Bull. Chem. Soc. Ethiop. **2024**, 38(2), 457-472.
DOI: https://dx.doi.org/10.4314/bcse.v38i2.13

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

EFFICACY OF FUMIGANT COMPOUNDS FROM ESSENTIAL OIL OF FEVERFEW (*CHRYSANTHEMUM parthenium* L.) AGAINST MAIZE WEEVIL (*SITOPHILUS zeamais* MOTS.): FUMIGANT TOXICITY TEST AND *IN-SILICO* STUDY

Fekade Beshah Tessema[1,2], Aweke Mulu Belachew[2], Yilma Hunde Gonfa[2,4], Tilahun Belayneh Asfaw[2,5], Zelalem Gizachew Admassie[6], Archana Bachheti[7], Rakesh Kumar Bachheti[2,8] and Mesfin Getachew Tadesse[2,3*]

[1]Department of Chemistry, College of Natural and Computational Science, Woldia University, PO Box 400, Woldia, Ethiopia
[2]Department of Industrial Chemistry, College of Natural and Applied Sciences, Addis Ababa Science and Technology University, PO Box 16417, Addis Ababa, Ethiopia
[3]Centre of Excellence for Biotechnology and Bioprocess, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia
[4]Department of Chemistry, College of Natural and Computational Science, Ambo University, PO Box 19, Ambo, Ethiopia
[5]Department of Chemistry, College of Natural and Computational Science, Gondar University, PO Box 196, Gondar, Ethiopia
[6]Plant Biotechnology Directorate, Bio and Emerging Technology Institute, Addis Ababa, PO Box 5954, Ethiopia
[7]Department of Environment Science, Graphic Era University, Dehradun-248002, Uttarakhand, India
[8]Department of Allied Science, Graphic Era Hill University, Society Area, Clement Town, Dehradun-248002, India

(Received December 23, 2022; Revised December 27, 2023; Accepted January 1, 2024)

ABSTRACT. Post-harvest insects are among the significant problems in the agricultural sector. The most accessible tools available for managing post-harvest arthropod-pests are fumigants because of the convenience of their applications and fast action in disinfecting. This study aimed to examine the fumigant toxicity of essential oil (EO) against maize weevil and identify the specific fumigants among the major components. The EO was extracted from aerial part of *Chrysanthemum parthenium* L. using Clevenger apparatus and was tested for fumigant toxicity. GC-MS was used to determine the chemical composition of EO. The major components were identified and screened virtually using Auto dock vina 1.2. in PyRx 0.8 platform. Dm AChE PDB ID: 6XYY was used as a target for molecular docking and malathion and pirimiphusmethyl were used as a reference for comparison. From the results of binding affinities, most of the major EO components showed better fumigant activity than the reference fumigants. More specifically 1,6-dioxaspiro[4,4]non-ene, β-farensen, bornyl-tiglate, γ-terpinene, *p*-cymene, bornyl-acetate, bornyl-isovalerate, terpinen-4-ol, *trans*-chrysanthenyl-acetate and α-phellandrene were found to be effective fumigants against maize weevil. The above findings suggest that the EO of the aerial part of *C. parthenium* can be a potential candidate for the development of novel natural fumigants for stored products.

KEY WORDS: Essential oil, Fumigants, Insecticides, Binding affinity, Maize weevil

INTRODUCTION

Post-harvest loss of cereal grain in sub-Saharan African countries is growing [1]. The use of synthetic chemicals for controlling post-harvest pests is known for its risk to human health [2]. For this and other related advantages like cost and ease of management, bio-pesticides are preferred [3]. Maize weevil (*Sitophilus zeamais* Mots.) is a corn pest that reduces production and damages storage quality [4]. In addition to causing significant losses due to the consumption of

*Corresponding author. E-mail: mesfin.getachew@aastu.edu.et

grains, maize weevil also results in elevated temperature and moisture conditions that lead to accelerated growth of molds, including toxigenic species [5] and allergen production [6]. The reduction in nutritional quality, weight, and germination rates of seeds are associated with food safety issues, including transmitting fungi and several types of bacteria. Towards these losses, synthetic insecticides have been used with all the limitations. Synthetic insecticides would be prohibited soon for many reasons associated with environmental issues. To fill such a gap, there is a need to develop selective insect control alternatives with fumigant actions from natural origin. *Chrysanthemum parthenium* (Syn. *Tanacetum parthenium*) belongs to the Asteraceae family and is known as a multi-purposed aromatic plant. It is used for ornament, flavor, and fragrance industries and medicinal [7], as part of herbal medicine in Turkey [8], Iran [9] China, and Japan [10]. Feverfew is its English name and widespread cultivation as a native plant of Europe. Different activities were reported for the species including anticancer [11], antibacterial, anti-inflammatory [12], for treating allergies, headaches and amenorrhea [7], and insecticidal activity [13].

Insecticidal activities are usually tested via repellence, adult mortality, anti-ovipositional, and growth inhibition. Powdered leaves of *Azadirachta indica* (Neem), *Cymbopogon citratus* (Lemon Grass), *Lantana camara* (Lantana), *Ocimum basilicum* (Basil) and *Tagetes erecta* (African marigold) have been tested as grain protectants against maize weevil. *A. indica* and *L. camara* had high repellence ratings against weevils for a longer period [2]. EO from *Carum carvi* fruit exhibited fumigant insecticidal activity against maize weevil [14]. EOs, which are mostly volatile and semi-volatile components of plants, have been shown to possess the potential to formulate fumigant insecticides with possible advantages, e.g. low mammalian toxicity, rapid degradation, and being locally available [15]. The essential oil may have the potential to be developed as a new natural fumigant for the control of stored product insects.

Volatile compounds of many plant extracts and essential oils consist of alkanes, alcohols, aldehydes, and terpenoids, particularly monoterpenoids, and exhibit fumigant activity [16]. EOs are known for antiseptic and antimicrobial activities [13] and insecticidal activities because of the lipophilic components that can easily penetrate the insect integuments [17]. β-Farnesene, camphor [18], bornyl acetate, α-pinene [19], and terpien-4-ol are known terpenoids for insecticidal activity. α-Pinene and 3-carene from cypress were assessed for contact and fumigant toxicities against the maize weevil, *S. zeamais*. Both compounds showed efficacies against adult and immature weevils [20]. EO components like γ-terpinene and terpinen-4-ol are known for fumigant and repellent activity rather than contact toxicity [21].

Terpenoids, in general having pleasant smells and being non-toxic to humans and other mammals, are among the suitable candidates for bio-pesticides [22]. The same report outlined that EO from *C. parthenium* (Syn: *Tanacetum parthenium* (L.) Sch. Bip.) aerial part showed no mortality rate against larvae of *Aedes aegypti*. EOs from *Artemisia lavandulaefolia* DC. Prodr. and *Artemisia sieversiana* Milld. from China tested for insecticidal activity against maize weevil showed contact toxicity with LD_{50} values of 55.2 and 112.7 mg/adult, respectively [18]. Fumigant toxicity is more commonly used for EOs than contact toxicity [6]. *Artemisia vestita* Wall was recommended as a fumigant insecticide against maize weevil [15]. From a comparative study of monoterpenes contact and fumigant toxicity: camphene and camphor showed toxicity against stored products insects [23]. Findings from a screening program of Chinese herbal medicine suggested that the essential oil of *Chenopodium ambrosioides* L. and its main active constituent, (*Z*)-ascaridole, is a potential natural fumigant [24]. *S. zeamais* adults showed the strongest tolerance to ρ-cymene [15].

Using compound elimination assay and chemical analysis, *trans*-cinnamaldehyde in cinnamon oil and terpinen-4-ol in tea tree and marjoram oils were identified as the major active components. Cinnamon oil was the most active in both contact/residual and fumigant bioassays and exhibited strong behavioural inhibitory activity [6]. *C. parthenium* EO showed replant activity against the Colorado potato beetle [25]. The volatility of EOs helps to present fumigant toxicity.

Their vapor action may also be auspicious for pest control because of their insecticidal properties and the fact that they can act as fumigants. In this context, many studies of the fumigant activity of essential oil vapors against various insects, especially those that attack stored products, suggest that such substances may often behave like conventional fumigants, exhibiting strong adverse effects on both the immature stages and adults [26]. Fumigant insecticides are considered the best alternative due to their non-residual formulation [17].

Few studies reported the insecticidal properties of *C. parthenium* extracts and EOs [27], and to our knowledge, no reports on the insecticidal activity against the maize weevil (*S. zeamais)* were available so far. There is no report on its potential insecticidal activity against storage pests and on the specific identification of responsible components. The observation of the fumigant toxicity of *C. parthenium* plant against cabbage insects initiated this study. This study aims to examine the fumigant toxicity activity of the essential oil against maize weevil and identify the specific fumigants among the major components of EO of *C. parthenium.*

EXPERIMENTAL

Chemicals and reagents

Ultrapure water (18.2MQ at 20 ºC water purification system, Purelab flex 4 Elga, USA) was used for hydro distillation. Anhydrous sodium sulfate (AR, BASF, India) was used to remove water residue from EO after separating from the distillate. Hexane (HPLC grade, LOBA Chemie, India) was used for diluting the EO and C_7-C_{25} alkanes (certified material, Trace CERT, China) were used as references in hexane to compute retention indexes of the EO components. Acetone (AR grade, BASF, India) was used for dilution, and malathion (Ethiolathion 50% EC, Addis Ababa) was purchased from the local market as a reference for the fumigant toxicity test.

Plant material

Aerial parts (stem and leaves) of *Chrysanthemum parthenium* L. were collected from a family garden at Debrebrhan (North Shewa), Ethiopia. The geographical location of Debrebrhan is at a longitude of 39°31'58.7928"E and latitude of 9°40'35.9724" N Elevation of 2,840 m. Herbarium samples were prepared and deposited at the national herbarium in Addis Ababa University, College of Science. After collecting, the areal part of the plant was allowed to dry in shade for about a week and ground using stainless steel grinder (700 g Electric Grains, China) to a fine powder. Then the powder was stored in a refrigerator at 4 ℃ before extraction. The fresh aerial parts of the plant were also chopped and used for extraction as a comparison for yield calculation.

Extraction

Hydro-distillation was conducted in a Clevenger-type apparatus loading 200 g air-dried and powdered aerial part of *C. parthenium* in 1 L of ultra-pure water for 3 h at the temperature of 70±5 ℃ [28]. The EO was separated from the distillate water and dried over anhydrous sodium sulfate. The density of the oil was calculated from the weight of its volume in a calibrated vessel at room temperature.

GC-MS analysis and identification of components

With a few adjustments, gas chromatography-mass spectrometry measurements were carried out following the method used by Helal *et al.* [29]. The GC/MS analyses were carried out with an Agilent 7890B series coupled with an Agilent 5977A mass spectrometer (electron impact ionization ESI, 70 eV), a 7863-automatic injector, a fused silica capillary column DB-5MS of 30

m × 0.25 mm and film thickness 0.25 μm (Agilent Technologies, Santa Clara, CA, USA). The linear gradient program of GC oven temperature was set to rise from 40 ℃ (4 min. hold) to 220 ℃ (15 min. hold) at 4 ℃/min. The injector and detector temperatures were set to 250 ℃ and 280 ℃, respectively. The samples (1 mL) were injected neat, with a split ratio of 1:10. Helium (99.995%) was used as carrier gas with 170 kPa column head pressure and 22.95 cm/s linear velocities (1 mL/min at constant flow). Mass spectra were acquired by automatic scanning in the mass range m/z 30 to 300 at 5.1 scan/s.

Identification of component compounds was done by their Kovat/Retention index and mass spectra with NIST 17, F & amp F Libraries, and published literature [13]. The Kovat/Retention index was determined using a reference to a series of n-alkanes (C_7-C_{25}) under identical experimental conditions. The compounds were identified by comparing with standards (when available) and with mass spectra libraries using MSD ChemStation E.01.00.237 data system, which includes the spectral libraries NIST databases and Fragrance & amp, Flavor. The relative amount of individual component compounds was computed based on GC integrator peak areas without correction factors.

Fumigant activity of EO against maize weevil

Test insect *S. zeamais*, two-week-old adults were obtained from the laboratory of Bio- and Emerging Technology Institute (BETin), Ethiopia. The food media used were whole maize grains and freshly harvested maize was purchased from the local market. The grains were equilibrated to a moisture content of approximately 14% before use. They were disinfected with ethanol for 3 min and then washed with water. After washing, they were allowed to dry with sunlight.

The fumigant activity of the essential oil against *S. zeamais* adults was tested as described by Erdogan and Mustafa [30] with slight modifications. Briefly, range-finding assumptions were made based on the recommended application concentration of the commonly used insecticide (5% malathion). The essential oil was dissolved in acetone and different concentrations (2.5, 5, 7.5, and 10%) were prepared [14]. A glass jar of size (5 cm diameter and 8 cm height) was collected and made ready for the assay. Small size sponge was cut and anchored inside the glass jar. 1 mL of each dilution was applied to the prepared small-sized sponge and placed inside the jar which was then filled with 200 g maize then 15 two weeks old adult insects were added. Similarly, 1 mL of acetone and 1 mL of 5% malathion were applied to their respective test sponge-containing jars as negative and positive standards, respectively. Finally, the jar was tightly covered. The experiment was done in triplicate. The mortality rate (%) was calculated by dividing the number of dead insects by the total number of insects in a jar and multiplying by 100 [31]. Results were recorded after 3 h and 12 h of application time.

In-silico study to screen fumigant components

Calculation of drug-likeliness properties of major components of EO

The SwissADME and admetSAR 2.0 (http://lmmd.ecust.edu.cn/admetsar2/), a free web tool was used to generate the physicochemical, medicinal, toxicity profile, and drug-likeness properties of the major components of EO. Lipinski's rule, also called the rule of five, was used to evaluate the drug-likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that may be active per oral [32].

Selection and preparation of protein structure

Drosophila melanogaster Meig. has proven suitable for selecting insecticide targets [33]. From an inverted virtual screening study, acetylcholinesterase (Dm AChE) was selected as the best

target for insecticidal activities [34]. The reason is that there is better consistency and better scores. In the same vein, targets for specific insecticidal activity are suggested [30]. A protein with PDB ID: 6XYY was selected among the proposed targets using resolution, Ramachandran plot, and Q mean values. The 3D X-ray crystal structure of update of AchE from *D. melanogaster* complex with tacrine derivative (9-(3-phenylmethylamino)-1,2,3,4-tetrahydroacridine) (PDB ID: 6XYY) was retrieved from the Protein Data Bank (PDB)(http://www.pdb.org). Next, all heteroatoms, ligands, and water molecules were removed from the receptor before the docking simulation. The refined structures were evaluated by several validation tools like PROCHEK and QMEAN to select the best structure and assess the quality. The accuracy and stereochemical features of the predicted structures were evaluated with PROCHECK by Ramachandran Plot analysis [35]. The best model was selected based on the overall quality factor and number of residues in the core, allowed, generously allowed, and disallowed regions. Verify3D, ERRAT, and QMEAN were used to analyse the selected model [36]. The protein was visualized by a Swiss-PDB viewer and made ready for docking.

Ligand structure preparation

The chemical structures of major components obtained from the result of GC-MS and selected for docking on the percentage abundance base (≥ 0.50%) were retrieved as SDF files from the PubChem database at NCBI (http://pubchem.ncbi.nlm.nih.gov/). The SDF files were converted into PDB file format using OPEN BABEL software. The prepared 3D structures of the compounds were saved in the pdb format and were finally prepared for docking. Finally, ligands were minimized and optimized by using UCSF Chimera. The native ligand was used for comparison and the commercial insecticides pirimiphusmethyl and malathion were used as reference ligands.

Docking procedure using PyRx/Autodock vina

All the docking calculations were performed using AutoDock Vina 1.2.0 [37] for Dm AChE and were modified by adding polar hydrogen and then kept rigid in the docking process. In contrast, all the torsional bonds of ligands were set free by the Ligand module in AutoDock Tools. The suitability of molecular docking parameters and algorithms to reproduce the native binding poses was first verified with a redocking experiment using co-crystal ligands.

Virtual screening of major component molecules (ligands) and reference molecules was performed into the active site of the Dm AChE by using AutoDock Vina software in PyRx platform (GUI version 0.8) [38]. The use of AutoDock vina is also based on the consistency of scores [29] and is fast computing [37] for screening purposes. Using PyRx software, the receptor 6XYY and all ligands were prepared, and then docking was performed using a grid vina search space whose coordinates were x = 22.9787, y = 62.2006, and z = 13.5587, and dimensions 55.3028, 60.3491, 63.7619 Å. These parameters were set to cover the entire 3-dimensional active site of the target. During docking, the Lamarckian Genetic Algorithm was selected with standard docking protocols. The ligand molecules were flexible throughout the docking study, and the macromolecule was kept rigid. Docking was performed to obtain a population of possible orientations and conformations for the ligand at the binding site. The best confirmation with the lowest binding energy poses or docking score than that of the positive control was chosen after the docking search was completed. The most favorable binding pose was chosen based on the lowest free energy of binding (ΔG) and the lowest inhibition constant (Ki), and was computed from the binding energies.

Visualizing docking results

The 3D visualizations and 2D Hydrogen-bond interactions of the complex receptor-ligand structure were performed using PyMol software [39] and LigPlot+ v.1.4.5 program [35],

respectively, to identify the interactions pose of a protein-ligand complex in each docking pose, including hydrophobic bonds, hydrogen bonds, and their bond lengths. The analysis of the complex structure of the ligand-protein interaction was performed using LigPlot+ v 1.4.5 [35].

RESULTS

Properties and yield of EO

The hydro distillation of the aerial part of *C. parthenium* (shown in Figure 1a) without the flower yielded 0.78% essential oil as an average of three runs. Both the dry and wet-based yield calculations were attempted and the dry-based yield was reported. As shown in Figure 1b, the colour of the oil was light green and its density was measured to be 0.98 g/mL. The oil bears a strong aroma odour same as the fresh plant.

Figure 1. Images of (a) *Chrysanthemum parthenium* L. and (b) EO from the aerial parts in a vial.

GC-MS analysis

GC-Chromatogram shows 59 components (Figure 2), 25 of which were identified, as shown in Table 1. The identifications of component molecules were based on comparisons with their Kovat indices, literature retention indexes, and MS spectral data from GC-MS known libraries (i.e., NIST). The major components included *trans*-chrysanthenyl-acetate (32.38%), camphor (25.58%), *p*-cymene (9.3%), *trans*-crysanthenol (9.04%), bornyl-tiglate (4.87), and α-phellandrene (1.81%). Additionally, the other identified components in lower abundances include camphene (1.78%), *cis*-chrysanthenol (1.66%), γ-terpinene (1.58%), and bornyl-acetate (1.23%). According to terpenes classification, the identified components include 72.57% of oxygenated monoterpenes, 14.47% of monoterpene hydrocarbons, 6.35% of oxygenated sesquiterpenes, 1.63% of sesquiterpenes. The total well-identified components out of the total were found to be 95.76%. The majority of the components were oxygenated monoterpenes. Among the 25 identified components, based on the strength of confirmation and % abundance (≥ 0.5%, 15 major components (Figure 3) were selected for virtual screening. Considering functional groups of the components identified hydrocarbons and alcohols were significant in terms of the number of components whereas, in terms of percentage abundance esters (38.98%), ketones (25.58%),

hydrocarbons (16.10%), and alcohols (14.09%) were significant to determine the properties and applications of the EO. As shown in Table 1, above large % of oxygenated monoterpene is associated with the sample collection time after flowering.

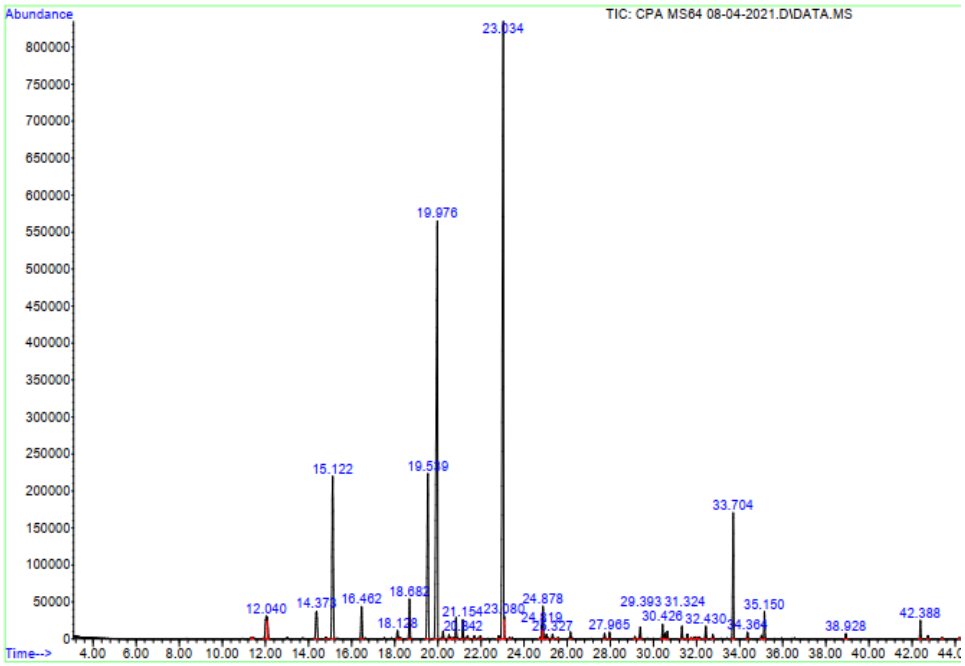

Figure 2. GC-MS chromatogram of essential oil from the aerial part of *C. parthenium*.

Table 1. Identified components of essential oil from the aerial part of *C. parthenium*.

No.	RT (min)	RI_L	RI_{Exp}	Abundance	Name
1	12.039	947	948	1.78	CAmphene
2	14.373	1007	1005	1.81	α-Phellandrene
3	15.122	1023	1023	9.3	*p*-Cymene
4	16.462	1056	1057	1.58	γ-Terpinene
5	18.682	--	1114	1.66	*cis*-Chrysanthenol
6	19.539	1132	1136	9.04	*trans*-chrysanthenol
7	19.976	1148	1148	25.58	(+)-2-Bornanone (D-camphor)
8	20.842	1171	1171	0.81	Borneol
9	21.154	1180	1179	0.7	Terpinen-4-ol
10	21.386	--	1184	0.11	4-Ethyl-1-methoxy-2-methyl-benzene
11	22.821	1227	1225	0.11	*p*-Cumenol
12	23.034	1235	1231	32.38	*trans*-Chrysanthenyl acetate
13	23.080	--	1232	0.45	Carveol
14	24.819	--	1281	0.45	E-4,9-Decadienol
15	24.878	1283	1283	1.23	Bornyl acetate
16	25.327	1297	1295	0.16	Thymol
17	27.965	1372	1370	0.2	Copaene
18	29.393	--	1418	0.46	Silphinene/α-guaiene
19	30.426	1453	1451	0.51	(E)-.β-faresene

20	31.324	1480	1479	0.44	(-)-Germacrene D
21	31.758	1495	1493	0.02	Zingiberene
22	32.430	1516	1515	0.5	Bornyl isovalerate
23	33.704	--	1558	4.87	Bornyl tiglate
24	35.150	--	1607	0.98	(1S,3aS,4S,5S,7aR,8R)-5-isopropyl-1,7a-dimethyloctahydro-1H-1,4-Methanoinden-8-ol
25	42.388	--	1875	0.63	1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiynylidene)-, (2E)-

RT (min) - Retention time in minute, RI_L - Retention index literature value, RI_{Exp} - Retention index experimental value.

For in silico investigation major components were selected from GC-MS chromatogram and spectral results. The most abundant components were 1,6-dioxaspiro[4,4]non-ene, β-farensen, bornyl-tiglate, γ-terpinene, *p*-cymene, bornyl-acetate, bornyl-iosvalerate, terpinen-4-ol, *trans*-chrysanthenyl-acetate, α-phellandrene, borneol, *cis*-chrysanthenol, camphor, *trans*-crysanthenol, and camphene. 9N-phenylmethylamine-tetrahydroacridine (native ligand) and pirimiphusmethyl and malathion were used as references.

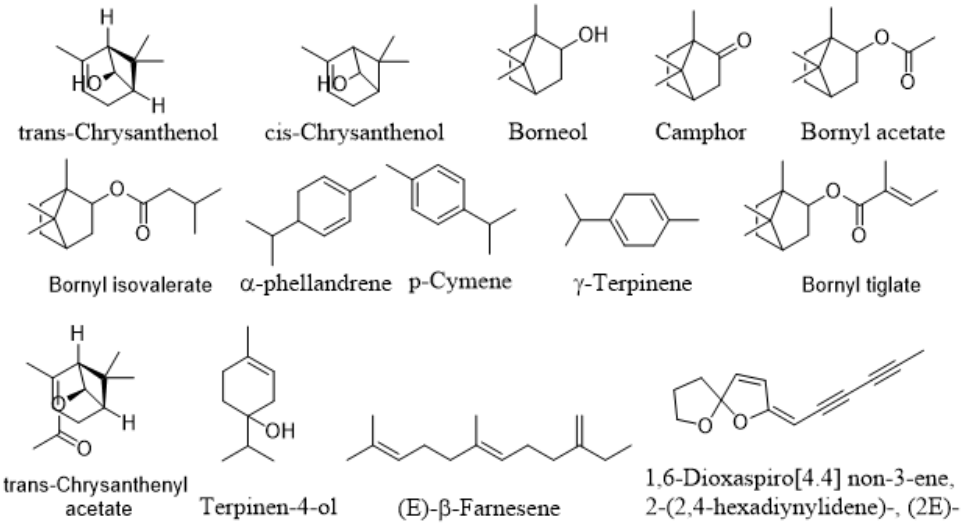

Figure 3. Structures of major components selected for virtual screening.

Fumigant toxicity test of EO against maize weevil

The setup for the test is shown in Figure 4a. Counting was done by rechecking as shown in the same Figure 4b. In all concentrations, including the pilot test (10%), all adults died 3 h after application which is the same as 5% Malathion (positive control) as shown in Table 2.

In-silico study to identify fumigant compounds among the major components of EO

From Ramachandran Plot analysis for *Dm AChE* (PDB-ID: 6XYY), the following values were observed: number of residues in core/ favoured regions – 404 (88.4%), additionally allowed – 49 (10.7%), generously allowed – 3 (0.7%) and disallowed regions – 1(0.2%). From Q_{mean} test, the Z score was equal to 0.25. These values are in the range of the best model for the purpose indicated. ADMET properties computed included drug likeliness, molar refractivity, and solubility of the

components of the EO. The physicochemical properties of the component's compounds used as ligands were found suitable as a drug with acceptable ADMET values which supports the no toxicity risk. Following Lipinski's rule of 5, all major components were suitable in their Drug-likeliness properties. These included molecular weight (< 500 Daltons), number of hydrogen bond donors (< 5), number of hydrogen bond acceptors (< 10), Log P (< 5), and molar refractivity (< 140). SwissADME Analysis generated the major components' physicochemical, medicinal, and toxicity profiles and found acceptable values and profiles.

Figure 4. (a) Sample jars for the fumigant toxicity test and (b) counting adult mortality.

Table 2. Result of fumigant toxicity test of EO against maize weevil.

Treatment	Code (Description)	Concentration (EO in acetone)	The average mortality rate at the time of application (1 mL) at 3 h
1	Lower Conc.	2.5%	100
2	Medium Conc.	5%	100
3	Higher Conc.	7.5%	100
4	Pilot Scale Conc.	10%	100
5	Acetone		0
6	5% malathion	5%	100

Selecting target proteins

The selection of the target protein for virtual screening was done by considering the results of PROCHECK server, resolution, and Ramachandran Plot results from https://saves.mbi.ucla.edu/ and Z score value from https://swissmodel.expasy.org/ q_{mean}/. Experimental data for Dm AChE (PDB ID: 6XYY) was retrieved as method: X-ray diffraction, resolution: 2.70 Å, R-value free: 0.234, R-value work: 0.172, R-value observed: 0.178, and space group: P 43 21 2. Unit cells: a = 95.81 Å / α = 90˚, b = 95.81 Å / β = 90˚, c = 162.03 Å / γ = 90˚

Auto dock vina result

For fumigant insecticidal activity, the scoring function for the best conformational positions; values of free binding energy, Root mean square deviation (RMSD), and inhibition constant (Ki) μM (micromolar) for a fixed conformational position of the ligands were calculated based on molecular docking analysis. The RMSD values obtained for the lowest energy poses were predicted for each component. The lowest binding energy was the native ligand found with the receptor protein. 1,6-dioxaspiro [4,4]non-ene, β-farensen, bornyl-tiglate, γ-terpinene, *p*-cymene, bornyl-acetate, bornyl-Isovalerate, terpinen-4-ol, *trans*-chrysanthenyl-acetate and α-phellandrene binded with lower binding energy than the two reference ligands. Borneol and *cis*-chrysanthenol were equivalently banded with Pirimiphusmethyl to the target protein. Camphor, *trans*-crysanthenol, and camphene were bonded better than Malathion. The insecticidal activity of *C. parthenium* essential oil major components can be listed in the order of activity as 9N-

phenylmethylamine-tetrahydroacridine (native ligand) > 1,6-dioxaspiro[4,4]non-ene > β-farensen > bornyl-tiglate > γ-terpinene > *p*-cymene, bornyl-acetate > bornyl-isovalerate > terpinen-4-ol > *trans*-chrysanthenyl-acetate > α-phellandrene > porneol > *cis*-chrysanthenol > pirimiphusmethyl > camphor > *trans*-crysanthenol > camphene > malathion (reference). The docking studies result of all the selected ligands (component compounds) is shown in Table 3.

Table 3. Auto dock vina results for protein (PDB ID: 6xyy) binding Affinity towards major components of essential oil from the aerial part of *Chrysanthemum parthenium.*

Ligand	Binding energy (kcal/mol)	RMSD (Å)	Ki (mM)	# of H bond	Amino Acids involved in interactions
9N-Phenylmethyl amine	-8.2	2.27	0.97	0	Trp321, Tyr324, Tyr71,Tyr73, Glu69, Asp375
1,6-Dioxaspiro [4,4]non-ene	-7.6	3.167	2.68	0	Trp271, Phe330, Gly151, Glu80, Ser238, Asn84, Phe440, Trp83, Tyr71, Tyr370, Phe371
β-Farensen	-6.9	1.992	8.74	0	Tyr370, Tyr71, Phe371, Trp83, Tyr374, Phe330, Glu80, Thr154, Asp375, His480, Tyr324
Bornyl-tiglate	-6.8	20.893	10.35	0	Trp321, Tyr71, Glu69, Tyr73, Tyr324, Arg70
γ-Terpinene	-6.8	1.762	10.35	0	Tyr71, Glu80, Trp472, Gly79, Asn84, Leu479, His480, Trp83, Tyr370
p-Cymene	-6.8	1.418	10.35	0	Trp83 Tyr370, Tyr71, Glu80, Gly79, Trp472, Asn84, Leu479, His480
Bornyl-acetate	-6.4	2.772	20.33	0	Trp83, Tyr370, His480, Tyr71, Glu80, Gly150, Thr154, Tyr374
Bornyl-isovalerate	-6.3	1.439	24.07	0	Arg70, Trp321, Tyr71, Tyr73, Tyr324
Terpinen-4-ol	-6.3	2.048	24.07	1	Tyr370, Trp83, Thr154, Tyr71, Glu80, Trp472, Tyr374
trans-Chrysanthenyl-acetate	-6.2	2.548	28.5	0	Arg70, Trp321, Glu69, Tyr71, Tyr73, Val318
α-Phellandrene	-6.2	1.426	28.5	0	Tyr71 , Tyr370, Trp472, Gly79, Glu80, Asn84, Leu479, His480
Borneol	-5.6	1.473	78.48	2	His438, Met346, Trp563, Pro443, Asn564, Glu446, Arg571
cis-Chrysanthenol	-5.6	26.289	78.48	2	Asn268, Arg571, His438, Pro270, Met346, Glu446, Cys442, Pro443, Trp563, Asn564
Pirimiphusmethyl	-5.6	4.545	78.48	0	Trp321, Tyr324, Glu69, Val318, Tyr71, Tyr73
Camphor	-5.5	25.285	92.9	0	Tyr71, Trp321, Tyr73, Arg70, Glu69
trans-Crysanthenol	-5.5	9.297	92.9	2	Trp563, Asn564, Pro443, Arg571, His438, Asn268, Pro270, Met346, Glu446, Cys442
Camphene	-5.2	9.593	154.2	0	Gly150, Trp83, Tyr71, Thr154, Tyr370, His480
Malathion	-4.4	31.401	594.9	3	Arg571, His438, Pro443, Pro568, Asn564, Glu446, Pro270, Trp563, Asn268

PDB – Protein Data Bank, RMSD - Root mean square deviation.

Apart from a few hydrogen bond interactions, most interactions were hydrophilic with the nearest residues; some of which were the same as the native ligand considered (Figure 5). The inhibitor constant, Ki, indicates how potent an inhibitor's value describes the binding affinity between the inhibitor and the target enzyme. It is the concentration required to produce half-maximum inhibition. Prior research in the area suggested that the smaller Ki, the greater the binding affinity and the smaller cost of medication would be needed to inhibit the activity of the target proteins (enzymes) [40].

Molecular docking studies showed that the identified compounds had binding energy values between −4.4 and −8.2 kcal mol^{-1}. The lowest binding energy was that of the native ligand. The Ki of docked components for *Dm AChE* was estimated between 0.97 – 549.9 μM. References were much lower both in binding energies and Ki values.

DISCUSSION

The yield reported herein was in the range of reported values (0.78%). The flower of *C. parthenium* yields 0.8% EO [9]. From the whole aerial part of *C. parthenium*, a 0.62% EO yield was reported from Turkey [41]. The minimum % yield of EO content set by British Herbal Phramcopia is 0.5% for dry herbs [42]. The current value was above this range and can be used for both economical and effective considerations in herbal formulations. From previous investigations on the oil of the aerial part of the plant in the same genus, the percentage of camphor ranged from 43% to 62% and chrysanthenyl acetate from 14% to 24% [11], whereas in the present case the percentage of acetate dominated over the one of camphor. This may affect the therapeutic effect of the EO. The chemotype of the species can be deduced to be either Aureum or Snowball from the percentage abundance of the two major components, i.e. camphor/ *trans*-chrysanthenyl acetate [10]. Reports indicated that the composition of EO mostly depends on geo-climatic location, the genetic composition of the species, growing conditions, and harvesting or collection time for aromatic plants [43]. Following the GC-MS analysis, the fumigant toxicity test was performed.

Fumigant toxicity was reported as the major route of insecticidal activity for EOs [5, 19]. From the fumigant toxicity test, 100% mortality of adults after 3 h similar to reference insecticides dictates the potential of the crude EO as a fumigant insecticide. The standard dose recommended for the malathion to be used as a pesticide is 100 g per quintal of maize (1 g malathion per 1000 g of maize). In this experimental case, the maximum dose of the essential oil in the 1 mL treatment was 10%, i.e. 0.1 mL (0.1 g) applied to 200 g of maize. The minimum dose of the essential oil in the 1 mL treatment was 2.5%, i.e. 0.025 mL (0.025 g) applied to 200 g of maize. Therefore, the dose of the treatment was 8 and 2 times less than the standard in the minimum and maximum applied dose of the essential oil, respectively. In a study using neem seed and leaves powder 3% (w/w) were observed to be efficient in controlling maize weevil [44]. Together with the safe nature of the EO to the environment and to humans [19], such efficacy encourages further study to come up with alternatives in controlling the weevils. Fumigant EO components are more likely to be friendly than the powder (contact) insecticides as the former minimizes residual toxicity risk [17]. The components of the EO are also expected to demonstrate a synergic effect for such significant fumigant activity. To explain the point of attention for such an effect, reports suggested boosting insecticidal activity due to synergic effects [26]. For the treatment of bulk grains or stored products fumigants are widely used due to their efficacy and low cost compared to other methods [20]. Following the experimental method for the fumigant toxicity test, the EO can be applied as a fumigant for the storages before and/or after packing using the effective optimized concentration. Further investigation may be needed for determining the lowest effective concentration for application as fumigants.

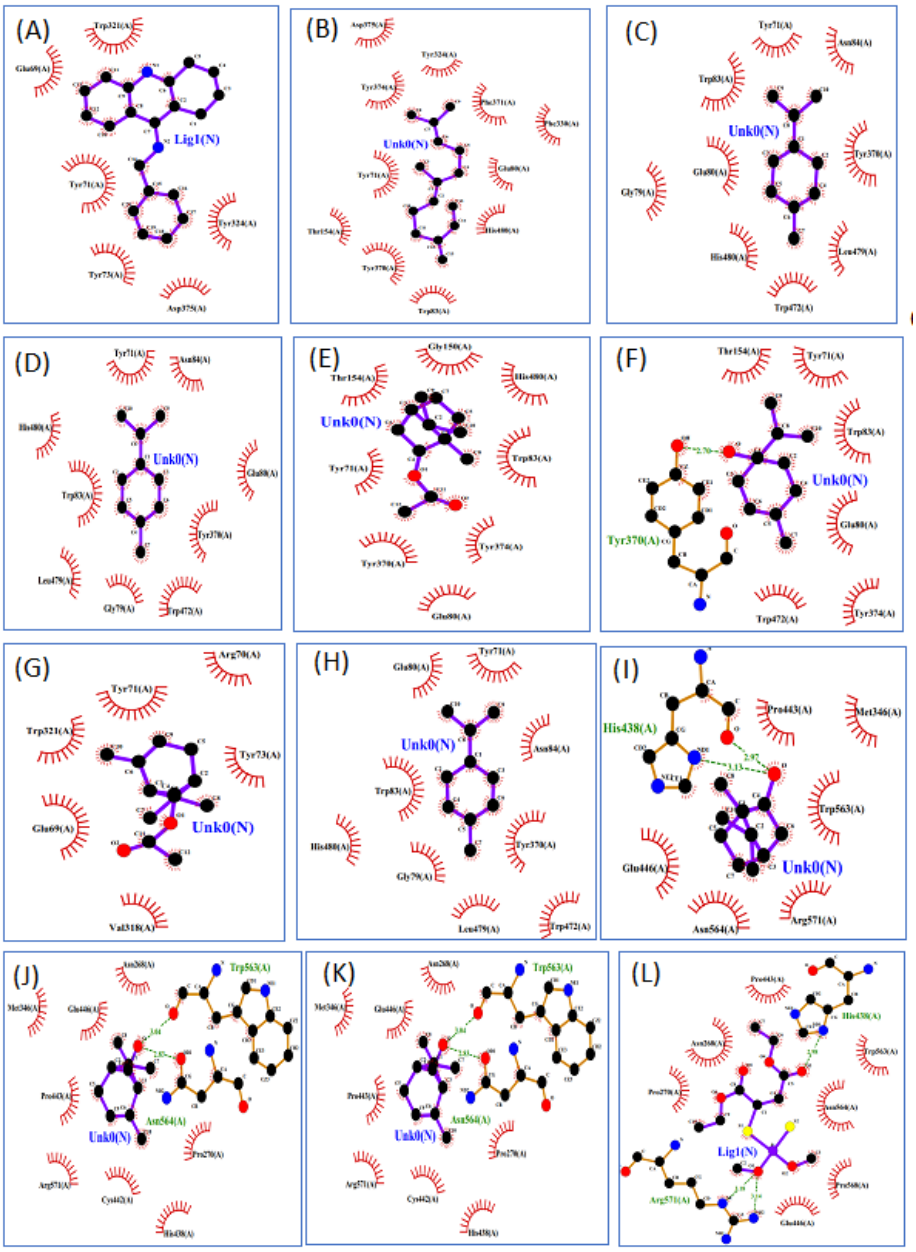

Figure 5. LigPlot+ diagrams for the ligands docked in Dm AChE (PDB ID: 6XYY): (A) 9N-phenylmethylamine-tetrahydroacridine -6xyy complex, (B) β-farensen-6xyy complex, (C) γ-terpinene-6xyy complex, (D) p-cymene-6xyy complex, (E) bornyl-acetate 6xyy complex, (F) terpinen-4-ol-6xyy complex, (G) *trans*-chrysanthenyl-acetate-6xyy complex, (H) α-phellandrene-6xyy complex, (I) borneol-6xyy complex, (J) *cis*-chrysanthenol-6xyy complex, (K) *trans*-crysanthenol-6xyy complex, (L) Malathion-6xyy complex. Note: Hydrogen bonding interactions and residue amino acid names were labeled in green.

The synergic effects of the components on the overall toxicity [17] of the EO should be studied. The toxic elements of herbs, i.e., α and β-thujone were not identified even in trace amounts. Teixeira da Silva also reviewed 0 % of this toxic component from same plant [45]. The absence of these toxic monoterpenes minimizes the risk of toxicity. Cytotoxicity test doesn't seem much important as the work is on storage pests and cereals which are not directly used by humans. Rather a residual effect may be important. The minimal residual effect is explained by the drug-likeness properties of the major components of the EO and the absence of toxic components. Meanwhile, there is a report on the toxicity of the essential oil of *Chrysanthemum parthenium* being insignificant at concentrations of 5–15% (v/v) [12]. This agrees with the physicochemical properties computed [17] and to identify the responsible components for the observed fumigant toxicity activity molecular docking study was performed.

The active site was retrieved for interaction between ligands and Dm AChE based on re-docking 9N-phenylmethylamine-tetrahydroacridine towards Dm AChE by using MetaPocket 2.0 online server [46]. Among the components as the best out of those studied, 1,6-dioxaspiro-[4,4]-non-ene showed the binding affinity −7.6 kcal mol^{-1} with the lowest Ki 2.68 μM. Similarly, the reference insecticides gave the binding affinity -5.6 kcal mol^{-1} and 4.4 kcal mol^{-1} with the Ki (78.8 μM and 549.9 μM), respectively. Apart from a few options for hydrogen bonding, most interactions involved were hydrophobic interactions (Figure 5, Table 3). Therefore, based on binding affinity, Ki, and type of interaction 1,6-dioxaspiro-[4,4]-non-ene, β-farensen, bornyl-tiglate, γ-terpinene, p-cymene, bornyl-acetate, bornyl-isovalerate, terpinen-4-ol, *trans*-chrysanthenyl-acetate and α-phellandrene had good potential to inhibit Dm AChE. It can be suitable for the development of a novel fumigant insecticide. After docking, the generation of schematic two-dimensional protein-ligand complex and visualization was done by LigPlot+ and for the native ligand (9N-phenylmethylamine-tetrahydroacridine), it was bonded with Dm AChE via hydrophobic interactions with the active site residues, including the amino acid residues Trp321, Tyr324, Tyr71, Tyr73, Glu69, Asp375 within 3 Å from the ligand (Table 3). From these residues, Tyr71 was involved in the interaction of most components indicating that most poses selected were with the main binding site of the receptor. The binding interactions of bornyl-tiglate, bornyl-isovalerate, *trans*-chrysanthenyl-acetate, and camphor also enclosed most of the residues involved in the interactions and showed common interaction patterns with the native ligand to the same implications. The effect will be significant as these are among the highest in % composition. Several studies found that the interaction of residues is an important mechanistic component of substrate-selective inhibition [47].

Terpinen-4-ol, borneol, *cis*-chrysanthenol, *trans*-crysanthenol, and malathion (reference) were compounds that formed hydrogen bonds with Arg371, Tyr370, His438, Trp563, and Asn564 with a bond length of less than 3.00Å as shown in Figure 3. Here, these observed H-bond interactions formed with Dm AChE residues seem to be comparatively more stable [48] than the other ligands, with slightly higher binding energies. The H-bond interaction was more responsible for the fumigant insecticidal activity of the EO. Hydrogen bonds play a significant role in ligand binding with receptors by influencing drug specificity, metabolization, and adsorption [47]. This is in agreement with the possible activities of terpinen-4-ol [6,22]. Considering functionalities of component compounds, esters, alcohols, and hydrocarbons [49] are among the most expected biological activities. Stronger fumigant toxicity of terpinen-4-ol (LC_{50} = 2.96 mg/L), and moderate toxicity of borneol and camphor (LC_{50} = 29.64 mg/L and 21.67 mg/L) [5], respectively, agreed with our findings which were -6.3 kcal/mol, -5.6 kcal/mol and -5.5 kcal/mol binding energies, respectively. From the results of binding affinities, most of the major components of the EO investigated showed better fumigant activity than the reference fumigants.

CONCLUSIONS

The findings from both the fumigant toxicity test and virtual screening suggest that the essential oil of *C. parthenium* can play an important role in stored grain protection and contributes to the need for alternative protection avoiding the risks associated with the use of synthetic insecticides. The fumigant activity of the essential oil and binding affinities of the major components are significantly promising as they show potential for development as possible natural fumigants for the control of stored product insects, specifically maize. 1,6-Dioxaspiro[4,4]non-ene, β-farensen, bornyl-tiglate, γ-terpinene, *p*-cymene, bornyl-acetate, bornyl-isovalerate, terpinen-4-ol, *trans*-chrysanthenyl-acetate and α-phellandrene were found to be effective fumigants against maize weevil. However, for the practical application of the essential oil/major components as novel fumigants, further studies on optimizing the minimum concentration of the essential oil and time of effectiveness on the development of formulations are necessary to improve the efficacy, and stability while reducing costs. To examine the contribution of each major component of the essential oils to the overall contact and/or fumigant toxicity, a compound elimination assay should be conducted.

ACKNOWLEDGMENTS

Authors acknowledge AASTU, Department of Industrial Chemistry and EBETin for the chance given to do this investigation. International Program in the Chemical Sciences (IPICS), Uppsala University for financial support.

REFERENCES

1. Medugu, M.; Okrkata, A.; Dunuwel, D. Management of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) using Nigerian raw diatomite. *J. Appl. Sci. Environ. Manage.* **2020**, 24, 1663–1669.
2. Parugrug, M.L.; Roxas, A.C. Insecticidal action of five plants against maize weevil, *Sitophilus zeamais* Motsch. (Coleoptera Curculionidae). *KMITL Sci. Technol. J.* **2008**, 8, 24–38.
3. Nwosu, L.C. Maize and the maize weevil: Advances and innovations in postharvest control of the pest. *Food Qual. Saf.* **2018**, 2, 145–152.
4. Arrahman, A.; Saenong, M.S. Controlling maize weevil in corn plants by improving cultivation technology and postharvest handling. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, 648.
5. Chu, S.S.; Du, S.S.; Liu, Z.L. Fumigant compounds from the essential oil of Chinese *Blumea balsamifera* leaves against the maize weevil (*Sitophilus zeamais*). *J. Chem.* **2013**, 2013, 289874.
6. Motschulsky, S. Insecticidal activity of 28 essential oils and a commercial product containing *Cinnamomum cassia*. *Insects* **2020**, 11, 474.
7. Ben-Erik, V.W.; Michael, W. *Medicinal Plants of the World: An Illustrated Scientific Guide to Important Medicinal Plants and Their Uses*, 2nd ed., Briza Publications: Pretoria, South Africa; **2017**.
8. Polatoğlu, K.; Demirci, F.; Demirci, B.; Gören, N.; Başer, K.H.C. Antibacterial activity and the variation of *Tanacetum parthenium* (L.) Schultz Bip. essential oils from Turkey. *J. Oleo Sci.* **2010**, 59, 177–184.
9. Shafaghat, A.; Larijani, K.; Salimi, F. Composition and antibacterial activity of the essential oil of *Chrysanthemum parthenium* flower from Iran. *J. Essent. Oil-Bearing Plants* **2009**, 12, 708–713.
10. Grzeszczuk, M.; Wesołowska, A. Composition of essential oils and some antioxidants in flowers of three *Chrysanthemum* cultivars. *J. Elem.* **2018**, 23, 1331–1342.
11. Kumar, V.; Tyagi, D. Chemical composition and biological activities of the essential oils of

genus *Tanacetum* - a review. *J. Pharmacogn. Phytochem.* **2013**, 2, 155–159.
12. Mohsenzadeh, F.; Chehregani, A.; Amiri, H. Chemical composition, antibacterial activity and cytotoxicity of essential oils of *Tanacetum parthenium* in different developmental stages. *Pharm. Biol.* **2011**, 49, 920–926.
13. Ghavam, M. *Tripleurospermum disciforme* (C.A.Mey.) Sch.Bip., *Tanacetum parthenium* (L.) Sch.Bip, and *Achillea biebersteinii* Afan.: Efficiency, chemical profile, and biological properties of essential oil. *Chem. Biol. Technol. Agric.* **2021**, 8, 1–17.
14. Fang, R.; Jiang, C.H.; Wang, X.Y.; Zhang, H.M.; Liu, Z.L.; Zhou, L.; Du, S.S.; Deng, Z.W. Insecticidal activity of essential oil of *Carum carvi* fruits from China and its main components against two grain storage insects. *Molecules* **2010**, 15, 9391–9402.
15. Chu, S.S.; Liu, Q.R.; Liu, L.Z. Insecticidal activity and chemical composition of the essential oil of *Artemisia vestita* from China against *Sitophilus zeamais*. *Biochem. Syst. Ecol.* **2010**, 38, 489–492.
16. Yang, Y.; Lee, H.; Clark, J.M.; Ahn, Y. Insecticidal activity of plant essential oils against *Pediculus humanus* Capitis (Anoplura: Pediculidae). *J. Med. Entomol.* **2004**, 41, 699–704.
17. Sfara, V.; Zerba, E.N.; Alzogaray, R.A. Fumigant insecticidal activity and repellent effect of five essential oils and seven monoterpenes on first-instar nymphs of *Rhodnius prolixus*. *J. Med. Entomol.* **2009**, 46, 511–515.
18. Liu, Q.R.; Chu, S.S.; Jiang, G.H.; District, H.; District, H.; District, H. Insecticidal activity and chemical composition of the essential oils of *Artemisia lavandulaefolia* and *Artemisia sieversiana* from China. *Chem. Biodivers.* **2010**, 7, 2040–2045.
19. Ebadollahi, A.; Mahboubi, M. Insecticidal activity of the essential oil isolated from *Azilia eryngioides* (PAU) Hedge et Lamond against two beetle pests. *Chil. J. Agric. Res.* **2011**, 71, 406–411.
20. Mbata, G.N.; Toews, M.D. Recent advances in postharvest pest biology and management. *Insects* **2021**, 12.
21. Ayvaz, A.; Sagdic, O.; Karaborklu, S.; Ozturk, I. Insecticidal activity of the essential oils from different plants against three stored-product insects. *J. Insect Sci.* **2010**, 10, 1–13.
22. Chantraine, J.; Laurent, D.; Ballivian, C.; Saavedra, G. Insecticidal activity of essential oils on *Aedes aegypti* larvae. *Phyther. Res.* **1998**, 12, 350–354.
23. Owolabi, M.S.; Ogundajo, A.L.; Alafia, A.O.; Ajelara, K.O. Composition of the essential oil and insecticidal activity of *Launaea taraxacifolia* (Willd.) Amin Ex C. Jeffrey growing in Nigeria. *Foods* **2020**, 9, 914.
24. Chu, S.S.; Feng Hu, J.; Liu, Z.L. Composition of essential oil of Chinese *Chenopodium ambrosioides* and insecticidal activity against maize weevil, *Sitophilus zeamais*. *Pest Manag. Sci.* **2011**, 67, 714–718.
25. Lazarevic, J.; Kostic, I.; Milanovic, S.; Šešlija Jovanović, D.; Krnjajic, S.; C¨alic, D.; Stankovic, S.; Kostic, M. Repellent activity of *Tanacetum parthenium* (L.) and *Tanacetum vulgare* (L.) essential oils against *Leptinotarsa decemlineata* (Say). *Bull. Entomol. Res.* **2021**, 111, 190–199.
26. Vera, S.S.; Zambrano, D.F.; Méndez-sanchez, S.C.; Rodríguez-sanabria, F.; Stashenko, E.E.; Luna, J.E.D. Essential oils with insecticidal activity against larvae of *Aedes aegypti* (Diptera: Culicidae). *Parasitol Res.* **2014**, 113, 2647–2654.
27. Erdogan, P.; Yildirim, A.; Sever, B. Investigations on the effects of five different plant extracts on the two-spotted mite *Tetranychus urticae* Koch (Arachnida: Tetranychidae). *Psyche J. Entomol.* **2012**, 2012, 125288.
28. Mirjalili, M.H.; Salehi, P.; Sonboli, A.; Vala, M.M. Essential oil composition of feverfew (*Tanacetum parthenium*) in wild and cultivated populations from Iran. *Chem. Nat. Compd.* **2007**, 43, 181–182.
29. Helal, I.E.; Galala, A.A.; Saad, H.-E.A.; Halim, A.F. Chemical composition and α-amylase inhibitory activity of *Apium leptophyllum* essential oils. *J. Am. Sci.* **2015**, 11, 204–209.
30. Erdogan, P.; Mustafa, Z. Fumigant activity of some essential oil against wheat weevil,

Sitophilus granarius L. (Coleoptera: Curculionidae). *J. Bacteriol. Mycol.* **2021**, 9, 57–60.
31. Suleiman, R.; Rosentrater, K.A.; Chove, B. Periodic physical disturbance : An alternative method for controlling *Sitophilus zeamais* (maize weevil) infestation. *Insects* **2016**, 7, 51.
32. Lipinski, C.A. Lead- and drug-like compounds: The rule-of-five revolution. *Drug Discov. Today Technol.* **2004**, 1, 337–341.
33. Perry, T.; Batterham, P.; Daborn, P.J. The biology of insecticidal activity and resistance. *Insect Biochem. Mol. Biol.* **2011**, 41, 411–422.
34. Vieira, T.F.; Araújo, M.F.; Fernandes, M.J.G.; Pereira, D.M.; Fortes, A.G.; Castanheira, E.M.S.; Gonçalves, M.S.T.; Sousa, S.F. *In Silico* Identification of Protein Targets Associated to the Insecticide Activity of Eugenol Derivatives, Proceedings of the Chemistry proceedings; **2021**, 3(138), 1–8.
35. Laskowski, R.A.; Swindells, M.B. LigPlot+: Multiple ligand–protein interaction diagrams for drug discovery. *J. Chem. Inf. Model.* **2011**, 51, 2778–2786.
36. Hasan, A.; Khan, A.; Datta, A.; Hasan, H. A comprehensive immunoinformatics and target site study revealed the corner-stone toward Chikungunya virus treatment. *Mol. Immunol.* **2015**, 65, 189–204.
37. Trott, O.; Olson, A. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2012**, 31, 455–461.
38. Dallakyan, S.; Olson, A. *Chemical Biology*, Hempel, J.E., Williams, C.H., Hong, C.C. (Eds.), *Methods in Molecular Biology*, Vol. 1263, Springer: New York; **2015**.
39. DeLano, W.L. The PyMol molecular graphics system. *Proteins Crystallogr.* **2002**, 30, 442–454.
40. Mulu, A.; Gajaa, M.; Woldekidan, H.B.; Wmariam, J.F. The impact of curcumin derived polyphenols on the structure and flexibility COVID-19 main protease binding pocket: A molecular dynamics simulation study. *Peer J.* **2021**, 9, 1–16.
41. Coban, F.; Ozer, H.; Mete, E. Essential oil composition of *Tanacetum parthenium* from Eastern Black sea. *Agric. Conspec. Sci.* **2019**, 84, 91–94.
42. Hendriks, H.; Bos, R. The essential oil of *Tanaceturn parthenium* (L.) Schultz-Bip . *Flavour. Fragr. J.* **1996**, 11, 367–371.
43. Long, C.; Sauleau, P.; David, B.; Lavaud, C.; Cassabois, V.; Ausseil, F.; Massiot, G. Bioactive flavonoids of *Tanacetum parthenium* revisited. *Phytochemistry* **2003**, 64, 567–569.
44. Erenso, T.F.; Berhe, D.H. Effect of neem leaf and seed powders against adult maize weevil (*Sitophilus zeamais* Motschulsky) mortality. *Int. J. Agric. Res.* **2016**, 11, 90–94.
45. Teixeira da Silva, J.A. Mining the essential oils of the Anthemideae. *African J. Biotechnol.* **2004**, 3, 706–720.
46. Nachon, F.; Rosenberry, T.L.; Silman, I.; Sussman, J.L. A second look at the crystal structures of *Drosophila melanogaster* acetylcholinesterase in complex with tacrine derivatives provides insights concerning catalytic intermediates and the design of specific insecticides. *Molecules* **2020**, 25, 1198.
47. Tanuja, J.; Priyanka, S.; Tushar, J.; Subhash, C. *In silico* screening of anti-inflammatory lompounds from Lichen by targeting cyclooxygenase-2. *J. Biomol. Struct. Dyn.* **2019**, 38, 3544–3562.
48. Narramore, S.; Stevenson, C.E.M.; Maxwell, A.; Lawson, D.M.; Fishwick, C.W.G. New insights into the binding bode of pyridine-3-Carboxamide inhibitors of *E. coli* DNA gyrase. *Bioorg. Med. Chem.* **2019**, 27, 3546–3550.
49. Djilani, A.; Dicko, A. *The Therapeutic Benefits of Essential Oils* in *Nutrition, Well-Being, and Health*, Bouayed, J., Bohn, T. (Eds.), Intech: Online Publication; **2012**; pp. 155–178.