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Efficacy of three *Tagetes* species for the suppression of lepidopteran pests in bagless apple orchards

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Background: The shift to bagless apple cultivation (i.e., fruit production without bagging) has increased the need for pest management during the summer and fall. The use of insect-repellent plants has become an effective biological control strategy in light of the increasing emphasis on reducing chemical pesticide applications and environmentally friendly integrated management strategies.

Methods: In this study, we evaluated the efficacy of three *Tagetes* species, marigold (*Tagetes erecta* L.), peacockweed (*Tagetes patula* L.), and Inca peacockweed (*Tagetes minuta* L.), intercropped in apple orchards for controlling major pests: the peach fruit moth (*Carposina sasakii* M.), fruit tree leaf roller (*Spononota lechriaspis* M.), and apple leaf miner (*Lithocolletis ringoniella* M.). The relationship between pest suppression and volatile organic compound (VOC) profiles was further examined using gas chromatography–ion mobility spectrometry combined with principal component analysis.

Results: The results indicated that all three *Tagetes* species reduced lepidopteran pest damage, with Inca peacockweed providing the most pronounced and stable control effect after four consecutive years of planting. Specifically, Inca peacockweed decreased the damage incidence to 1.8%, 1.5%, and 2.4% for peach fruit moth, apple leaf miner, and fruit tree leaf roller, respectively, within a 3 m radius, and the level of control declined with increasing distance from the *Tagetes* rows. VOC profiling revealed that ketones were the dominant compounds in marigold and peacockweed, whereas esters predominated in Inca peacockweed; VOC diversity was greater in flowers than in leaves.

Conclusion: Overall, this work provides a scientific foundation for optimizing ecological planting designs and offers new perspectives for the development of plant-based pest control strategies in sustainable apple production systems.

KEYWORDS

biological pest control, insect repellent plants, lepidopteran pests, marigold, *Tagetes*, volatile organic compounds

1 Introduction

With an area of 2 million hectares and a production of 47,571,800 tons in 2022, apples are the most widely cultivated deciduous fruit tree in China and play a vital role in rural revitalization. Apple production is currently transitioning toward bagless cultivation, and this has resulted in changes in the occurrence of orchard pests. This transition is driven primarily by rising labor costs and the scarcity of seasonal workers, as the traditional fruit-bagging practice is highly labor-intensive. In addition, bagless cultivation enhances fruit flavor and coloration by improving light exposure, which helps meet the increased demand for high-quality produce. Consequently, pest management strategies are needed to support labor-saving cultivation and the green development of the apple industry (Zhai et al., 2018; Li et al., 2021).

The peach fruit moth (*Carposina sasakii* M.), a member of the Lepidoptera fruit-boring moth group, is one of the most economically significant pests in orchards between June and September. It oviposits on apple fruit in early summer (June), and its larvae damage the fruit upon hatching (Sun et al., 2018). The fruit tree leaf roller (*Spilonota lechriaspis* M.), a member of the Lepidoptera leaf-roller group, primarily attacks the newest leaves of apple trees; damage begins in late spring (May) and peaks in mid-to-late summer (July–August) (Wang et al., 2016). The apple leaf miner (*Lithocolletis ringoniella* M.), a member of the Lepidoptera micro-moth group, causes serpentine or blotch-type mines on leaves, leading to premature defoliation and yield reduction. In addition, the orange fruit fly (*Bactrocera dorsalis* H.), one of the five most destructive fruit fly species worldwide within the family Diptera, has recently caused damage in apple orchards in northern China (Aketarawong et al., 2014; Guo et al., 2019). While this dipteran pest was observed in the experimental area, the present study focuses exclusively on the three predominant lepidopteran pests due to their consistent and significant economic impact throughout the experimental period.

Overreliance on chemical pesticides in orchards has resulted in the well-documented “3Rs” challenge: pesticide residues, pest resistance, and pest resurgence (Tang et al., 2022). Residues pose environmental and food-safety risks; resistance diminishes insecticide effectiveness; and resurgence occurs when natural enemy populations collapse following chemical applications. Contemporary orchard management emphasizes maintaining pest populations below economic thresholds through ecological regulation rather than complete eradication. Practices such as orchard mulching, augmentative release of natural enemies, and other biodiversity-enhancing measures are therefore essential for preserving ecological balance and producing high-quality fruit (Zhai et al., 2018; Li et al., 2021).

The genus *Tagetes* (Asteraceae) includes marigold (*Tagetes erecta* L.), peacockweed (*Tagetes patula* L.), *Tagetes minuta* L. (Inca peacockweed), fine-leaved marigold (*Tagetes tenuifolia* K.), sweet marigold (*Tagetes lucida* C.), and related species, most of which are annual herbs. Their stems and leaves have ornamental value and contain volatile bioactive compounds with sedative,

decongestive, antibacterial, and insecticidal properties. These compounds are widely used in cosmetics, functional foods, and medical healthcare applications (Liu et al., 2015; Mohn et al., 2017). Owing to their broad-spectrum biological activity and environmental safety, *Tagetes* species have recently attracted attention as green pest-management tools in agriculture. For example, after one year of marigold rotation, the incidence of soil-borne tobacco disease was reduced from 63.3% to 31.6% (Huang Feiyan et al., 2022) (Huang et al., 2022). In a study on the remediation of polluted farmland in mining-impacted areas of Yunnan, Liu Jinbo et al. found that marigold can serve as a suitable species for the reuse of contaminated soils (Sun et al., 2020). Guo Yanjun et al. further showed that returning crushed marigold stems, leaves, and roots to the soil effectively suppressed citrus root nematodes (Guo et al., 2023).

The present study was conducted in apple orchards located in the coastal region of Shandong Province, a major apple-producing area in northern China. Our objectives were to clarify the natural pest-control effects of *Tagetes* species, establish marigold-planted and control areas between orchard rows, systematically monitor and compare the occurrence dynamics and damage incidence of key pests, and analyze the chemical fingerprinting characteristics of marigold volatiles in relation to observed pest-control effects. This work was motivated by the limited research available on the role of *Tagetes* in pest suppression in apple orchards. Gas chromatography–ion mobility spectrometry (GC-IMS) was employed to qualitatively and quantitatively characterize volatile organic compounds (VOCs) from the main organs (flowers and leaves) of marigold intercropped between rows. Characteristic VOC profiles were constructed to identify key active compounds. Principal component analysis (PCA) and other multivariate statistical methods were then applied to (1) assess variability in VOC fingerprints across growth stages and environmental conditions; (2) analyze correlations between characteristic VOC profiles and changes in pest damage incidence across corresponding periods or regions; and (3) identify marker compounds or compound combinations closely associated with strong pest-control effects. By integrating chemical-ecology analyses with field monitoring, this study provides theoretical and empirical support for developing green pest-control technologies in apple orchards based on the allelopathic properties of *Tagetes* species. The combined evaluation of volatile chemical characteristics and pest-suppression data will help optimize planting, advance ecological control models, and improve the applicability of *Tagetes*-based strategies within integrated pest management systems for apple orchards.

2 Materials and methods

2.1 Test conditions and materials

The experiment was conducted at the Beizhai Experimental Base of the Qingdao Academy of Agricultural Sciences in Laoshan District, Qingdao City, Shandong Province, China (36°14'18.071"

N, 120°32′21.502″ E) between April and November 2020. The site is located in a hilly region characterized by sandy loam soils and well-managed commercial orchards. The apple orchard was planted with the cultivar ‘Qinfu 1’; the trees were 14 years old, arranged in five rows with four inter-row intervals, and each row was 25 m in length. Tree spacing was 4 m × 6 m. In recent years, the area has experienced many overcast and rainy days during summer, and the annual rainfall has exceeded 800 mm. Meteorological data (daily temperature, precipitation, and wind speed) for the experimental period were obtained from a weather station located within 1 km of the site.

Marigold seeds, commercially available as “Marigold Seed,” were purchased annually from Jiangsu Litian Agriculture Co., Ltd. Sowing was carried out in April of each year. Dwarf plants were selectively retained each growing season for seed production, and their seeds were sown around April 10 in the following spring. Inca peacockweed was collected from wild populations in the Laoshan mountainous area; seeds were collected and stored annually and then sown on or around April 10 in the subsequent spring. Pest species were identified morphologically by entomologists at the Qingdao Academy of Agricultural Sciences using standard taxonomic keys, and voucher specimens were deposited in the academy’s laboratory. High-resolution images showing typical pest damage symptoms and the inter-row planting pattern of marigolds are provided in the [Supplementary Material \(Supplementary Figure S6\)](#).

2.2 Experimental design and methods

The trial comprised four treatments: a blank control (CK, no *Tagetes* planting), marigold planting (T1, *Tagetes erecta*), peacockweed planting (T2, *Tagetes patula*), and Inca peacockweed planting (T3, *Tagetes minuta*). The experiment was performed in a randomized complete block design (RCBD) with three blocks (replicates). Each block contained four plots, each of which was randomly assigned to one of the four treatments (CK, T1, T2, T3). A minimum 5 m buffer zone was maintained between adjacent plots to minimize cross-treatment interference, and each treatment plot was 10 m in length.

Plant management: The three *Tagetes* species were sown on April 10. When the plants reached a uniform height of approximately 30 cm, they were mowed to a height of 10–15 cm. This practice was adopted to encourage bushier growth, increase total leaf area for volatile emission, and prevent the plants from overgrowing the inter-row space and potentially shading the apple trees. This mowing regimen was maintained throughout the growing season as needed.

2.3 Sampling and analysis

Assessments were conducted at four distances (1, 2, 3, and >3 m) from the outer edge of the *Tagetes* planting row toward the center of the adjacent apple tree row. For each treatment plot, three

representative apple trees adjacent to the planted *Tagetes* row were selected as sample trees. Samples were taken from the middle and outer canopy of these sample trees. For each distance interval (1, 2, 3, and >3 m) within a plot, 100 fruits (for fruit-boring pests) or 100 leaves/shoots (for leaf miners/rollers) were randomly sampled from the middle and outer canopy of these three sample trees. Damage incidence was calculated as (Number of damaged samples/Total number of samples) × 100%. For significance testing, Tukey’s and Dunnett’s multiple comparison tests were used. Tukey’s test was applied for pairwise comparisons among all treatment groups, and Dunnett’s test was used to compare each treatment group against the control (CK). Differences with P-values between 0.05 and 0.01 were denoted by one asterisk (*), and those between 0.01 and 0.001 were denoted by two asterisks (**).

2.4 Insect source for behavioral assays

The peach fruit moth larvae and adults used in the behavioral assays (Section 2.3) were obtained from a laboratory colony established from individuals collected in untreated orchards. The insects were reared on an artificial diet under controlled conditions (25 ± 1°C, 70 ± 5% relative humidity, 16:8 h L:D photoperiod) for multiple generations. Larvae used in the tests were of the third instar.

3 Results

3.1 Effect of different *Tagetes* species on the damage incidence of three lepidopteran pests

The damage incidence of three key lepidopteran pests was significantly reduced by intercropping with *Tagetes* species ([Figure 1](#)). The primary lepidopteran pests affecting apple productivity in the study orchard were the fruit tree leaf roller moth, the apple leaf miner, and the peach fruit moth.

After four consecutive years of planting *Tagetes* species, the damage incidence of the peach fruit moth remained below 5% in all *Tagetes* treatments. For example, in 2022, the damage incidence of the peach fruit moth in the control group (CK) was 7.7%, which was significantly higher (**P < 0.01) than in T1 (marigold, 1.0%), T2 (peacockweed, 1.4%), and T3 (Inca peacockweed, 0.2%). In 2023, the damage incidence in the Inca peacockweed treatment (T3) was 0%, which was significantly lower than that of the control group (2.8%) according to Dunnett’s test (P < 0.05).

Apple leaf miner (*Lithocolletis ringoniella*) damage decreased annually from 2020 to 2023, and the introduction of all three *Tagetes* species in 2020 markedly reduced damage incidence in the first year (P < 0.01). Over the four-year period, damage incidence in the marigold (T1) and Inca peacockweed (T3) treatments remained below 3.0%. In 2023, the damage incidence in the control group was 5.7 times higher than in T1, 3.1 times higher than in T2, 2.2 times

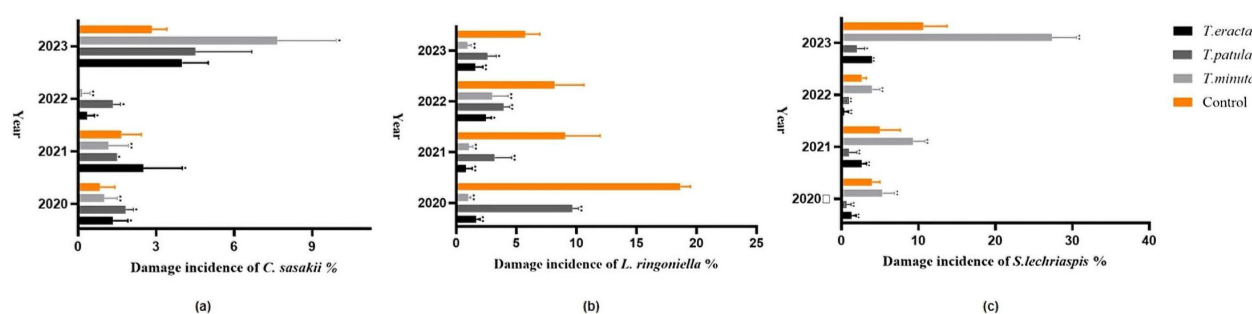


FIGURE 1

Effect of different *Tagetes* species on the damage incidence of three lepidopteran pests across four consecutive years (2020–2023). (a) Peach fruit moth (*Carposina sasakii*); (b) Apple leaf miner (*Lithocolletis ringoniella*); (c) Fruit tree leaf roller (*Spilonota lechriaspis*). Data are presented as mean $\pm$ SEM ($n = 3$). Asterisks indicate significant differences from the control (CK) group within the same year according to Dunnett's test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

higher than in T3, and 6.3 times higher than the four-year average across all *Tagetes* treatments.

Damage caused by the fruit tree leaf roller moth showed marked interannual variability. In 2022, the damage incidence in the control group (CK) was 27.3%, whereas all three *Tagetes* treatments had damage incidences below 10.0%. All treatment groups differed significantly from the control, with Inca peacockweed (T3) showing the lowest damage incidence at 4.0%. Overall, while all three *Tagetes* species reduced lepidopteran pest damage, Inca peacockweed exhibited the most consistent and pronounced combined inhibitory effect over the four-year planting period.

3.2 Radius of protection against three lepidopteran pests by different *Tagetes* species

The “radius of protection” was defined as the maximum distance from the outer edge of the *Tagetes* planting row at which pest damage incidence remained significantly lower ($P < 0.05$, Dunnett's test) than in the control plot. All measurements were taken within the designated treatment plots, and the “>3 m” category represents the farthest sampling point within a plot toward the apple tree row; it does not extend into the buffer zone between different treatments.

The pest suppression effect of *Tagetes* plants was distance-dependent, with the strongest effects observed closest to the planting rows (Figure 2, Table 1). There were clear differences among the three *Tagetes* species in their ability to reduce peach fruit moth damage compared with the blank control; all three provided protection against peach fruit moth at distances greater than 3 m. The lowest damage incidence (0.0%) was observed between 1–2 m and 2–3 m from the planting rows. Within the 2–3 m range, Inca peacockweed showed the strongest inhibition, with a fruit damage incidence of only 0.2%, much lower than that observed in marigold (2.0%) and peacockweed (1.0%) (Table 1). Under Inca peacockweed treatment, the peach fruit moth damage incidence at >3 m was 0.6%, which was substantially lower than that in the marigold treatment (2.5%). The protective radius against the fruit tree leaf

roller moth varied among the three *Tagetes* species and was approximately 1 m for marigold (*T. erecta*), 3 m for peacockweed (*T. patula*), and >3 m for Inca peacockweed (*T. minuta*). Consistent with this distance-dependent pattern, damage incidence was 0% within the 0–1 m range for all treatments and gradually increased with distance from the *Tagetes* rows. Inca peacockweed again demonstrated the strongest inhibitory effect, resulting in significantly lower damage incidence than marigold beyond 3 m and lower damage than peacockweed at 1–2 m. For apple leaf miner, the protection radius was 2 m for marigold and peacockweed and 3 m for Inca peacockweed, with all three *Tagetes* species showing some level of suppression. However, Inca peacockweed achieved the greatest reduction in apple leaf miner damage compared with the other two species.

3.3 Behavioral responses of peach fruit moth larvae and adults to marigold and Inca peacockweed

The peach fruit moth larvae showed a 14.4% selection rate for Inca peacockweed, corresponding to a control effect of 85.6%, and a 23.3% selection rate for marigold, with a control effect of 76.7% (Figure 3). Adult peach fruit moths exhibited a 58.9% selection rate for marigold, with a control effect of 41.1%, and a 54.4% selection rate for Inca peacockweed, with a control effect of 45.6%. Considering both larval and adult responses, the overall control effect of Inca peacockweed on peach fruit moth was stronger than that of marigold, and the control effect of Inca peacockweed on peach fruit moth larvae was particularly significant.

3.4 Comparative analysis of VOC profiles in various marigold species

3.4.1 GC-IMS component analysis

The GC-IMS spectra revealed distinct volatile profiles between species and tissues (Supplementary Figures S1, S2; Supplementary Material). Using GC-IMS, we identified VOCs in the flowers and

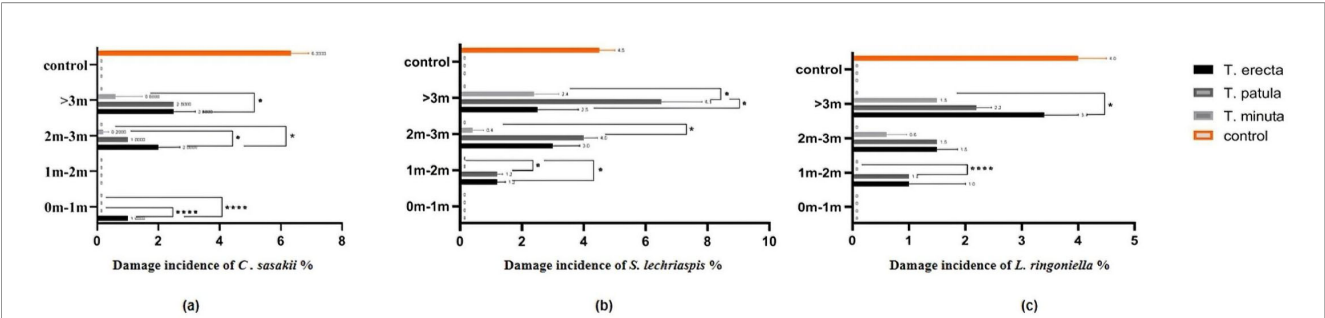


FIGURE 2 Distance-dependent effect of Tagetes species on the damage incidence of three lepidopteran pests (data from 2022 and 2023). (a) Peach fruit moth; (b) Apple leaf miner (*Lithocolletis ringoniella*); (c) Fruit tree leaf roller moth. Data are presented as mean $\pm$ SEM (n = 3). Asterisks indicate significant differences from the control (CK) group within the same year according to Dunnett's test: * P < 0.05, ** P < 0.01, *** P < 0.001.

leaves of marigold and Inca peacockweed. Flavor Spec[®] software was used to generate three-dimensional plots (Supplementary Figure S1) and top-view plots (Supplementary Figure S2), where the X-axis represents ion drift time (DT), the Y-axis represents gas chromatographic retention time (RT), and the Z-axis represents peak area. Each peak (or point) corresponds to an individual compound, and the color intensity reflects its relative concentration.

The volatile fractions of the two *Tagetes* species differed markedly, with Inca peacockweed exhibiting a broader range of VOCs than marigold (Supplementary Figures S1a, b). Within each species, substantial differences were also observed between tissues, with flowers containing noticeably more VOCs than leaves. For

compounds with similar drift times (DT) and retention times (RT), the peak areas likewise differed significantly between the two species.

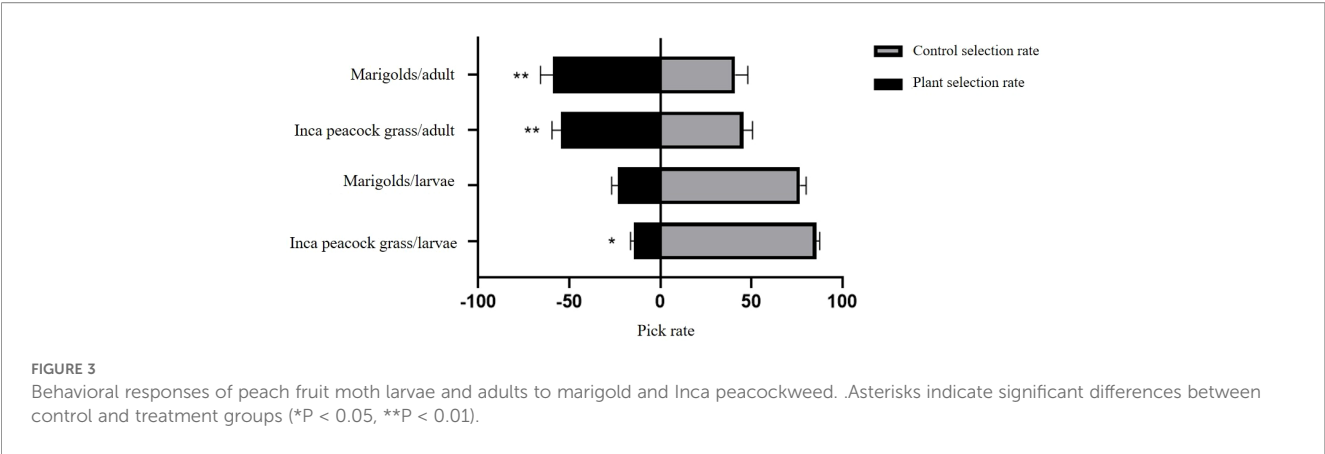
3.4.2 Qualitative and quantitative analysis of VOCs

Using the GC×IMS Library Search, 109 VOCs were detected (Figure 4). Among these, 46 were qualitatively identified based on their retention indices in the NIST database, their ion migration times in IMS, and the exclusion of dimer signals. These identified compounds included 10 alcohols, 8 esters, 8 ketones, 7 aldehydes, 3 terpenes, 3 acids, 3 furans, 3 thioethers, and 1 alkane. Overall, alcohols, esters, ketones, and aldehydes were the dominant VOCs in

TABLE 1 Radius of protection of three *Tagetes* species against different lepidopteran pests.

	Damage incidence ratio								
	<i>C.sasakii</i>			<i>L.ringoniella</i>			<i>S.lechriaspis</i>		
	Significant	Summary	P value	Significant	Summary	P vale	Significant	Summary	P vale
<i>T. erecta</i>									
control vs.0m-1m	Yes	**	0.0086	Yes	**	0.0091	Yes	*	0.0114
control vs.1m-2m	Yes	**	0.0061	Yes	*	0.0343	No	ns	0.1278
control vs.2m-3m	Yes	*	0.0203	No	ns	0.3826	No	ns	0.0803
control vs.>3m	Yes	**	0.0062	No	ns	0.3938	No	ns	0.7015
<i>T. patula</i>									
control vs.0m-1m	Yes	**	0.0061	Yes	**	0.0091	Yes	*	0.0114
control vs.1m-2m	Yes	**	0.0061	Yes	*	0.0285	Yes	*	0.0202
control vs.2m-3m	Yes	**	0.0086	No	ns	0.7555	Yes	*	0.0288
control vs.>3m	Yes	*	0.0165	No	ns	0.3938	No	ns	0.1076
<i>T. minuta</i>									
control vs.0m-1m	Yes	**	0.0061	Yes	**	0.0091	Yes	*	0.0114
control vs.1m-2m	Yes	**	0.0061	Yes	**	0.0091	Yes	*	0.0114
control vs.2m-3m	Yes	**	0.0052	Yes	**	0.0013	Yes	*	0.0449
control vs.>3m	Yes	**	0.0021	No	ns	0.1502	Yes	*	0.0288

P-values from Dunnett's test comparing each treatment-distance combination to the control are shown. *P < 0.05, **P < 0.01, ns, not significant.



the two marigold species. Monoterpenes, mainly limonene, β -rhodiolene, and α -pinene, were the predominant terpenoids and constituted key volatile constituents in most of the plants.

The analysis revealed a set of core VOCs that were consistently present across all four sample types (Table 2). Key among these ubiquitous compounds were E,E-2,4-octadienal, acetone, and butyl

sulfide, each of which exceeded 1% in multiple samples, suggesting their potential role as common chemical signatures of these *Tagetes* species. Inca peacockweed flowers and leaves contained a broader range of volatile substances than those of marigold. Inca peacockweed-specific volatiles included Z-3-hexenyl acetate, amyl acetate, isoamyl acetate, 2,3-butanediol, and propyl 2-

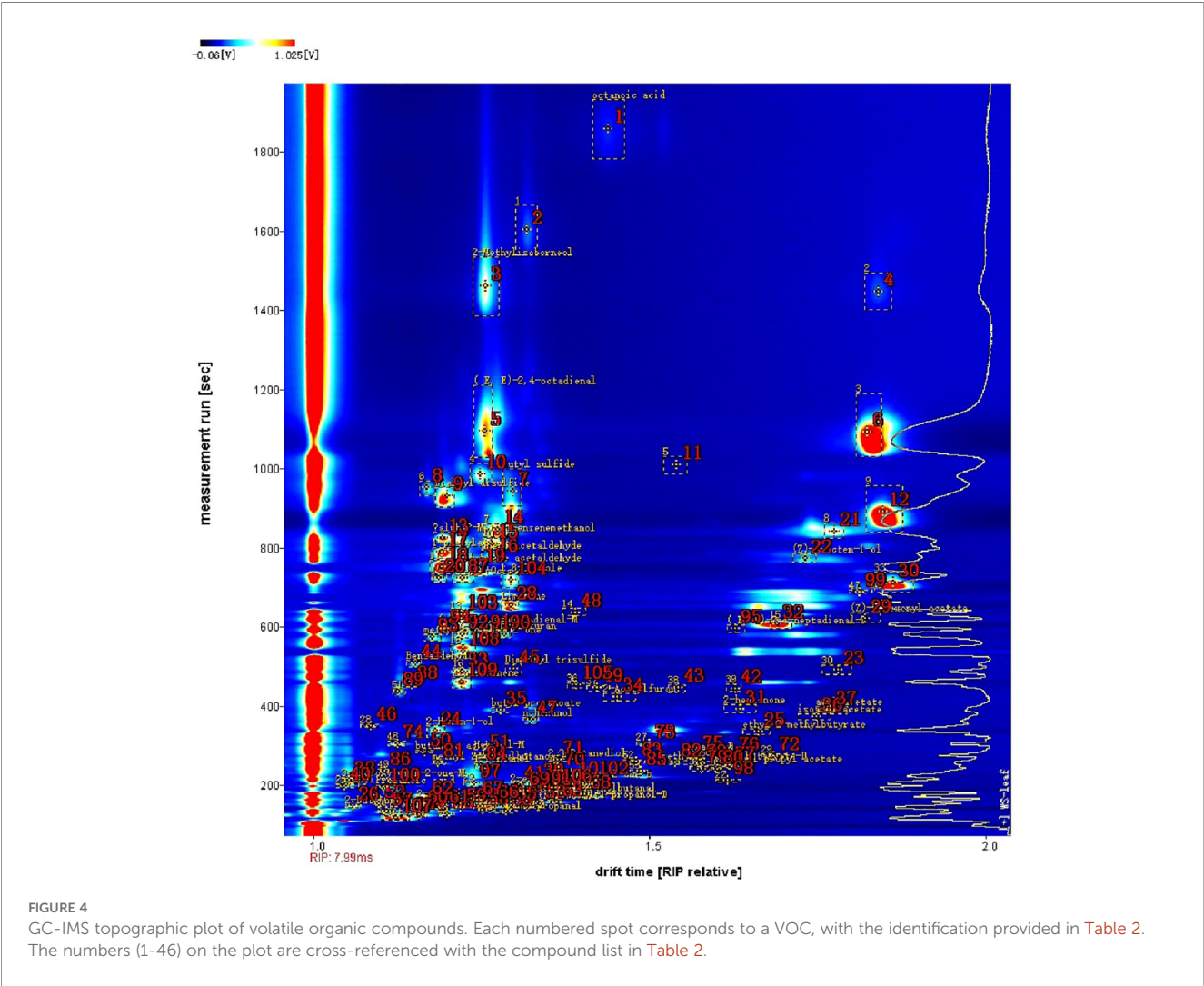


TABLE 2 Analysis of VOCs in flower and leaf tissues of marigold and Inca peacockweed.

No.	Chemical compound	CAS#	Molecular formula	RI	Relative content (%) in flowers-WS	Relative content (%) in leaves-WS	Relative content (%) in flowers-QC	Relative content (%) in leaves-QC
1	octanoic acid	C124072	C8H16O2	1206.2	5.3884%	1.7621%	1.0229%	0.4537%
2	2-Methylisoborneol	C2371428	C11H20O	1163.3	1.9404%	5.3965%	0.5451%	1.2203%
3	(E, E)-2,4-octadienal	C30361285	C8H12O	1111.9	9.9023%	6.8611%	6.7784%	8.4370%
4	Butyl sulfide	C544401	C8H18S	1085.7	1.8022%	1.2069%	2.1357%	3.3850%
5	Diallyl disulfide	C2179579	C6H10S2	1083.2	0.9365%	2.8629%	0.2144%	0.1847%
6	α -Methylbenzenemethanol	C98851	C8H10O	1061.8	0.7064%	0.9030%	0.1254%	0.0475%
7	phenylacetaldehyde	C122781	C8H8O	1050.6	0.4789%	1.1372%	1.9823%	3.0227%
8	1-phenylethanol	C98851	C8H10O	1053.6	0.9994%	2.0493%	0.2203%	0.1218%
9	(Z)-3-octen-1-ol	C20125842	C8H16O	1050.3	0.0493%	0.4347%	0.1082%	0.0321%
10	2-Hexen-1-ol	C2305217	C6H12O	856.4	0.7893%	0.8611%	0.2082%	0.3691%
11	ethyl 2-methylbutyrate	C7452791	C7H14O2	853.8	0.1311%	0.0512%	2.0164%	0.1973%
12	2-Butanone	C78933	C4H8O	605.7	0.1590%	0.1394%	0.0288%	0.1233%
13	Limonene	C138863	C10H16	1020.8	1.8506%	1.1432%	0.7599%	1.0080%
14	(Z)-3-Hexenyl acetate	C3681718	C8H14O2	1011.2	0.0791%	0.0890%	1.2625%	0.8010%
15	2-heptanone	C110430	C7H14O	898.5	2.1155%	0.1485%	0.1883%	0.4450%
16	2-Acetylfuran	C1192627	C6H6O2	918.2	0.0903%	0.0401%	0.1244%	1.0796%
17	butyl propanoate	C590012	C7H14O2	896.9	0.0580%	0.1360%	0.1830%	0.0451%
18	isoamyl acetate	C123922	C7H14O2	884.5	0.4788%	0.0505%	2.6133%	1.7312%
19	amyl acetate	C628637	C7H14O2	896.1	0.2786%	0.0573%	2.0164%	0.4302%
20	3-hydroxybutan-2-one	C513860	C4H8O2	728.6	2.5660%	2.0018%	0.3628%	0.1471%
21	2-ethyl furan	C3208160	C6H8O	707.5	0.9241%	0.1754%	0.2250%	0.9378%
22	Benzaldehyde	C100527	C7H6O	965.2	0.2011%	0.1770%	0.1459%	0.1794%
23	Dimethyl trisulfide	C3658808	C2H6S3	958.4	0.1259%	0.0792%	0.3867%	0.1901%
24	n-Hexanol	C111273	C6H14O	878.4	0.0597%	0.1695%	0.1005%	0.0625%
25	butanoic acid	C107926	C4H8O2	808	0.0188%	0.0121%	0.0886%	0.0236%
26	Hexanal	C66251	C6H12O	801.7	1.9564%	0.1395%	0.7329%	1.3575%
27	Ethyl Acetate	C141786	C4H8O2	618.8	0.1779%	0.0887%	0.0398%	0.0562%
28	2-methylpropanal	C78842	C4H8O	568.8	0.4026%	0.2657%	0.4366%	0.9302%
29	1-propanol	C71238	C3H8O	572.9	0.1790%	0.0628%	0.1279%	0.2189%
30	2,3-butanedione	C431038	C4H6O2	588.6	0.3847%	0.5174%	0.1526%	0.1074%
31	2-methyl-1-propanol	C78831	C4H10O	640.5	0.5708%	0.4757%	0.1268%	0.4247%
32	3-methylbutanal	C590863	C5H10O	672.6	1.1289%	0.9045%	0.9198%	1.6317%
33	2,3-Butanediol	C513859	C4H10O2	787.6	0.0435%	0.0390%	0.4501%	0.0780%
34	2-Methyl propyl acetate	C110190	C6H12O2	773.3	0.0726%	0.0174%	0.9383%	0.1908%
35	methyl 2-methylbutanoate	C868575	C6H12O2	779.3	0.5580%	0.1037%	0.8602%	0.4015%
36	1-Pentanol	C71410	C5H12O	771.6	0.2396%	0.1303%	0.0589%	0.0997%

(Continued)

TABLE 2 Continued

No.	Chemical compound	CAS#	Molecular formula	RI	Relative content (%) in flowers-WS	Relative content (%) in leaves-WS	Relative content (%) in flowers-QC	Relative content (%) in leaves-QC
37	β -Ocimene	C13877913	C10H16	1038.1	0.2051%	0.6143%	0.1411%	0.0803%
38	2-pentyl furan	C3777693	C9H14O	999.3	0.5082%	0.2481%	0.2909%	0.1372%
39	methyl-5-hepten-2-one	C110930	C8H14O	997.2	0.7956%	0.3256%	0.1658%	0.3151%
40	(E, E)-2,4-heptadienal	C4313035	C7H10O	1003.5	0.0677%	0.0737%	0.0366%	0.3981%
41	3-Pentanone	C96220	C5H10O	692.8	0.2910%	0.1312%	0.0660%	0.3731%
42	Propanoic acid	C79094	C3H6O2	706	0.1127%	0.1487%	0.0419%	0.0443%
43	1.8-Cineole	C470826	C10H18O	1036.9	1.8739%	1.1447%	0.4896%	0.9139%
44	2-Pentanone	C107879	C5H10O	702.8	1.4137%	1.2732%	0.4068%	0.8576%
45	acetone	C67641	C3H6O	524.4	3.2107%	2.4398%	1.8782%	2.2241%
46	α -Pinene	C80568	C10H16	940.2	0.9212%	1.2896%	0.4484%	0.2900%

(WS refers to marigold; QC refers to Inca peacockweed).

“WS, Marigold (*Tagetes erecta*); QC, Inca peacockweed (*Tagetes minuta*).”

methylacetate, whereas marigold-specific components included 2,3-butanedione, diallyl disulfide, 1-phenylethanol, α -methylbenzyl alcohol, and several other minor constituents.

The most abundant compound across all four sample types was E,E-2,4-octadienal, accounting for 6.86%, 9.90%, 8.44%, and 6.78% in marigold flowers, marigold leaves, Inca peacockweed flowers, and Inca peacockweed leaves, respectively. This was followed by acetone (2.44%, 3.21%, 2.22%, and 1.88%) and dibutyl sulfide (1.21%, 1.80%, 3.39%, and 2.14%), which may represent characteristic constituents of *Tagetes* species. To identify the most abundant volatiles, which are often responsible for the dominant olfactory profile and key drivers of biological activity (Zhu et al., 2021), we applied a threshold of $\geq 1\%$ relative content, albeit minor compounds may also contribute synergistically to repellent effects (López et al., 2011). Among compounds exceeding this threshold, several showed pronounced interspecific differences: the relative content of 2-methylisoborneol was 6.0-fold higher in marigold flowers than in Inca peacockweed flowers, and limonene was 3.0-fold higher. Conversely, phenylacetaldehyde and butyl sulfide were 4.4-fold and 1.8-fold higher, respectively, in Inca peacockweed flowers than in marigold flowers. In addition, several constituents were both $\geq 1\%$ and specific to marigold, including diallyl disulfide (2.86% in leaves), 1-phenylethanol (2.05% in leaves), α -pinene (1.29% in leaves), 3-hydroxy-2-butanone (2.00% in leaves, 2.57% in flowers), 2-pentanone (1.27% in leaves, 1.41% in flowers), 1,8-eucalyptol (1.14% in leaves, 1.87% in flowers), 2-heptanone (2.12% in flowers), and 2-butanone (1.09% in flowers). In Inca peacockweed leaves, distinct constituents with $\geq 1\%$ relative content included isoamyl acetate (1.73% in leaves, 2.61% in flowers), 2-acetylfuran (1.08% in leaves), ethyl acetate (1.53% in flowers), ethyl 2-methylbutanoate (2.02% in flowers), pentyl acetate (2.02% in flowers), and Z-3-hexenyl acetate (1.26% in flowers). Overall, ketones predominated among marigold-specific constituents, whereas esters were more characteristic of Inca peacockweed,

with flowers generally exhibiting greater diversity and expression of VOCs than leaves.

3.4.3 PCA of VOCs

The characteristic differences among samples are illustrated in Figure 5. Marigold flowers and leaves were closely clustered but clearly separated from Inca peacockweed flowers and leaves. All four sample types were well discriminated in the PCA plot, and replicates clustered tightly, indicating substantial differences between the two plant species and similarly pronounced differences between flowers and leaves of Inca peacockweed, consistent with the fingerprinting results. The first and second principal components explained 51% and 31% of the total variance, respectively, demonstrating that these components captured most of the information contained in the volatile profiles of the four samples.

4 Discussion

One of the key approaches for implementing green control, an eco-friendly pest management strategy, against agricultural pests and diseases involves expanding the cultivation of candidate plant species to determine their potential biological control functions. China is rich in plant resources, and many species have demonstrated potential for use in biological control (Zhang and Chen, 2014). In recent years, research on biocontrol technologies in apple orchard management has increased. Laffon et al. (2022) reported the biocontrol effects of two aromatic plants, French marigold and basil (*Ocimum basilicum*), on codling moth in apples (Laffon et al., 2022). Owing to their unpleasant odor, which is repellent to many herbivores (López et al., 2011), marigolds are naturally resistant to a range of pests and diseases, particularly mites and root-knot nematodes (Sadia et al., 2013; Massuh et al., 2017). Inca peacockweed produces a variety of

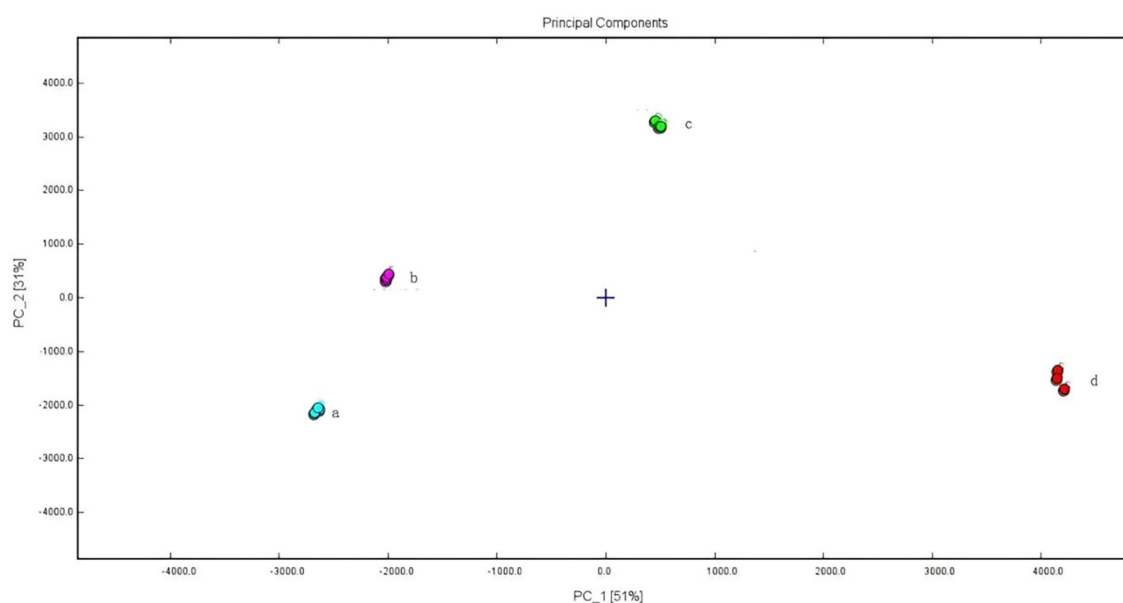


FIGURE 5

PCA of VOCs from marigold leaves (a), marigold flowers (b), Inca peacockweed leaves (c), and Inca peacockweed flowers (d).

bioactive compounds with insecticidal and repellent properties and has been used in countries such as South Africa and India (Wanjala and Wanzala, 2016). Most research on Inca peacockweed in China has focused on its ecological impacts; for example, Zhang Ruihai et al. (2019) investigated its distribution, risk status, and biological characteristics and classified it as an invasive species (Zhang et al., 2019). Yun Lingling et al. demonstrated that Inca peacockweed increased soil bacterial diversity, whereas Inca peacockweed combined with marigold reduced bacterial richness (Yun et al., 2020).

The superior pest suppression offered by *T. minuta* must be balanced against its classification as an invasive species in China (Zhang et al., 2019). To mitigate the risk of unintended spread from orchard environments, we recommend implementing strict containment protocols. These include: (1) mowing plants before seed set to prevent propagation via seeds, (2) establishing physical root barriers at the plot edges to restrict vegetative spread, and (3) conducting regular monitoring in adjacent areas for volunteer plants. We advocate that the cultivation of *T. minuta* should be integrated with local invasive species management guidelines and that future breeding efforts focus on developing sterile cultivars that retain the strong VOC-mediated pest repellence without the associated invasion risk.

While previous studies in apple orchards have primarily focused on the release of natural enemies (Zhang et al., 2021) or the application of microbial agents (Zhu et al., 2021), our approach leverages plant diversity to enhance ecosystem resilience. The “push” strategy provided by *Tagetes* species offers a continuous, preventive measure compared with the periodic application of biocontrol agents. However, its efficacy may be affected by abiotic factors such as wind and rain, which could help explain the limited impact on *Bactrocera dorsalis*, whose peak activity coincides with the windy autumn months.

The row spacing of most orchards is 5–6 m. Our results demonstrate that *Tagetes* species exert species-specific control effects on different pests within this distance. Inca peacockweed is particularly effective at controlling lepidopteran pests such as peach fruit moth, fruit tree leaf roller moth, and apple leaf miner within 3 m in apple orchards. Although marigold can partially suppress lepidopteran pests, Inca peacockweed exhibited the most consistent and broad inhibitory effect after several years of planting. Its control efficacy against both peach fruit moth larvae and adults was superior to that of marigold, and the reduction in larval infestation was especially pronounced. These findings provide a valuable reference for reducing chemical pesticide inputs and advancing biocontrol technologies. Overall, inter-row planting of *Tagetes* species, particularly Inca peacockweed, significantly reduced damage by key lepidopteran pests.

GC-IMS analysis further showed that Inca peacockweed has a distinct volatile profile enriched in esters, compounds frequently reported as insect repellents and antifeedants (López et al., 2011). For example, isoamyl acetate and Z-3-hexenyl acetate, which were abundant in Inca peacockweed flowers (Table 2), have been shown in other systems to disrupt host-finding and oviposition behaviors in lepidopteran pests by masking host plant volatiles or acting as direct repellents. This mechanism likely underlies the marked reduction in peach fruit moth and leaf roller damage observed near *T. minuta* plantings. Furthermore, the presence of β -ocimene, a well-known herbivore-induced plant volatile that can attract natural enemies such as lacewings and parasitic wasps, suggests that indirect biological control via enhanced natural enemy activity may also contribute to the pest suppression observed in this study.

Marigolds use VOCs, which are typically associated with plant biomass, to repel pests (Senato et al., 2004). In this study, ketones were identified as the primary specific constituents of marigold,

whereas esters were predominant in Inca peacockweed. GC-IMS profiling showed that both the diversity and concentration of VOCs were higher in flowers than in leaves for both species, and that the differences between marigold and Inca peacockweed were more pronounced in floral tissues. This may reflect the presence of flower-specific components with stronger insect-repellent activity. These findings have implications for the development of plant-derived pesticides and for the targeted extraction of key repellent compounds. In the verification experiments, we focused primarily on the major VOCs in leaves because, under orchard conditions, the flowering period occurs later than the critical window for controlling most lepidopteran pests.

Overall, *Tagetes* species are rich in volatile alcohols, esters, ketones, and aldehydes, with important ecological and biological implications. Alcohols such as linalool, which can deter aphids, are often involved in plant defense and can either attract natural enemies or repel herbivores (Cantó-Tejero et al., 2022). Esters, largely derived from alcohols and acids, function as “distress signals” released upon herbivore damage; they can attract natural enemies of pests and thereby help reduce pest populations (Yang et al., 2023). Marigold’s distinctive active components show strong photoactivated toxicity, damaging insect cell membranes and mitochondria and markedly suppressing both aboveground pests and soil-dwelling nematodes. Low-molecular-weight aldehydes, which are highly repellent (e.g., to fruit flies and mites) and readily diffusible, can form an “odor barrier” in orchards (Germinara et al., 2024). Because alcohols, esters, and aldehydes (such as apple esters) occur naturally in fruits and vegetables, they are generally safe for non-target organisms (including natural enemies and pollinators such as bees) and are compatible with the principles and regulatory requirements of green pesticides (Wang et al., 2024).

The unique VOCs released by marigold reflect its distinct chemical–ecological functions and the fundamental mechanisms underlying pest control in apple orchards. Diallyl disulfide interferes with the olfactory orientation of insects (e.g., codling moth oviposition), while 2,3-butanedione disrupts the insect respiratory system; together, these actions confer antibacterial effects and reduce the risk of disease–vector pest transmission. Phenylethanol acts in a dose-dependent manner, attracting parasitic wasps (natural enemies) at low concentrations but repelling herbivorous pests at higher concentrations. α -Methylbenzyl alcohol enhances the control of waxy scale pests, such as scale insects, and increases the penetration of other active ingredients, including ketones. When plants are damaged, they release β -ocimene, which attracts predatory natural enemies such as lacewings and lady beetles to aggregate and feed. These specialized compounds highlight the superiority of inter-row marigold planting for pest management in apple orchards and provide precise targets for the development of green prevention and control technologies for “plant-based defense-driven natural enemy conservation.” Principal component analysis (PCA) based on GC-IMS data further showed that the composition of VOCs in the marigold inter-row planting area differed significantly from that in the control area, with PC1 and PC2 contributing 51% and 31% of the variance, respectively, indicating that marigold-derived volatiles create a distinct chemical microenvironment within the orchard.

The economic viability of this approach is a crucial consideration for its adoption. The costs associated with *Tagetes* seed purchase, establishment, and mowing management must be weighed against the potential savings from reduced pesticide applications. Although a formal economic analysis was beyond the scope of this study, our data suggest that the substantial reduction in pest damage could translate into higher marketable yield and improved fruit quality, potentially enabling access to premium markets. Our analysis focused on VOCs with a relative content exceeding 1%, a reasonable threshold for identifying the most abundant candidates underlying the observed repellent effects. While this approach successfully highlighted key differences, such as the ester-rich profile of *T. minuta*, future work involving dose–response bioassays with pure compounds is essential for confirming the efficacy of these major constituents and for exploring potential synergistic effects with minor components. Long-term cost–benefit analyses will also be necessary to provide growers with clear economic guidelines.

In addition to economic feasibility, the practical integration of *Tagetes* species must be evaluated in the context of different orchard production systems. A key factor for broad adoption is compatibility with mechanized operations. In highly mechanized orchards where wide-track tractors are used for mowing, spraying, and harvesting, companion plants in the inter-row may be damaged by traffic or interfere with machinery. To mitigate these conflicts, planting layouts may need to be adapted, such as by confining *Tagetes* to dedicated, non-traffic strips directly beneath the tree canopy or along the orchard perimeter, thereby preserving the driving alleys for equipment. Future research should therefore focus on optimizing planting configurations and management practices that maximize the pest-suppression benefits of companion plants while maintaining the operational efficiency of commercial, mechanized orchards.

This study has several limitations that indicate directions for future work. First, as a single-site study, the efficacy of *Tagetes* intercropping needs to be validated across diverse geographical regions and apple production systems. Second, the specific mechanistic roles of the key VOCs identified here should be confirmed through laboratory olfactometer assays and field applications of synthetic volatiles.

According to our findings, Inca peacockweed has a pronounced effect on controlling common orchard pests, highlighting its value as a biological control resource and its potential to contribute substantially to the development of green agriculture. Qualitative analysis of marigold VOCs further revealed unique bioactive molecular structures that may exert inhibitory effects on additional pest species. Based on these results, we propose an optimized strategy of “double-row dense planting” (row spacing ≤ 3 m) using highly volatile Inca peacockweed varieties as a technical paradigm for ecological pest prevention and control in orchards. Moreover, the extraction of marigold tissues enriched in key insect-repellent substances to formulate low-toxicity or innocuous sprays provides both new material and new concepts for orchard biological control and for the development of plant-derived pesticides, opening promising avenues for future research on their biocontrol potential.

5 Conclusion

In conclusion, our findings show that intercropping with *Tagetes* species, especially Inca peacockweed, is an effective strategy for suppressing major lepidopteran pests in bagless apple orchards. The superior performance of *T. minuta* is closely associated with its distinctive VOC profile, which is rich in floral esters that exert strong repellent and behavior-disrupting effects on pests such as the peach fruit moth. We identified specific bioactive compounds (e.g., isoamyl acetate, Z-3-hexenyl acetate) and outlined plausible mechanisms by which they interfere with host-finding and oviposition, thereby providing a chemical–ecological basis for the observed pest suppression. Our results provide a practical, eco-friendly management strategy in which double-row dense planting of *T. minuta* within a ≤ 3 m range can function as an effective pest barrier in orchards.

However, several limitations should be acknowledged. The study was conducted at a single site, and the robustness of the observed effects under different climatic conditions, soil types, and orchard systems requires evaluation. Future work should isolate and test key VOCs in controlled behavioral assays, assess the economic feasibility of large-scale implementation, and examine the long-term impacts of *Tagetes* intercropping on soil health and overall orchard biodiversity. Despite these uncertainties, our findings lay the groundwork for the development of novel plant-derived repellents, provide a concrete pathway for reducing pesticide dependence while maintaining yield, and contribute meaningfully to the advancement of sustainable apple production systems.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JS: Conceptualization, Data curation, Investigation, Methodology, Software, Writing – original draft. HW: Conceptualization, Software, Writing – original draft. MjZ: Data curation, Investigation, Writing – original draft. RZ: Software, Supervision, Validation, Writing – review & editing. MmZ: Formal Analysis, Methodology, Visualization, Writing – review & editing. YM: Funding acquisition, Methodology, Project administration, Resources, Writing – review & editing. SC: Formal Analysis, Supervision, Visualization, Writing – review & editing. CZ: Formal Analysis, Supervision, Writing – review & editing. TH: Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – review & editing. XS: Formal Analysis, Funding acquisition, Resources, Supervision, Validation, Visualization, Writing – review & editing. SL: Funding acquisition, Resources, Supervision, Writing – review & editing.

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Conflict of interest

Authors SC and TH were employed by company Chengdu Tepu Biotech Ltd Co.

Authors CZ was employed by company Shouguang Daily Media Co., Ltd.

Authors SL was employed by company Qingdao Brightmoon Seaweed Group Co., Ltd.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2025.1670233/full#supplementary-material>

SUPPLEMENTARY FIGURE 1

Three-dimensional topographic images of VOCs from marigold leaves (a), marigold flowers (b), Inca peacockweed leaves (c), and Inca peacockweed flowers (d).

SUPPLEMENTARY FIGURE 2

Ion migration chromatogram (top-view) of VOCs from marigold leaves (a), marigold flowers (b), Inca peacockweed leaves (c), and Inca peacockweed flowers (d).

SUPPLEMENTARY FIGURE 3

Gallery plot of VOCs from marigold leaves (a), marigold flowers (b), Inca peacockweed leaves (c), and Inca peacockweed flowers (d).

SUPPLEMENTARY FIGURE 4

Marigold planted in the orchard.

SUPPLEMENTARY FIGURE 5

Inca peacockweed planted in the orchard.

SUPPLEMENTARY FIGURE 6

From left to right, the typical damage symptoms of fruit tree leaf roller, apple leaf miner, and peach fruit moth are shown.

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