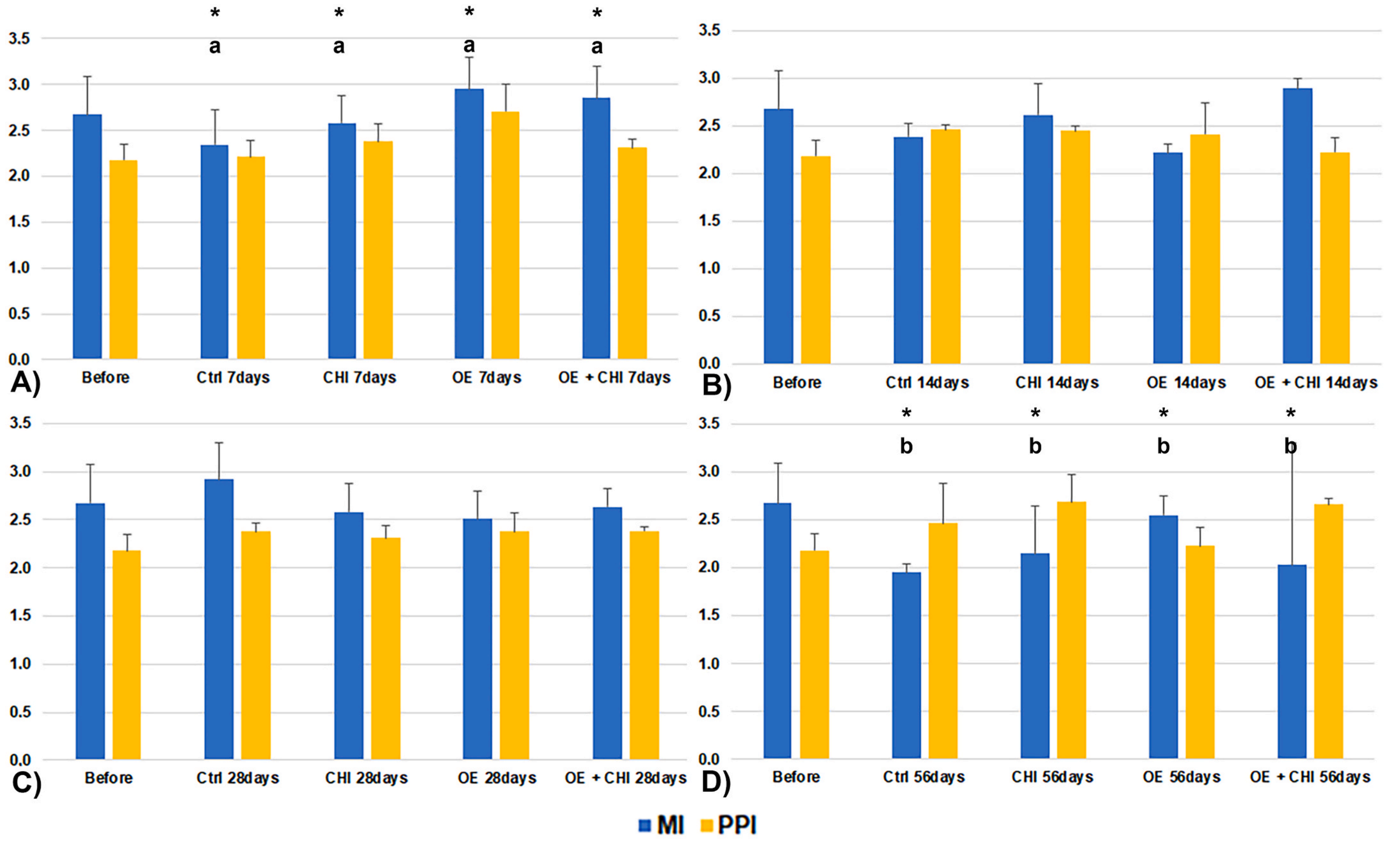

Fig. 6. Average values of Maturity index (MI) and plant parasitic index (PPI) (±SD) from baseline condition (before) and each variant condition *i.e.,* control soil plots with untreated seeds (Ctrl), plots with seeds coated only with chitosan (CHI), plots with seeds coated only with basil essential oil (EO), soil plots with seeds coated with basil essential oil and chitosan (EO + CHI): (A) after 7, (B) 14, (C) 28, (D) 56 days after sowing. One asterisk (*) followed by different letters represented the significance differences of MI equal to $p < 0.05$ in the pairwise comparisons according to the Tukey's test.

Table 2

Average values of Enrichment Index (EI), Channel Index (CI), Structure Index (SI) and Basal Index (BI) (±SD) from baseline condition (before) and each variant condition i.e., control soil plots with untreated seeds (Ctrl), plots with seeds coated only with chitosan (CHI), plots with seeds coated only with basil essential oil (EO), soil plots with seeds coated with basil essential oil and chitosan (EO + CHI) in each time interval i.e., after 7, 14, 28, 56 days after sowing. Asterisks followed by different letters represented the significance differences of the indexes (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; n.s. = no significant difference) in the pairwise comparisons according to the Tukey's test.

Index	Treatments (T)					Periods (P)				Results of ANOVA		
	Before	Ctrl	OE + CHI	OE	CHI	7 days	14 days	28 days	56 days	T	P	T x P
EI	46 ± 8	62 ± 13*a	58 ± 18*a	43 ± 11*b	55 ± 14	52 ± 14***a	45 ± 7***a	50 ± 7***a	72 ± 17***b	$p < 0.05$	$p < 0.001$	$p < 0.05$
CI	82 ± 20	54 ± 27	64 ± 35	60 ± 26	59 ± 27	65 ± 28**a	75 ± 26**a	62 ± 11**a	35 ± 31**b	n.s.	$p < 0.001$	n.s.
SI	49 ± 18	52 ± 15	66 ± 15	55 ± 16	54 ± 21	59 ± 13	46 ± 11	61 ± 9	61 ± 26	n.s.	n.s.	n.s.
BI	36 ± 12	26 ± 9	23 ± 12	32 ± 9	28 ± 10	27 ± 7**a	37 ± 6**a	27 ± 4**a	19 ± 13**b	n.s.	$p < 0.001$	n.s.

5. Conclusions

Essential oils (EOs) are widely regarded as promising green alternatives to synthetic products in crop protection due to their high volatility and biodegradability, and presumed low risk to the environment, including minimal impact on non-target organisms. However, before their routine application in the field, it is necessary to evaluate their potential effects on free-living bioindicator organisms.

This study demonstrated that a high concentration of *Ocimum basilicum* EO (25% v/v) combined with chitosan in the coating of chickpea seeds did not produce significant negative effects on nematode alpha diversity nor on functional metrics. The taxonomic community structure, often regarded as the most sensitive indicator of nematode community changes, revealed that time was the primary factor influencing the system overall, including controls. This result is more likely related to the natural progression of the crop system rather than to any direct impact from the EO seed coating.

These findings align closely with the principles of the European Green New Deal. Nevertheless, further research is essential to evaluate alternative EOs, different application methods, and various formulations. Chitosan-based EO coatings represent just one of many strategies under investigation to mitigate the high volatility of EOs. Moreover, depending on the target organism, varying application frequencies may be necessary, which could exacerbate unintended biocidal effects on free-living organisms. Consequently, these potential variations must be rigorously tested on soil bioindicator targets to ensure the preservation of biodiversity and natural capital.

CRediT authorship contribution statement

Catani Linda: Writing – original draft, Formal analysis. **Grassi Eleonora:** Writing – original draft, Methodology, Data curation. **Guidi**

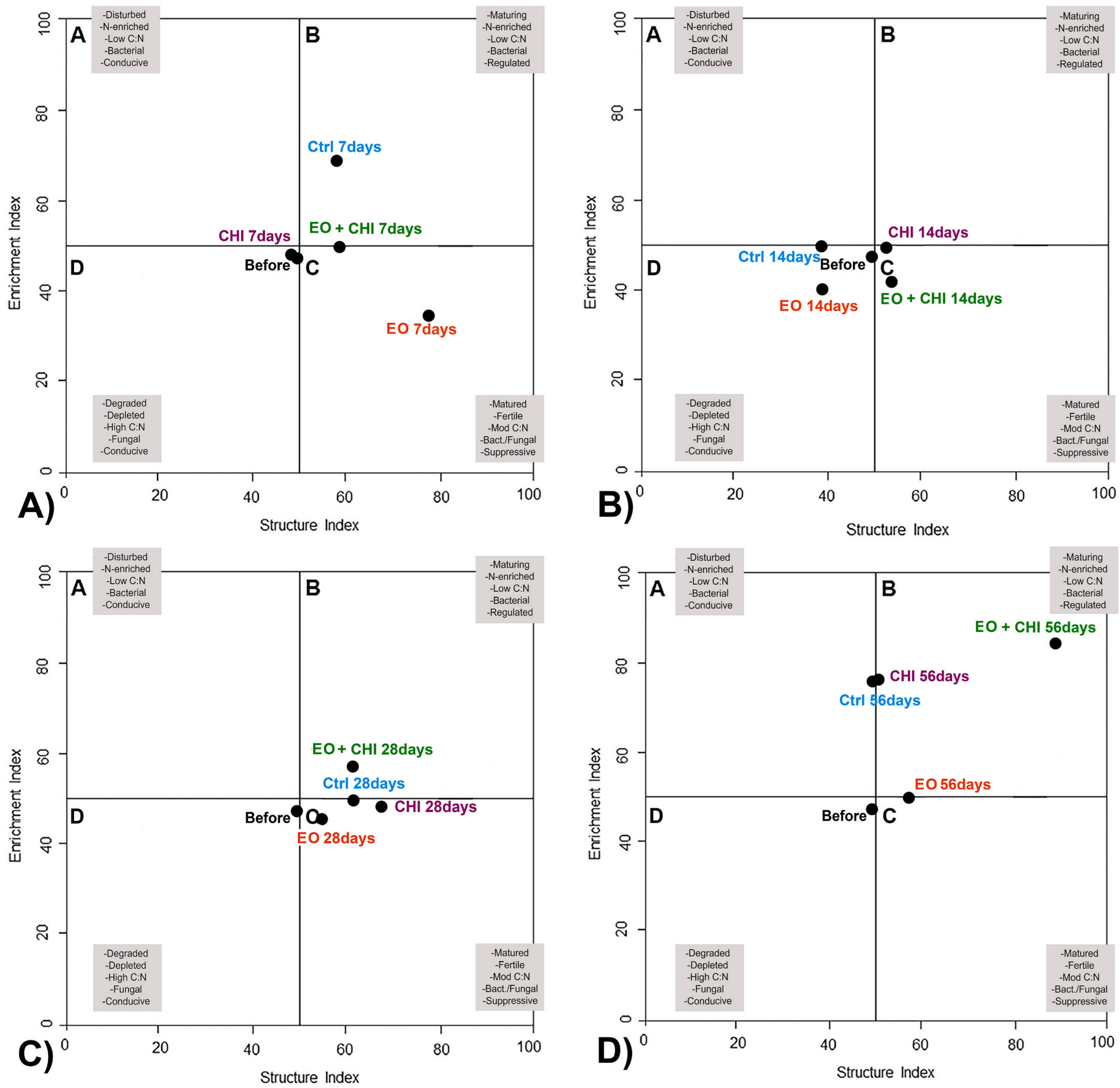

Fig. 7. Plots of enrichment *vs.* structure indices of the nematode community assembled for periods: (A) after 7, (B) 14, (C) 28, (D) 56 days after sowing. Data points represent mean values in baseline condition (before) and each variant condition *i.e.,* control soil plots with untreated seeds (Ctrl), plots with seeds coated only with chitosan (CHI), plots with seeds coated only with basil essential oil (EO), soil plots with seeds coated with basil essential oil and chitosan (EO + CHI).

Loretta: Visualization. **Farina Priscilla:** Writing – original draft, Methodology, Formal analysis. **Tani Camilla:** Methodology, Data curation. **Conti Barbara:** Writing – original draft, Validation, Methodology, Funding acquisition, Conceptualization. **Annibaldi Anna:** Visualization, Formal analysis. **Girolametti Federico:** Visualization, Formal analysis. **Ascrizzi Roberta:** Writing – original draft, Formal analysis. **Flamini Guido:** Writing – original draft, Formal analysis. **Da Costa Monteiro Luana:** Writing – original draft, Validation. **Semprucci Federica:** Writing – original draft, Validation, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.125898.

Data availability

Data will be made available on request.

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