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Chemical composition and bioactivity of essential oils against *Ephestia kuehniella* and their impact on beneficial parasitoid *Bracon hebetor*

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ABSTRACT

The present work aimed to evaluate the fumigant toxicity of essential oils extracted from five aromatic plants against *Ephestia kuehniella* (Zell.) and its impact on the parasitoid *Bracon hebetor* (Say). The chemical composition of all the essential oils showed that they were mainly composed of oxygenated monoterpenes. Among all the tested oils, *Eucalyptus camaldulensis* (Dehn.) oil was the most effective against both *E. kuehniella* larvae and adults with LC_{50} of 165.06 and 0.20 µL/L air, respectively. The next most toxic oil was *Pelargonium graveolens* (L.) oil. The mortality rate was affected by the exposure time and the concentration; *E. camaldulensis* reached 100% larval mortality at 300 µL/L air after 60 h. Oils had differential impact on *B. hebetor*, *E. camaldulensis* killed all the larvae within 24 h, while there was no mortality in *Ocimum basilicum* (L.) till day 5. The enzyme inhibitory effects of *E. camaldulensis* oil on acetylcholinesterase (AChE) and Glutathione S-transferase (GST) in *E. kuehniella* larvae were 64.46 and 51.45% respectively, which was the highest among the tested oils. These results imply that although *E. camaldulensis* and *P. graveolens* oils have potential as fumigants against *E. kuehniella*, their application could harm non-target beneficial insects. *O. basilicum* oil might be more suitable for integrated pest management strategies because it had a potent effect on insect moths and was less toxic to *B. hebetor*.

Keywords: Essential oils; Fumigant toxicity; Mediterranean flour moth; Beneficial insects; Integrated pest management; Enzyme inhibition

1. Introduction

Insect pests can damage stored-grain products, causing weight loss, reduced volume, impairment of germination, contamination by insect faeces and body parts, and deterioration of quality. The metabolism of insect infestation increases temperature and humidity in stored-grain products, which further induces fungal development and stimulates the germination of stored grains (Moazeni *et al.*, 2014). Among stored-product insects, the Mediterranean flour moth, *E. kuehniella* Zell. (Lepidoptera: Pyralidae) is distributed worldwide. This insect is one of the most notorious pests that spoil stored products, including cereal grains, flour, and other types of processed foods, incurring tremendous economic losses worldwide in the agriculture and food processing industries (Pandır and Baş, 2016). The larvae of *E. kuehniella* causes contamination of the stored products, poor quality of the products, and health risks to consumers (Abdelmalek *et al.*, 2017). Larvae and their silken webs make the products unsuitable for consumption and cause huge losses to farmers and food producers (Shams Salehi *et al.*, 2016).

The following strategies were suggested as having potential for managing this pest. In this regard, fumigation is very effective in the control of insect pests in stored products. Methyl bromide has been used commonly as a fumigant for pest control in the post-harvesting period (Yang *et al.*, 2020). However, methyl bromide is a toxic chemical that can lead to failure in the central nervous system and

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respiratory system, besides affecting the lungs, eyes, and skin. It evaporates quickly into the air and is therefore most lethal at the fumigation site (de Souza *et al.*, 2013). The growing concern for environmental conservation as well as the international market demand for pesticide-free food called for the development of safer pest control techniques. Hence, researchers try to look for safer substitutes to the conventional insecticides. Several fumigants have been tried out in the control of stored-product pests and these include methyl bromide. Methyl bromide was being replaced by essential oils for post-harvest insect control due to their potential as biocontrol agents (Tenne and Karunaratne, 2018). The effectiveness of oils in managing stored-product pests because of their low toxicity to mammals and a good environmental balance is making many people focus on the oils (Isman, 2000; Abdelmaksoud *et al.*, 2024). Previous studies have revealed that the essential oils possess contact and fumigant toxicity, antifeedant, repellent, and growth inhibitory effects against insect pests (El-Bakry *et al.*, 2016; Aouadi *et al.*, 2020). *C. nardus* L. (Poales: Poaceae), *E. camaldulensis* Dehnh. (Myrtales: Myrtaceae), *O. basilicum* L. (Lamiales: Lamiaceae), *P. graveolens* L'Hér. (Geraniales: Geraniaceae), and *T. vulgaris* L. (Lamiales: Lamiaceae) have been identified as fumigant and repellent essential oils against stored-product pests (Campolo *et al.*, 2018; Subramanya *et al.*, 2022; Bincy *et al.*, 2023).

Braconidae family, with species such as *Bracon brevicornis* Wesm. and *Bracon hebetor* Say, is an important family of parasitic wasps widely used in biological control. These wasps are effective in controlling a broad spectrum of pests, especially lepidopteran pests such as *E. kuehniella* Zell.*, Galleria mellonella* L.*, Corcyra cephalonica* Stainton*, Plodia interpunctella* Hübner, and *Ectomyelois ceratoniae* Zell. (Lettmann *et al.*, 2021). The integrated pest management programs must be known and careful when applying volatile oil with any other biological agent in the control program.

The purpose of this study was to assess the insecticidal efficacy of *C. nardus, E. camaldulensis, O. basilicum, P. graveolens*, and *T. vulgaris* essential oils against the larvae and adults of *E. kuehniella* and their impact on AChE and GST enzymes. Also, the effect of these essential oils on the non-target organism, *B. hebetor* parasitoid wasp, will be evaluated.

2. Materials and Methods

2.1. Plant materials

Leaves of five plant species, *C. nardus* (citronella), *E. camaldulensis* (red gum), *O. basilicum* (Basil), *P. graveolens* (rose geranium), and *T. vulgaris* (thyme) were brought from the National Research Centre's farm of medicinal and aromatic plants during flowering time.

2.2. Preparation of the essential oils

After being cleaned with tap and distilled water, the harvested plants were allowed to dry in the shade for five days at room temperature (26 ± 1 ˚C). Each plant's 2,000 grams of leaves were combined with 4000 milliliters of distilled water and put through a Clevenger apparatus hydro distillation process. The resultant herbal oils were kept in swarthy containers at a low temperature of 4 ˚C until they were utilized in toxicity tests and chemical analysis. They were dehydrated using anhydrous sodium sulfate.

2.3. Chemical constituent analysis of the essential oils

The chemical compositions of the essential oils were determined using gas chromatography-mass spectrometry (GC-MS) along with a reference database of natural products. The obtained plant essential oils were diluted with diethyl ether. A volume of 1µl of the dilution was injected into the GC-MS (Trace GC Ultra) with ISQ single quadrupole mass spectrometry and TG-5MS fused silica capillary column (30 m x 0. 25 mm x 0. 1 mm film thickness). A GC/MS detection method using electron ionization with a power level of 70 electron volts was used in the analysis. The carrier gas was helium gas, and the flow speed was maintained at 1 mL min^{-1}. The injector and MS transfer line temperature were set at 280 ˚C. The temperature of the oven was set at 50 ˚C for 2 min, then increased at a rate of 7 ˚C min^{-1} to 150 ˚C, then at a rate of 5 ˚C min^{-1} to 270 ˚C and was maintained at this temperature for 2 min before being ramped up at a rate of 3 ˚C min^{-1} to a final temperature of 5 ˚C min^{-1} (hold at 10 min). The compounds were identified by comparing the RI of GC peaks obtained with a homologous series of n-alkanes (C8-C20) to those reported in the literature (Adams, 2017). The mass spectra of these components were also searched against the GC/MS system's NIST 08 and WILLY 7 library data.

2.4. Insect rearing

2.4.1. Stock culture of *Ephestia kuehniella*

The Mediterranean flour moth, *E. kuehniella* was cultured on wheat flour (*Triticum aestivum* L.) in plastic boxes under a condition of darkness at 25 ± 1 ºC and relative humidity of 65 ± 5%. The eggs were incubated in one plastic container measuring 25 cm in length, 15 cm in width, and 10 cm in height; the container was filled half with a 1:1 mixture of wheat flour and bran. In each jar, powdered yeast (5 g) was also included. The rearing conditions were 26 ± 1 °C and 75% RH in complete darkness. For bioassays, one-day-old of both larvae and moths were collected from the rearing colony and used.

2.4.2. Stock culture of *Bracon hebetor*

The stock culture of *B. hebetor* was obtained from infested flour collected from the flour mills' warehouse. The parasitoid *B. hebetor* wasps were reared on the fifth instar larvae of *G. mellonella* at 25 ± 2 °C, 60 ± 5% RH, and under a photoperiod of 16:8 (L: D) h. One drop of honey was provided to the adult wasps as a food source.

2.5. Fumigant toxicity bioassay

2.5.1. Effect of the tested oils on *E. kuehniella*

The toxicity of the essential oils against *E. kuehniella* larvae and adults was carried out using the fumigant toxicity method, as explained by Huang *et al.* (2000), with slight modifications. Fumigation experiments were conducted using glass jars with a volume of 380 mL for larvae and 1000 mL for adults. Filter paper pieces (2×3 cm) were placed at the lower surface of the screw caps of the glass jars. The selected essential oils were applied to the filter paper pieces at the dilutions of 100-900 µL/L air and 0.1-0.8 µL/L air for larvae and adults, respectively. To avoid direct contact of insects with the applied oils, the inner side of the jar's neck was coated with Vaseline. The jars with the insects were sealed by their screw caps after placing 10 insects in each of them. All the treatments and the control were carried out three times. The mortality was documented hourly until death. Insects were considered dead if there was no leg or antennal movement.

A second experiment was carried out to determine 50% lethal concentrations. A series of dilutions were made after a concentration-setting trial to assess the mortality of insects. Ten larvae and adult insects were placed separately in glass jars with screw lids, as done in the first experiment. The oil amounts tested on *E. kuehniella* were 38, 76, 114, 190, 266, and 342 µL, equivalent to 100, 200, 300, 500, 700 and 900 µL/L air for larvae. The oil concentrations applied to moths were 0.1, 0.3, 0.5, 0.8, 1, and 1. 2 µL/L air. The nonexposed control insects were maintained under similar conditions. All the concentrations were repeated three times. The number of dead and live insects in each bottle was determined 48 h after the start of exposure. LC_{50} values were determined by using Probit analysis.

Another bioassay assessed the LT_{50} values at 300, 500, 700, and 900 µL/L and 0.1, 0.3, 0.5, and 0.8 µL/L of the tested essential oils on larvae and adults, respectively. The mortality was determined by an hourly check on the insects until all the insects were dead.

2.5.2. Effect of the tested oils on the natural enemies

Newly emerged adults of the parasitoid *B. hebetor* were exposed to the LC_{50} values of the tested oils, resulting from treating *E. kuehniella* adults. Each concentration of tested oils was applied to filter paper strips. Treated filter papers were placed at the top of 1000 mL plastic cup jars. Ten newly emerged parasitoid adults were placed in the cup and then sealed with air-tight lids. There was no direct contact between the oil and the insects. In the control jars, oil was not applied to the filter papers. Each experiment was replicated three times for each tested oil. Mortality was determined 24, 72, 120, and 168 h after the exposure.

2.6. Enzymes assessment

2.6.1. Insect tissue preparation

The treated insects were homogenized in distilled water (1 g insect body/5 mL water) for 3 minutes. The homogenates were then centrifuged at 3000 rpm for 15 minutes, and the resulting supernatant was used as the enzyme solution or stored at -20 ºC for later use in biochemical tests. A control experiment was conducted using the supernatant from an untreated insect.

2.6.2. Acetylcholinesterase (AChE) activity

The activity of AChE was estimated in the supernatant (as an enzyme solution). Substrate specificity was determined with three thioesters (Weber, 1966). A 10 µl aliquot of supernatant was added to 1. 5 mL of 5, 5- dithiobis-2- nitrobenzoic acid (DTNB) in 52 mM phosphate buffer of pH 7. 2. Following the incubation, 50 µl of 156 mM solution of a thioester acetyl thio-choline iodide was added. The enzyme activity was measured as the change in optical density caused by the reaction of DTNB to 5-thio-2-nitrobenzoic acid as described by Ellman *et al.* (1961). The reaction was followed at 405 nm, and the values were corrected for the spontaneous hydrolysis of the substrate. The change in enzyme activity ratio was determined by dividing the average enzyme activity.

2.6.3. Glutathione S-transferase (GST) activity

The activity of GST was assayed in the supernatant and was measured spectrophotometrically using CDNB (1-chloro-2,4-dinitrobenzene) and glutathione by the method of Habig *et al.* (1974). This process is associated with the increase of absorbance at 340 nm. The rate of increase is directly proportional to the GST in the sample.

2.7. Data analysis

Data were analyzed using analysis of variance (ANOVA) on SPSS 25. 0 computer program; Duncan's Multiple Range Test was used to compare the means. The LC_{50}, LT_{50}, and slope values were estimated by statistical analysis using Finney's method (Finney, 1971). If the control mortality is between 5% and 20%, the mortalities of treated groups were adjusted based on Abbott's formula (Abbott, 1925). The insect mortality was analyzed using Probit analysis to determine the LC_{50} and LC_{90} values using the SPSS 25. The values of LC_{50} were regarded as significantly different if the 95% confidence limits were not included in each other.

3. Results

3.1. Chemical composition of the essential oils

Table 1 presents the chemical constituents of volatile organic compounds across five aromatic plants: *C. nardus, E. camaldulensis, O. basilicum, P. graveolens,* and *T. vulgaris*. Oxygenated monoterpenes were the major components of all the essential oils. *C. nardus* oil consisted of oxygenated monoterpenes of 62.99%. The major compounds were citronellal (17.94%), elemol (12.27%), and citronellyl acetate (11.92%).

Meanwhile, *E. camaldulensis* oil had monoterpene hydrocarbons of 28.41% and oxygenated monoterpenes of 42.46%, while the major compounds were identified as 1,8-cineole, 32.73% and γ-Terpinene 10.84%. *O. basilicum* oil contained a high percentage of oxygenated monoterpenes, 65.58%, and a moderate percentage of sesquiterpene hydrocarbons, 28.08%. Notably, estragole (48.34%) and linalool (12.56%) were the major identified chemicals. Furthermore, *P. graveolens* contained 84.88% of oxygenated monoterpenes, of which citronellol (25.39%) and geraniol (20.25%) were the most abundant. In comparison, *T. vulgaris* oil was composed of 58.49% oxygenated monoterpenes and 38.13% monoterpene hydrocarbons. The major constituents identified in that oil were thymol (35.76%), ρ-cymene (16.93%), and γ-terpinene (11.62%).

Table 1: GC-MS analysis of aromatic compounds in different plant species

Compounds	RT	RI_{exp}	RI_{lit}	*Cymbopogon nardus*	*Eucalyptus camaldulensis*	*Ocimum basilicum*	*Pelargonium graveolens*	*Thymus vulgaris*
Anisole	4.94	913	913	6.07	–	–	–	4.78
α-Pinene	5.35	932	932	–	9.25	3.35	–	0.14
δ-3-Carene	7.36	1003	1001	1.22	–	–	–	–
α-Phellandrene	7.49	1007	1002	0.73	0.20	–	–	3.25
α -Terpinene	7.67	1013	1014	–	8.12	–	–	–
ρ-Cymene	7.83	1019	1020	–	–	–	–	16.93
Limonene	7.99	1025	1024	2.38	–	–	–	6.19
1,8-Cineole	8.1	1027	1026	–	32.73	4.68	–	–

Compounds	RT	RI_{exp}	RI_{lit}	*Cymbopogon nardus*	*Eucalyptus camaldulensis*	*Ocimum basilicum*	*Pelargonium graveolens*	*Thymus vulgaris*
γ-Terpinene	8.98	1053	1054	–	10.84	–	–	11.62
Linalool	10.62	1096	1095	–	–	12.56	8.19	2.67
Octadienol	11.31	1115	1113	0.89	–	–	–	–
Trans-Verbenol	12.22	1139	1140	–	1.06	–	–	–
Camphor	12.25	1140	1141	–	–	–	–	0.72
Isopulegol	12.43	1144	1145	3.31	–	–	–	–
Citronellal	12.71	1151	1148	17.94	–	–	–	–
Pinocarvone	13.07	1160	1160	–	1.76	–	–	–
Borneol	13.29	1165	1165	9.74	–	–	–	–
α-Terpineol	13.95	1180	1186	–	–	–	–	5.75
Myrtenol	14.55	1194	1194	–	2.44	–	–	–
Estragole	14.77	1198	1195	–	–	48.34	–	–
Citronellol	15.71	1222	1223	–	–	–	25.39	3.13
Citral	16.12	1233	1235	–	–	–	2.00	–
Geraniol	16.85	1450	1249	8.16	–	–	20.25	–
Linalool acetate	17.16	1258	1254	–	4.34	–	–	–
Thymol	18.71	1292	1289	–	0.13	–	–	35.76
Citral	19.83	1319	1316	0.52	–	–	–	–
Methyl thujate	19.86	1320	1318	–	–	–	–	5.24
δ-Elemene	20.64	1339	1335	2.58	–	1.79	–	–
Citronellyl acetate	21.21	1354	1350	11.92	–	–	–	–
Neryl acetate	21.51	1360	1359	–	–	–	5.31	–
α-Ylangene	22.21	1376	1373	2.09	–	–	1.89	–
Geranyl acetate	22.4	1381	1379	–	–	–	3.93	–
β-Damascenone	22.47	1382	1383	–	–	–	3.97	–
β-Bourbonene	22.64	1386	1387	0.55	–	–	3.65	–
Caryophyllene	23.55	1407	1408	–	2.17	1.25	–	–
α-cis-Bergamotene	23.81	1414	1411	–	–	9.59	–	–
α-Guaiene	24.71	1437	1437	–	2.10	1.75	–	–
α-Himachalene	25.18	1449	1449	–	–	–	1.42	–
α-Humulene	25.24	1450	1452	0.54	–	0.24	0.95	–
Dodecen-1-ol	26.15	1473	1469	–	–	–	7.58	–
γ-Muurolene	26.44	1480	1478	2.81	–	2.82	3.63	–
α-Cyclogeranyl acetate	26.52	1482	1480	4.44	–	–	–	–
α-Amorphene	26.73	1486	1483	1.96	–	–	–	–
γ-Bisabolene	28.43	1530	1529	–	–	–	0.79	–
Citronellyl butanoate	28.45	1531	1530	–	–	–	3.35	–
α-Cadinene	28.71	1538	1537	4.78	–	10.64	–	0.47
Elemol	29.1	1548	1548	12.27	–	–	–	–
Spathulenol	30.34	1579	1577	–	16.27	–	0.96	–
Caryophyllene oxide	30.52	1584	1582	–	1.50	0.32	–	0.71
Globulol	30.81	1591	1590	–	1.91	–	–	–
α-Acorenol	32.25	1630	1632	1.52	–	–	–	–
α-Eudesmol	33.11	1654	1652	1.13	1.07	–	–	0.83
α-Cadinol	33.13	1655	1652	–	1.74	0.93	–	–
Citronellyl tiglate	33.53	1665	1666	–	–	–	1.32	–

Compounds	RT	RI_{exp}	RI_{lit}	*Cymbopogon nardus*	*Eucalyptus camaldulensis*	*Ocimum basilicum*	*Pelargonium graveolens*	*Thymus vulgaris*
Geranyl tiglate	34.79	1698	1696	–	–	–	2.79	0.44
Farnesol	34.93	1715	1714	1.09	–	–	–	–
Valencene	37.23	1769	1767	–	–	–	0.80	–
Rimuene	41.73	1901	1896	–	–	–	–	0.67
Monoterpene hydrocarbons				4.33	28.41	3.35	0	38.13
Oxygenated monoterpenes				62.99	42.46	65.58	84.88	58.49
Sesquiterpene hydrocarbons				15.31	4.27	28.08	12.33	0.47
Oxygenated sesquiterpenes				16.01	22.49	1.25	0.96	2.21
Total				98.64	97.63	98.26	98.17	99.3

RT: Retention time, RI_{exp}: Calculated retention indices determined using the homologous series of n-alkanes (C8-C20), RI_{lit}: Retention indices in the literature.

3.2. Fumigant toxicity of the essential oils on *E. kuehniella*

The fumigant toxicity depended on the oil concentration and the exposure time of the fumigant. For instance, the mortality of *E. kuehniella* larvae was 100% at 300 µL/L air and exposure time of 60 h for *E. camaldulensis* oil, while the concentration of 700 µL/L air needed only 12 h to cause 100% mortality (Fig. 1). However, the lowest concentration of *C. nardus* essential oil at 900 µL/L air and the highest exposure time of 60 h had the fumigant toxicity of 86.67 % against *E. kuehniella* larvae.

Among all the tested oils, *E. camaldulensis* oil was the most toxic to the larvae of *E. kuehniella* with a calculated LC_{50} of 165.06 µL/L followed by *P. graveolens* oil with LC_{50} of 255.52 µL/L (Table 2). On the other hand, *C. nardus* and *O. basilicum* oils had the lowest toxicity levels with the LC_{50} of 691.84 and 632.95 µL/L, respectively.

Furthermore, all essential oils displayed a strong activity on the insect adult. Notably, *E. camaldulensis* oil exhibited 100% mortality on *E. kuehniella* moth at lower concentration (0.8 µL/L) and contact time of 48 hours (Fig. 2). Even at the highest concentration of *C. nardus* oil used in this study at 0.8 µL/L and the longest exposure time of 60 h, the toxic effect elicited was only 63.3% mortality.

E. camaldulensis oil displayed the highest efficacy with an LC_{50} of 0.20 µL/L air against *E. kuehniella* moth (Table 3), indicating its potential as a highly effective fumigant for controlling this pest. *P. graveolens*, *T. vulgaris*, and *O. basilicum* followed closely, with LC_{50} of 0.23, 0.28, and 0.33 µL/L air, respectively. Conversely, *C. nardus* exhibited the lowest efficacy with an LC_{50} of 0.79 µL/L air. The examined essential oils exhibit notably higher toxicity against *E. kuehniella* moth (LC_{50} values were between 0.20 and 0.79 µL/L) than the larvae (LC_{50} values ranged from 165.06 to 691.84 µL/L).

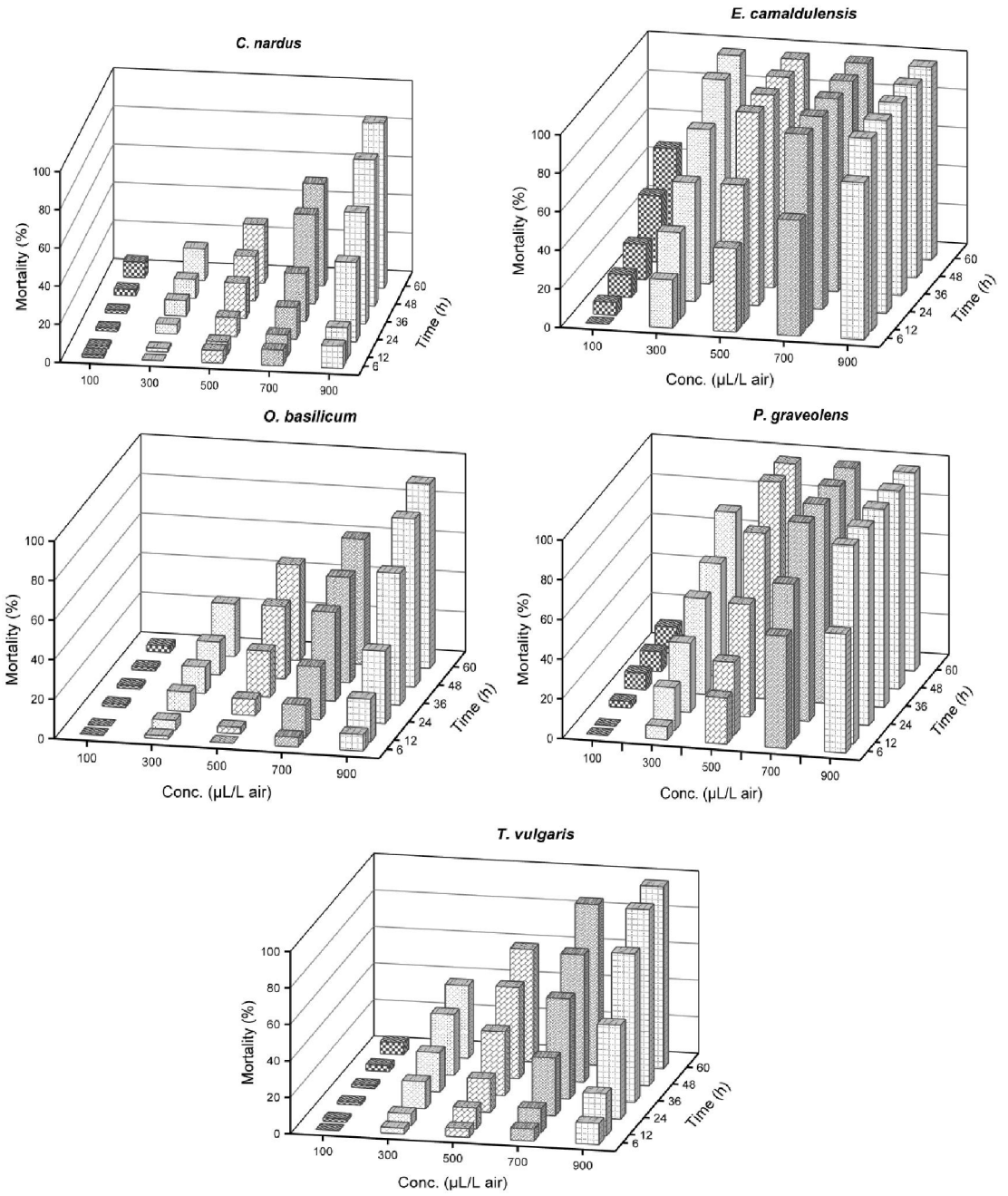

Fig. 1: Percent mortality of different essential oils on *E. kuehniella* larvae.

Table 2: Fumigant toxicity of essential oils against *Ephestia kuehniella* larvae.

Essential oils	LC_{50}[a] (µL/L air)	95% Confidence limits (µL/L air)		Slop[b] ± (SE)	Intercept[c] ± (SE)	(χ^2)[d]
		Lower limit	Upper limit			
Cymbopogon nardus	691.84	614.06	766.49	5.59 ± 1.31	-15.90 ± 3.75	1.72
Eucalyptus camaldulensis	165.06	145.87	183.75	4.90 ± 0.74	-10.86 ± 1.69	10.37
Ocimum basilicum	632.95	540.22	714.71	4.59 ± 0.97	-12.87 ± 2.76	8.43
Pelargonium graveolens	255.52	233.37	278.83	5.60 ± 0.77	-13.50 ± 1.87	8.57
Thymus vulgaris	432.08	352.81	496.05	3.97 ± 0.68	-10.48 ± 1.88	8.07

Note: [a] Concentration causing 50% mortality after 48 h of treatment, [b] Slope of concentration mortality regression line [c] Intercept of the regression line, [d] Chi-square value

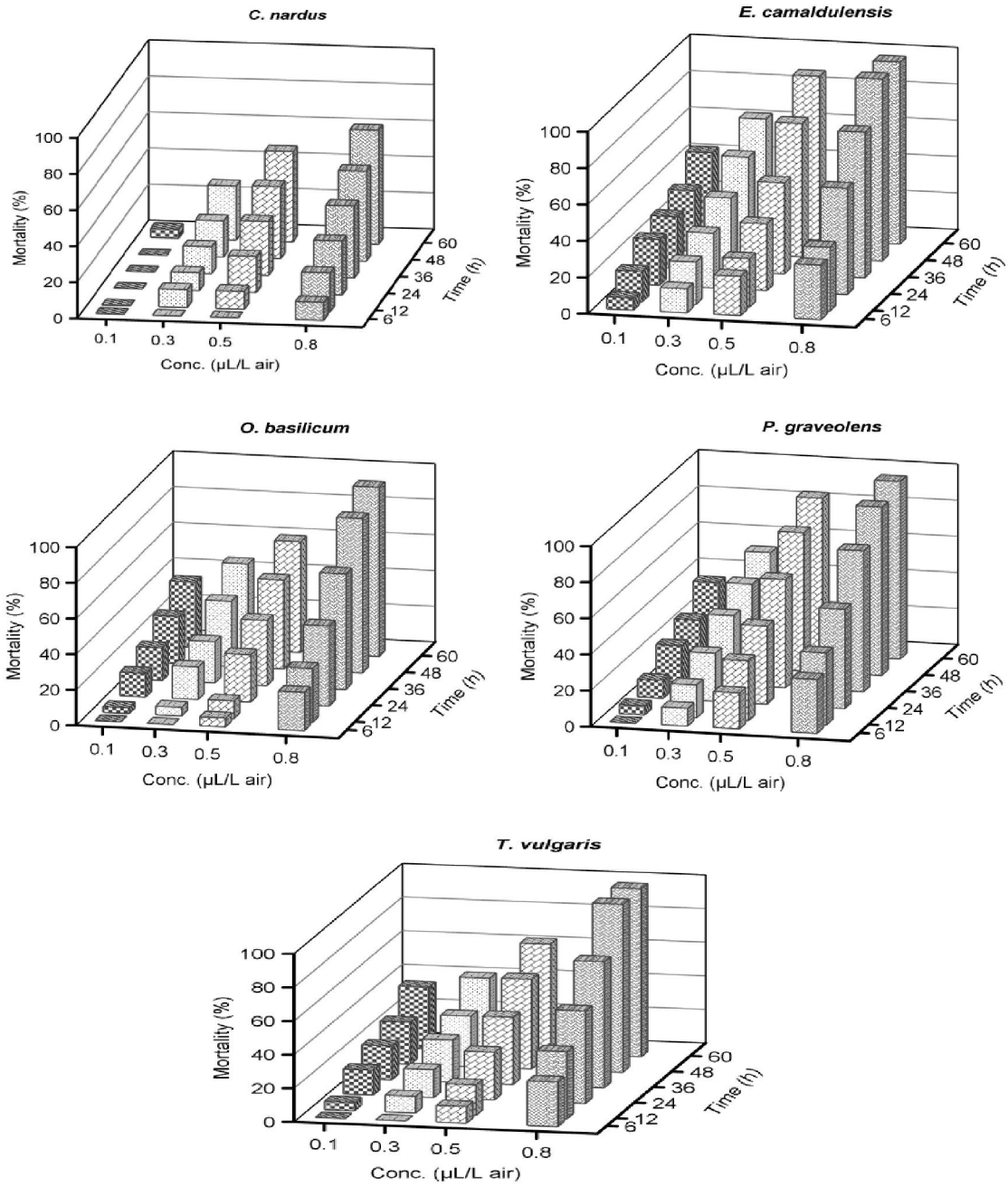

Fig. 2: Percent mortality of different essential oils on *E. kuehniella* moth.

Table 3: Fumigant toxicity of the tested essential oils against the moth of *Ephestia kuehniella.*

Essential oils	LC_{50}[a] (µL/L air)	95% Confidence limits (µL/L air)		Slop[b] ± (SE)	Intercept[c] ± (SE)	(χ^2)[d]
		Lower limit	Upper limit			
Cymbopogon nardus	0.79	0.57	1.06	1.80 ± 0.30	0.17 ± 0.11	6.21
Eucalyptus camaldulensis	0.20	0.13	0.26	1.98 ± 0.39	1.38 ± 0.24	7.22
Ocimum basilicum	0.33	0.22	0.48	1.61 ± 0.37	0.77 ± 0.21	7.59
Pelargonium graveolens	0.23	0.16	0.30	2.17 ± 0.39	1.37 ± 0.24	5.08
Thymus vulgaris	0.28	0.17	0.44	1.94 ± 0.40	1.05 ± 0.23	14.63

Note: [a] Concentration causing 50% mortality after 48 h of treatment, [b] Slope of concentration mortality regression line [c] Intercept of the regression line, [d] Chi-square value

The data given in Table 4 indicates the lethal time (LT_{50}) of five essential oils at various concentrations against larvae of the Mediterranean flour moth, *E. kuehniella*. The results presented in the tables prove that as the concentration of each essential oil increases, the value of LT_{50} decreases. *E. camaldulensis* oil had the highest mortality rate against *E. kuehniella* larvae, with LT_{50} varying from 3.54 h at 900 µL/L air to 13.35 h at 300 µL/L air. *P. graveolens* oil was ranked second, with LT_{50} between 5.25 and 34.97 h across the tested concentrations. Meanwhile, *O. basilicum* and *T. vulgaris* oils had lower toxicity levels than the other oils, with LT_{50} values varying from 23.51 to 199.72 h and 18.24 to 92.01 h, respectively. *C. nardus* oil, although having a decrease in LT_{50} values with increasing concentration, is relatively the least effective of the oils.

Table 4: LT_{50} values of different concentrations of *tested* essential oils against *E. kuehniella* larvae.

Essential oils	Concentrations (µL/L air)	LT_{50} (h)	95% Confidence limits (h)		Slop ± (SE)	Intercept ± (SE)	(χ^2)
			Lower limit	Upper limit			
Cymbopogon nardus	300	228.54	129.83	904.94	1.73 ± 0.38	-4.10 ± 0.61	0.92
	500	217.84	122.54	716.18	1.10 ± 0.20	-2.59 ± 0.31	4.21
	700	67.05	42.68	237.51	1.57 ± 0.19	-2.88 ± 0.28	11.75
	900	25.77	19.69	33.50	2.38 ± 0.18	-3.36 ± 0.27	10.43
Eucalyptus camaldulensis	300	13.35	7.48	19.20	2.48 ± 0.18	-2.79 ± 0.24	20.94
	500	7.17	5.13	8.91	3.81 ± 0.39	-3.26 ± 0.39	8.55
	700	5.25	4.35	5.87	5.27 ± 0.95	-3.80 ± 0.80	3.19
	900	3.54	1.63	4.57	4.11 ± 1.12	-2.26 ± 0.94	1.59
Ocimum basilicum	300	199.72	120.12	560.77	1.39 ± 0.25	-3.21 ± 0.39	1.39
	500	63.53	55.70	76.55	2.89 ± 0.34	-5.21 ± 0.54	2.28
	700	42.70	37.13	50.57	1.93 ± 0.19	-3.14 ± 0.28	1.71
	900	23.51	17.14	31.14	2.83 ± 0.20	-3.88 ± 0.29	16.02
Pelargonium graveolens	300	34.97	30.65	40.52	1.92 ± 0.18	-2.97 ± 0.27	3.42
	500	14.40	7.10	22.19	2.76 ± 0.19	-3.20 ± 0.25	33.86
	700	5.79	3.51	7.48	3.37 ± 0.39	-2.57 ± 0.39	7.67
	900	5.25	4.35	5.87	5.27 ± 0.95	-3.80 ± 0.80	3.19
Thymus vulgaris	300	92.01	70.70	139.80	1.67 ± 0.22	-3.28 ± 0.34	1.39
	500	50.32	43.46	60.64	2.01 ± 0.20	-3.43 ± 0.31	6.11
	700	29.72	22.72	39.28	2.69 ± 0.20	-3.96 ± 0.30	12.99
	900	18.24	12.14	25.05	3.32 ± 0.21	-4.19 ± 0.29	24.54

Data in Table 5 provides LT_{50} values for different concentrations of five essential oils against the *E. kuehniella* moth. *E. camaldulensis* and *P. graveolens* oils were the most effective against the adult

moths with the LT_{50} values varying from 13.70 h to 92.72 h. *O. basilicum* and *T. vulgaris* oils were found to be moderately toxic with LT_{50} ranging between 14.21 and 94.15 h, while *C. nardus* oil was the least effective, with LT_{50} values ranging from 45.35 h at 0.8 µL/L air to 126.88 h at 0.3 µL/L air. Notably, the lowest concentration of 0.1 µL/L air was ineffective for *C. nardus* oil. In general, *E. camaldulensis* oil was found to be the most effective against the larvae and moth of *E. kuehniella* followed by *P. graveolens* oil. On the other hand, the least effective was *C. nardus* oil against both life stages of the pest.

Table 5: LT_{50} values of different concentrations of *tested* essential oils against *E. kuehniella* moth.

Essential oils	Concentrations (µL/L air)	LT_{50} (h)	95% Confidence limits (h)		Slop ± (SE)	Intercept ± (SE)	(χ^2)
			Lower limit	Upper limit			
Cymbopogon nardus	0.1	-	-	-	-	-	-
	0.3	126.88	90.64	233.19	1.80 ± 0.28	-3.79 ± 0.45	2.50
	0.5	61.15	52.02	76.43	2.10 ± 0.23	-3.76 ± 0.36	3.50
	0.8	45.35	37.87	57.32	1.50 ± 0.17	-2.48 ± 0.25	3.32
Eucalyptus camaldulensis	0.1	92.25	66.29	161.54	1.20 ± 0.18	-2.36 ± 0.27	2.09
	0.3	43.03	35.72	54.76	1.41 ± 0.16	-2.30 ± 0.24	5.38
	0.5	24.34	11.42	50.66	1.79 ± 0.16	-2.49 ± 0.23	29.67
	0.8	13.70	4.94	22.89	2.51 ± 0.18	-2.85 ± 0.24	40.70
Ocimum basilicum	0.1	88.20	70.62	126.25	2.17 ± 0.30	-4.23 ± 0.47	1.49
	0.3	65.05	55.64	81.15	2.36 ± 0.27	-4.29 ± 0.43	2.53
	0.5	47.48	41.24	56.60	2.02 ± 0.20	-3.39 ± 0.31	2.23
	0.8	18.90	10.23	29.41	2.17 ± 0.17	-2.77 ± 0.24	25.05
Pelargonium graveolens	0.1	92.72	73.10	136.59	2.08 ± 0.29	-4.10 ± 0.45	2.32
	0.3	55.49	44.81	75.13	1.39 ± 0.17	-2.43 ± 0.26	1.36
	0.5	21.32	14.49	29.76	1.81 ± 0.16	-2.40 ± 0.23	10.97
	0.8	13.87	6.43	21.35	2.19 ± 0.17	-2.50 ± 0.23	25.02
Thymus vulgaris	0.1	94.15	73.36	141.45	1.95 ± 0.27	-3.85 ± 0.42	2.59
	0.3	80.57	64.42	113.56	1.85 ± 0.23	-3.52 ± 0.37	5.21
	0.5	43.61	37.03	53.55	1.64 ± 0.17	-2.69 ± 0.26	4.19
	0.8	14.21	4.87	24.08	2.45 ± 0.18	-2.83 ± 0.24	41.69

3.3. Effect of the tested oils on *B. hebetor*

The toxicity of various essential oils on the adult stage of *B. hebetor*, a natural enemy widely used in biological control, was determined using data presented in Table 6 after 1, 3, 5 and 7 days of treatment. The control group did not record any mortality up to the 7th day. *E. camaldulensis* oil was highly toxic with 100% mortality from day one. Although this potency can be useful in pest control, it poses a threat to beneficial species such as *B. hebetor*, which is a biocontrol agent. *T. vulgaris* also exhibited high toxicity with the mortality rate rising to 73% and above by day 5. The toxicity of *P. graveolens* oil and *C. nardus* oils was moderate with a gradual rise in mortality rate within the 7-day test period. This slow impact may enable a more sustainable control of pests since *B. hebetor* can still exist and effectively play its part as a natural enemy of pests. Interestingly, *O. basilicum* oil had no mortality up to the fifth day and therefore, it could be suitable for biological control management strategies that include the use of essential oils.

Table 6: Toxicity of essential oils to the parasitoid wasp *Bracon hebetor.*

Treatment	Days after treatment ± SE			
	1	3	5	7
Control	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	6.67 ± 6.67c
Cymbopogon nardus	6.67 ± 6.67b	26.67 ± 17.64b	26.67 ± 17.64b	33.3 ± 24.03 bc
Eucalyptus camaldulensis	100.0 ± 0.0a	100.00 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
Ocimum basilicum	0.0 ± 0.0b	0.0 ± 0.0b	26.67 ± 17.64b	26.67 ± 17.64bc
Pelargonium graveolens	6.67 ± 6.67b	13.3 ± 6.67b	26.6 ± 6.67b	33.3 ± 6.67bc
Thymus vulgaris	6.67 ± 6.67b	33.33 ± 24.04b	73.3 ± 17.64a	73.3 ± 17.64ab
F	69.6	8.990	8.364	5.497
Sig.	0.000	0.001	0.001	0.007

3.4. Effect of essential oils on AChE and GST enzymes

The effect of different essential oils on the AChE enzyme in *E. kuehniella* larvae are shown in Table 7. The AChE activity of the control group was 88.62 µmole/mL/g tissue, which is the initial level. *E. camaldulensis* oil had the highest AChE inhibition of 64.46%, which indicates the compound's high neurotoxic impact on the larvae of the insects. *P. graveolens* and *T. vulgaris* oils also possessed a high percentage of AChE inhibition (51.42 and 47.06%, respectively). On the other hand, *O. basilicum* oil had moderate effect with (38.05%). The lowest inhibition was recorded by *C. nardus* oil (29.72%). The degree to which these essential oils affected AChE was proportional to the observed insecticidal impact in the previous tables. It can be concluded that neurotoxicity of these essential oils particularly *E. camaldulensis* and *P. graveolens* is a major factor that contributes to their insecticidal effect on *E. kuehniella*.

Table 7: Effect of essential oils on AChE enzyme of *Ephestia kuehniella* larvae.

Treatment	AChE activity	% inhibition
Cont.	88.62 ± 2.89a	0e
Cymbopogon nardus	62.22 ± 2.17b	29.72 ± 2.32d
Eucalyptus camaldulensis	31.28 ± 2.26e	64.46 ± 3.70a
Ocimum basilicum	54.68 ± 1.98c	38.05 ± 3.91c
Pelargonium graveolens	43.01 ± 1.63d	51.42 ± 1.66b
Thymus vulgaris	46.92 ± 1.96d	47.06 ± 1.38b
F value	81.99	76.06
P	< 0.0001	< 0.0001

The impact of the essential oils on the GST enzyme in *E. kuehniella* larvae is presented in Table 8. The control group had a GST activity of 94.29 U/mL/g. *E. camaldulensis* oil again stood

out, recording the highest GST inhibition (51.45%), which means that it has the potential to influence the detoxification processes. *P. graveolens* oil also exhibited a good inhibitory activity against GST (47.71%). *O. basilicum* oil had moderate inhibition with 37.98% and the lowest inhibition was recorded by *C. nardus* oil at 21.1%. These essential oils are capable of inhibiting GST activity, and this would reduce the ability of the insect to metabolize and detoxify toxic compounds and hence increasing their susceptibility to the insecticidal effects of the oils. These results are in conformity with the insecticidal efficacy of the tested oils on *E. kuehniella*.

Table 8: Effect of essential oils on GST enzymes of *Ephestia kuehniella* larvae.

Treatment	GST activity	% inhibition
Cont.	94.29 ± 2.44a	0e
Cymbopogon nardus	74.42 ± 5.56b	21.10 ± 5.36d
Eucalyptus camaldulensis	45.73 ± 1.51d	51.45 ± 1.67a
Ocimum basilicum	58.48 ± 3.33c	37.98 ± 3.07c
Pelargonium graveolens	49.30 ± 1.50cd	47.71 ± 0.85ab
Thymus vulgaris	53.98 ± 0.86cd	42.71 ± 0.73bc
F value	38.24	54.14
P	< 0.0001	< 0.0001

4. Discussion

The GC-MS analysis of the selected plant species; *C. nardus, E. camaldulensis, O. basilicum, P. graveolens*, and *T. vulgaris*, showed presence of various volatile organic compounds. These observed differences in the chemical composition of the aromatic compounds of the different plant species may be due to genetic differences, environmental factors, and developmental stages of the plants (Abdel-Aziz *et al.*, 2022; Abdelmaksoud *et al.*, 2023). The abundance of oxygenated monoterpenes, for instance, citronellal, linalool, and 1,8-cineole in the studied plant species corresponds to other works that have analyzed the composition of the essential oils of these plants (Baser and Buchbauer, 2009). These compounds are reported to exhibit several biological activities such as antimicrobial, antioxidant, and insecticide properties that make the therapeutic and aromatic uses of the plant species relevant (Čmiková *et al.*, 2023). Citronellal, a major constituent of C. nardus oil, is well-documented for its insect-repellent efficacy (Isman and Machial, 2006). Borneol, another compound identified in *C. nardus*, has been found to exhibit insecticidal and repellent effects on some stored-product insects, including *Tribolium castaneum* (Herbst.) and *Rhyzopertha dominica* (F.) (Ukeh and Umoetok, 2011). The existence of geraniol, which has fumigant toxicity against stored-product pests (Chen and Viljoen, 2022), also increases the possibility of *C. nardus* as a botanical insecticide. *E. camaldulensis* oil containing 1,8-cineole and γ-terpinene can potentially manage stored-product insects (Abdelgaleil *et al.*, 2021). A high percentage of estragole was observed in *O. basilicum* oil. It has been identified that estragole has insecticidal and repellent activity against a number of insect pests, such as red flour beetle and rice weevil (Bedini *et al.*, 2016). Thymol was the major constituent in *T vulgaris* oil. Research has revealed that the fumigant toxicities of thymol are very high and can control different stored-product insects (Jankowska *et al.*, 2017).

The data obtained showed differences in the fumigant toxicity of the studied essential oils against *E. kuehniella,* with variations in the toxicity of the same essential oil between the larval and adult stages. This stage-specific susceptibility is in harmony with other works on the effectiveness of essential oils against stored-product pests (Rajendran and Sriranjini, 2008). *E. camaldulensis* oil had the highest level of insecticidal efficacy against both stages, which is in concordance with the earlier studies on the insecticidal potential of *Eucalyptus* species against various stored-product pests (Danna *et al.*, 2024). This essential oil is highly toxic due to its main components, which include 1,8-cineole and α-pinene, whose insecticidal effects have been confirmed by Aouadi *et al.* (2020). Notably, the LC_{50} values of all the tested essential oils were significantly lower in adult moths than the larvae, and the values were about 1000-fold lower. These findings were in accordance with Aouadi *et al.* (2020), who mentioned that adults of *E. kuehniella* were more sensitive to the fumigation effect of essential oils than larvae. Such higher susceptibility in adults might be due to variations in respiratory systems, cuticle permeability, or metabolic rates between the developmental stages (Slimane *et al.*, 2014). *P. graveolens* oil showed significant toxicity against both the life stages, and the adult's effectiveness was as good as *T. vulgaris* oil. Fumigant toxicity of *O. basilicum* and *C. nardus* oils was comparatively lower against *E. kuehniella* larvae. However, their efficacy was higher against adult moths, especially for *O. basilicum*. This implies that even though these oils could not significantly reduce larvae's mortality rate, they could be useful in controlling the adult moths or as part of an integrated pest management system. The differences in the levels of toxicity observed in this study for the various essential oils could be

attributed to differences in their chemical compositions and how their components may either enhance or reduce each other's toxicity (Bakkali *et al.*, 2008).

The LT_{50} values of the essential oils against *E. kuehniella* also had a decrease in response to the type and concentration of the essential oil used. LT_{50} values reduced with the increase in concentration which shows that the essential oils are more toxic at higher concentrations. In all the concentrations, *C. nardus* essential oil had the highest LT_{50} values on larvae and moths thereby showing low toxicity. On the other hand, the LT_{50} values for *E. camaldulensis* essential oil was the lowest, implying that it was the most toxic. Our findings were in line with Aouadi *et al.* (2020) who reported that the LT_{50} values of *Mentha rotundifolia* essential oil on *E. kuehniella* adult was 20.76, 5.30, and 1.03 h at concentrations 1.31, 3.28, and 13.16 µL/L air, respectively, and *Myrtus communis* oil was 105.09, 22.41, and 20.19 h at the same concentrations.

The findings of the present study revealed that *E. camaldulensis* oil was the most toxic to the adults of *B. bebetor* where the 100% mortality was recorded from day 1. This high toxicity aligns with the potent insecticidal effect on both the moths and the larvae of *E. kuehniella*. However, this result is somewhat worrying when considering the use of *E. camaldulensis* oil in pest management programs in which *B. hebetor* is a natural enemy, as this would seriously affect biological control (Desneux *et al.*, 2007). Among the tested oils, *O. basilicum* oil proved to be least toxic and, therefore, possibly the most suitable for *B. hebetor* in pest control. This is in concordance with other studies that have indicated that some botanical insecticides are relatively safer to some natural enemies (Nabil *et al.*, 2013). Nonetheless, the impacts of botanical insecticides on natural enemies may depend on formulation, concentration, and application method (Soares *et al.*, 2019). Knowledge of these factors can help in the development of effective pest management practices that do not harm natural enemies and support crop yield (Ochieng *et al.*, 2022). This study shows that the tested essential oils have differential inhibitory activity on AChE and GST enzymes in the larvae of *E. kuehniella*. Thus, the inhibition of AChE by essential oils results in the accumulation of acetylcholine in cholinergic synapses, paralysis, and death in insects (Shahriari *et al.*, 2018). The suppression of GST, an important detoxifying enzyme, also indicates that the essential oils might hinder the insect's capacity to metabolize and expel toxic substances (Felix *et al.*, 2021).

5. Conclusion

This work aimed to establish the efficacy of essential oils of five aromatic plants against *E. kuehniella*, a major stored-product pest, their impact on the target pest's essential enzymes, and the non-target parasitoid, *B. hebetor*. *E. camaldulensis* oil was the most effective insecticide against the larval and adult stages of *E. kuehniella,* with the lowest LC_{50} and LT_{50} values. *P. graveolens* oil exhibited the second highest toxicity, and *C. nardus* oil was the least effective in the present investigation. The action mechanism of the essential oils seems to be the inhibition of AChE and GST enzymes. The inhibition rates for both enzymes by *E. camaldulensis* oil were the highest, which was in accordance with its higher insecticidal efficacy. This double inhibition may explain its efficiency since it affects the nervous system and the detoxification processes of *E. kuehniella*. However, the findings also revealed that *E. camaldulensis* oil killed all the adult *B. hebetor* within 24 h and hence should not be used in integrated pest management. However, the toxicity of *O. basilicum* oil was much lower on *B. hebetor*, which might make it more appropriate for use with biocontrol measures. For future studies, these oils should be formulated in a way that increases their selectivity and the application techniques that would allow for their effectiveness against target pests without affecting the non-target beneficial insects.

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