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Essential Oil Composition of Aerial Part of *Pluchea ovalis* (Pers.) DC., Silver Nanoparticles Synthesis, and Larvicidal Activities against Fall Armyworm

Yilma Hunde Gonfa ^{1,2}, Fekade Beshah Tessema ^{1,2} , Archana Bachheti ³, Mesfin Getachew Tadesse ^{1,2}, Ebrahim M. Eid ^{4,5} , Sami Abou Fayssal ^{6,7} , Bashir Adelodun ^{8,9} , Kyung Sook Choi ⁹ , Ivan Širić ¹⁰ , Pankaj Kumar ¹¹  and Rakesh Kumar Bachheti ^{1,2,*} 

- ¹ Department of Industrial Chemistry, College of Applied Sciences, Addis Ababa Science and Technology University, Addis Ababa P.O. Box 16417, Ethiopia
 - ² Nanotechnology Centre of Excellence, Addis Ababa Science and Technology University, Addis Ababa P.O. Box 16417, Ethiopia
 - ³ Department of Environment Science, Graphic Era University, Dehradun 248002, Uttarakhand, India
 - ⁴ Biology Department, College of Science, King Khalid University, Abha 61321, Saudi Arabia
 - ⁵ Botany Department, Faculty of Science, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt
 - ⁶ Department of Agronomy, Faculty of Agronomy, University of Forestry, 10 Kliment Ohridski Blvd, 1797 Sofia, Bulgaria
 - ⁷ Department of Plant Production, Faculty of Agriculture, Lebanese University, Beirut 1302, Lebanon
 - ⁸ Department of Agricultural and Biosystems Engineering, University of Ilorin, PMB 1515, Ilorin 240003, Nigeria
 - ⁹ Department of Agricultural Civil Engineering, Kyungpook National University, Daegu 37224, Republic of Korea
 - ¹⁰ University of Zagreb, Faculty of Agriculture, Svetosimunska 25, 10000 Zagreb, Croatia
 - ¹¹ Agro-Ecology and Pollution Research Laboratory, Department of Zoology and Environmental Science, Gurukula Kangri (Deemed to Be University), Haridwar 249404, Uttarakhand, India
- * Correspondence: rkbachheti@gmail.com or rakesh.kumar@aastu.edu.et



Citation: Gonfa, Y.H.; Tessema, F.B.; Bachheti, A.; Tadesse, M.G.; Eid, E.M.; Abou Fayssal, S.; Adelodun, B.; Choi, K.S.; Širić, I.; Kumar, P.; et al. Essential Oil Composition of Aerial Part of *Pluchea ovalis* (Pers.) DC., Silver Nanoparticles Synthesis, and Larvicidal Activities against Fall Armyworm. *Sustainability* **2022**, *14*, 15785. <https://doi.org/10.3390/su142315785>

Academic Editors: Vesna Tumbas Šaponjac, Vanja Šeregelj, Jelena J. Vulić and Peng Zhang

Received: 22 September 2022

Accepted: 23 November 2022

Published: 27 November 2022

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Abstract: In this study, essential oil of the aerial part of *Pluchea ovalis* (POEO) was isolated and employed for the synthesis of AgNPs (POEO-AgNPs). Then, larvicidal activities of POEO and PEO-AgNPs were evaluated against the larvae of fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith); (Lepidoptera: Noctuidae). The potential medicinal values of *P. ovalis* and the lack of scientific reports on the applications of essential oils and nanoparticles of the plant species from the ecology of Ethiopia motivated the authors to carry out this research activity. The hydrodistillation technique was used for the isolation of POEO. Characterization of samples was done using gas chromatography/mass spectrometry (GC/MS), ultraviolet–visible spectroscopy (UV–Vis), scanning electron microscope-energy dispersive X-ray spectroscopy (SEM-EDX), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and zeta nanoanalyzer instruments. GC/MS analysis showed that sesquiterpenes (91.27%) are the dominant chemical constituents of POEO. The characteristic UV–Vis spectra absorption of POEO-AgNPs is observed at 428 nm. SEM imaging reveals that POEO-AgNPs have a dominantly spherical shape. A strong peak of EDX at 3.0 keV shows the existence of Ag element in POEO-AgNPs. XRD analysis determines the diffraction peaks of POEO-AgNPs at 2θ of 38.2°, 44.1°, 64.6°, and 77.8° which are indexed to (111), (200), (220), and (311), respectively. The average particle size and surface potential of POEO-AgNPs are 132 nm and −64.7 mV, respectively. POEO-AgNPs were stored at room temperature and 4 °C and showed good stability for about 6 months without aggregation or dissolution. The larvicidal activity was tested at 500, 250, and 125 µg/mL of POEO solution and 100% (full strength = 0.083 g/mL), 50%, and 25% POEO-AgNPs solution against 2nd instar larvae of *S. frugiperda* for 3 consecutive days. LC₅₀ and LC₉₀ are determined as 154.88 and 11,749.00 µg/mL for POEO and 69.18 and 1318.26% for POEO-AgNPs solutions, respectively. This finding will benefit the applications of POEO and POEO-AgNPs for a sustainable eco-friendly crop pest management method.

Keywords: *Pluchea ovalis*; essential oils; green silver nanoparticles; bioactivity; larvicides

1. Introduction

In many maize- and cabbage-producing countries, currently, fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is spreading rapidly and poses a serious loss of production, threatening the food security and income of the majority of smallholder farmers [1,2]. The larva of fall armyworm is the most invasive stage to destroying crop production of about 350 species from 76 families which is responsible for the loss of 70% of yield in the overall economy [3,4]. As a crop protection approach, current fall armyworm control widely depends on synthetic pesticides. However, the prolonged overuse of synthetic chemicals causes toxicity problems in the environment [5]. Therefore, it is highly important to substitute non-ecofriendly chemicals with green and biocompatible pesticides obtained from sustainable natural sources [6].

Plant extracts and their synthetic products have a great role in controlling pests of crops during the pre-harvesting and post-harvesting periods [7]. For instance, essential oils (EOs) of plants and their chemical constituents have important biological applications in treating various diseases, including crop pests [8–10]. A complex mixture of chemical constituents of EOs uses as promising larvicides due to their greenness, higher biological activities, and extraction at lower cost technology [11]. Currently, due to the cost and toxic properties of synthetic pesticides and some insects having developed resistance to them, EOs and their products are recommended as excellent alternative larvicidal and pupicidal agents for the management of crop pests [12–14]. In the agricultural sector, nanomaterials were also reported for their potential pesticide and insecticides activities [15,16]. Green nanoparticles (NPs) have been applied for the pest-controlling strategy to reduce the usage of harmful chemicals and the generation of toxic side products [17–19]. NPs have wide applications in agricultural fields, such as the control of crop pests and disease management [20,21]. Some studies reported that NPs are nanocarriers as well as active biopesticide agents. The dominant studies on the plant extracts mediated NPs synthesis based larvicidal activity evaluation targeted the management of mosquitoes [22]. Green silver nanoparticles (AgNPs) were also reported as potential nano-insecticides and nano-pesticides [23].

The genus *Pluchea* is a flowering plant in the family. The aerial parts of *Pluchea* plants have traditionally important medicinal values [24]. About 80 plant species of the genus *Pluchea* family are mainly distributed in Africa, Asia, Australia, and Northern and Southern America [25]. Previous literature showed that some species of these plants are extinct; however, only 16 species of the plants were reported to possess traditional uses in several countries of Asia and Northern America [26]. *Pluchea ovalis* (Pers.) DC. is woolly camphorweed. It belongs to the *Asteraceae* family, comprised of about 1600–1700 genera. The plant species is highly rich in sesquiterpene lactones used as bio-inhibitor [27–29]. It is an indigenous plant in Ethiopia. Extracts of petroleum ether, chloroform, ethanol, and aqueous of *P. ovalis* displayed significant growth inhibitory of fungi by using the agar well diffusion assay technique [30]. In some studies, EO of *P. ovalis* was reported to contain the main chemical components such as *p*-cymene, 2,5-dimethoxy-*p*-cymene, limonene, β -phellandrene, isocomene, β -maaliene, δ -cadinene, β -caryophyllene, and α -cadinol [31]. Volatile compounds of pet-ether extracts of the aerial part of *P. ovalis* were identified using GC/MS analysis, constituting dominant sesquiterpene hydrocarbons where α -selinene, humulene oxide, β -eudesmol, and dehydrosanssura were reported as the four leading constituents [32].

Even though the essential oil of *P. ovalis* is a rich source of sesquiterpenes that is responsible for larvicidal activity [33], there were no reports on the plant species nanoparticle synthesis and their evaluation against crop pests. The essential oil of *P. ovalis* and its AgNPs are potentially used as green and sustainable natural products for the management of

insect pests. Therefore, the purpose of this research activity was to isolate the essential oil of the aerial part of *P. ovalis* (POEO), synthesize its AgNPs (POEO-AgNPs), and finally, evaluate their larvicidal activity. The scope of research is to investigate the larvicidal effects of various concentrations of POEO and POEO-AgNPs against 2nd instar larvae of fall armyworms.

2. Materials and Methods

2.1. Plant Material Collection, Authentication, and Preparation

Plant sample (*Pluchea ovalis* (Pers.) DC.) was collected from Addis Ababa Science and Technology University Compound, Addis Ababa, Ethiopia, located at the latitude of 8° 88' North and longitude of 38° 80' East and elevation 2143 m above sea level (m.a.s.l.), in November 2020. A voucher specimen (YH04) was authenticated by Mr. Melaku Wondafrash and deposited at the National Herbarium (ETH), Plant Biology and Biodiversity Management Department, Addis Ababa University, Addis Ababa, Ethiopia. The plant sample was dried under shade for about a month and was ground using an electric grinder. A 2.5 kg powder of plant sample was stored in the brown glass bottle until further analysis.

2.2. Extraction of Essential Oil from *P. ovalis*

Extraction of EO from a powder of aerial part of the plant species (dry weight) was carried out by hydro-distillation technique in a Clevenger-type apparatus for 3 h based on the procedure from European Pharmacopeia (Phar. Eur. Supplement 7.0) and coded as POEO (*P. ovalis* essential oil). To separate POEO from the aqueous phase, it was dried over anhydrous Na₂SO₄ and then stored in a non-transparent plastic bottle in the refrigerator before further use.

2.3. Physicochemical Properties

The density, refractive index, and optical rotation of POEO were determined by density meter (A. Kruss Optronic GmbH, Hamburg, Germany), refractometer (A. Kruss Optronic GmbH, Hamburg, Germany), and optical spectrometer (ADP600 series, Bellingham+Stanley, Royal Tunbridge Wells, UK) instruments.

2.4. Instrument Analyses

GC/MS analysis was employed to identify chemical components of POEO based on the procedure reported by Goyal and co-workers [30]. Dilution of POEO was performed in n-hexane (1:10) and analyzed using GC/MS (Thermo Fisher Scientific Inc., Waltham, MA, USA), which was equipped with a DB-5 (30 m × 0.25 mm i.d., 0.25 µm film thickness) capillary separation column. GC/MS operating conditions comprised the following: oven temperature from 50 to 250 °C, at increments of 5 °C/min, held for 5 min; injector temperature, 220 °C; transfer line 250 °C; carrier gas, Helium at 1.0 mL/min constant flow rate; injection volume of the sample, 1 µL; split ratio, 1:20. Ionization of POEO chemical components was carried out at electron impact 70 eV mode, at an *m/z* range of 5–210. POEO constituents were identified by comparing their experimental Kovat's indices (KIs) relative to normal alkanes of the C₇–C₂₅ and mass (*m/z*) peaks with the data from the NIST database, Wiley spectral libraries, and literature reviews. FTIR analysis was applied to determine the major functional groups that exist in the chemical constituents of essential oil [34]. FTIR spectra were recorded using an FTIR spectrophotometer (iS50 ABX, Thermo SCIENTIFIC, Waltham, MA, USA). A drop of POEO was used with the parameters: resolution: 4 cm^{−1}, spectra range: 400–4000 cm^{−1}, and the number of scans: 32. Origin 2018 software was used to process FTIR spectra.

2.5. Biosynthesis of Silver Nanoparticles

Synthesis of POEO-mediated AgNPs was carried out based on the procedure reported by Paula et al. [35] and using optimized reaction conditions. The solution of 0.1 M HCl or

0.1 M NaOH was used to adjust the pH of the AgNO₃ solution. Color change visualization and UV–Vis spectroscopic method were employed for monitoring AgNP formation.

2.6. Characterization of POEO-AgNPs

Some previously reported procedures were used with some minor modifications for the characterization of POEO-AgNPs. FTIR analysis was used for the characterization of the surface chemistry of synthesized AgNPs. The information about functional groups of chemical compounds of EO of the plant species mainly responsible for reduction, capping, and stabilization processes were determined using FTIR spectroscopy at the frequency range of 4000–400 cm^{−1} [36]. The source of characteristic Surface Plasmon Resonance (SPR) information about POEO-AgNPs synthesis was determined using a UV–Vis spectrophotometer (JASCO V-770, Tokyo, Japan) at the wavelength of 300–700 nm and AgNO₃ solution was used as a reference to adjust the baseline [37]. Surface morphology and elemental composition of POEO-AgNPs were analyzed [38] using the combined SEM-EDX (SSX-550 SEM-EDX, Shimadzu Corp., Kyoto, Japan) with Sigma 300 operated at 20 kV. Crystalline structures of POEO-AgNPs were determined based on the method reported by earlier literature reviews [39] using an X-ray diffractometer (Malvern Panalytical, Melvern, UK) with Cu K α as a radiation source, 40 kV and 30 mA and in the angular range 30–80°. Zeta-nano analyzer (Nano Series HT, Malvern, UK) was applied to measure the average size and surface potential of POEO-AgNPs according to the method provided by Ramdath and co-workers [40]. The physicochemical stability of POEO-AgNPs was evaluated based on the procedure report using the samples deliberately stored at two different conditions: at room temperature and 4 °C. The stability changes were periodically studied using UV–Vis spectroscopy [41] for about 6 months.

2.7. Larvicidal Activity Assay

Larvae of fall armyworm, *S. frugiperda*, were obtained from Ambo Agricultural Research Center, Entomology Laboratory, located at the latitude of 8°57' N and longitude of 37°52' E, the elevation of 2225 m above sea level (m.a.s.l). Based on the standard procedure provided by Alves et al. [13] and under controlled conditions at 25 ± 1 °C, 80 ± 2% RH, and 12 h:12 h (L:D) photoperiod, larvae were reared on the artificial diet up to 1st instar stage and then, they were transferred to fresh leaves of maize until they reached 2nd instar stage. In solution preparation, 0.01% of Tween-80 (purity, 99.99%) was prepared for the dissolution of POEO in the sterilized distilled water. Various concentrations of POEO solution were prepared by using the serial dilution method. Three test concentrations of POEO solution, 500, 250, and 125 µg/mL, were prepared based on the concentration reported previously for another plant essential oil with optimum larvicidal activity, 250 µg/mL as reference. Three different concentrations of POEO-AgNPs solution were also prepared in sterilized distilled water for the evaluation of their larvicidal activity. Full strength (100%), 50%, and 25% concentrations of POEO-AgNPs (0.083 g/mL) were used for the experiment. The solution of Dursban (synthetic pesticide) was applied as a positive control. Tween-80 solution and distilled water were also employed as negative controls. For treatments, the experiments were performed in three replications. Young fresh leaves of maize were provided as larvae food on the Whatman no. 1 filter papers which were placed in the plastic Petri dishes (15 cm × 15 cm × 30 cm). Then, 10 homogenous 2nd instar larvae of fall armyworm were transferred into each Petri dish. Doses of 3 mL of each solution of POEO (500, 250, and 125 µg/mL), POEO-AgNPs (100, 50, and 25%), and negative control (0.01% Tween-80 and Distilled water) and a 1 mL dose of Dursban solution were applied topically on the bodies of larvae using micropipette uniformly. Larvae were placed on the leaves of maize in Petri dishes and covered with the lids properly. Finally, mortality data were recorded by counting the number of dead larvae every 24 h for three consecutive days after the time of exposure.

2.8. Statistical Analysis

The dead larvae data were recorded as mean $\pm$ SD of three replicates experiment using ANOVA (IBM SPSS statistics 26 software). Mortality percentage and Probit analysis were performed using Microsoft Excel to calculate larvae mortality%, LC₅₀, and LC₉₀. Differences between data were assumed to be statistically significant for $p < 0.05$.

3. Results and Discussion

3.1. Extraction of Essential Oil of *P. ovalis*

In this study, a powder of the aerial part of *P. ovalis* (dry weight) was hydrodistilled for about 3 h using Clevenger-type apparatus. The yellow and denser EO of *P. ovalis* (POEO) was collected. Then, POEO was separated and dried from the aqueous phase over anhydrous Na₂SO₄ and the total percentage yield of essential oil was determined as 0.80%.

3.2. Instrumental Analyses of Essential Oil of *P. ovalis*

The determined physicochemical properties of POEO, namely specific gravity at 20 °C, refractive index (nD) at 20 °C, and optical rotation ($^{\circ}\alpha$) at 25.5 °C are 0.2874, 1.4338, and -1.05 , respectively. POEO showed complete solubility in 90% ethanol. GC/MS chromatogram of POEO indicates chemical constituents were eluted in the retention time range of 26.835–44.225 min, in which four of the peaks represent 55% of the total identified peak area percentage (Figure 1). A total of 37 chemical constituents were identified by using GC/MS analysis (Table 1). The determined chemical constituents were dominated by sesquiterpenes (91.27%) which contain 51.84% oxygenated and 39.43% non-oxygenated hydrocarbons. The major chemical component of POEO is junenol, which accounts for 20.73% of the total area percentage of EO composition, and followed by α -cadinol (15.54%), δ -cadinene (12.93%), (+)- β -selinene (5.86%), τ -muurolol (4.70%), β -gurjuren (4.17%), cubebol (3.00%) γ -cadinene (2.99%), and epizonarene (2.69%) that account for 72.61% of the area percentage of EO composition (Figure 2). Determination of the chemical constituents of EO is vital for the study of their larvicidal activity because the biological properties of phytochemicals depend on their chemical structures and synergetic effects [12].

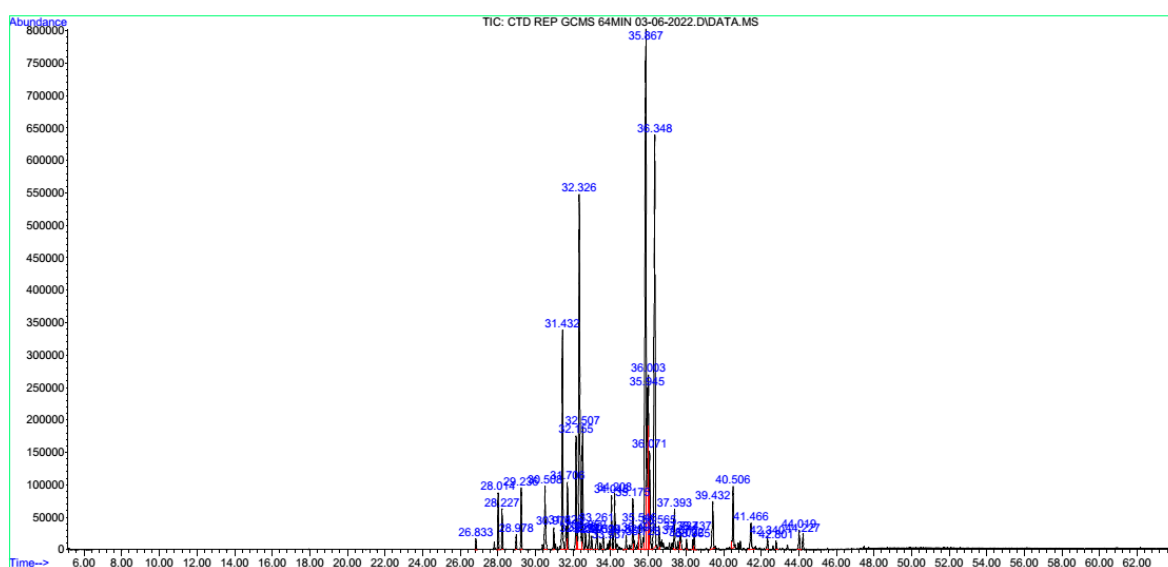


Table 1. GC-MS analysis of essential oil obtained from the aerial part of *P. ovalis*.

RT (min)	KI _{exp}	KI _{lit}	Compound Name	Area%
26.835	1342	1338	Presilphiperfol-7-ene	0.25
28.016	1377	1376	Modephene	1.42
28.225	1383	1382	α -Isocomene	1.06
28.978	1405	1412	β -Isocomene	0.34
29.235	1414	1413	Caryophyllene	1.45
30.506	1455	1451	9-epi- β -Caryophyllene	1.73
30.973	1469	1468	γ -Muurolene	0.49
31.430	1483	1484	(+)- β -Selinene	5.86
31.625	1489	1489	β -Guaiene	0.62
31.706	1492	1491	α -Muurolene	1.67
32.154	1506	1506	γ -Cadinene	2.99
32.220	1509	1510	2-Isopropyl-5-methyl-9-methylene [4.4.0]dec-1-ene	0.28
32.325	1512	1512	δ -Cadinene	12.93
32.506	1518	1518	Cubebol	3.00
32.659	1524	1523	(+)- δ -Cadinene	0.49
32.992	1530	1534	α -Calacorene	0.35
33.259	1535	1545	β -Pinone	0.88
33.597	1544	1554	β -Calacorene	0.43
33.935	1556	1597	Ziza-6(13)-ene	0.21
34.821	1567	1600	β -Oplopenone	0.32
35.173	1570	1608	β -Epicubenol	1.43
35.492	1576	1636	Muurola-4,10(14)-dien-1 β -ol	0.31
35.544	1596	1621	α -Epicubenol	0.79
35.730	1608	1646	11,11-Dimethyl-4,8-bis (methylene)bicyclo [7.2.0] undecan-3-ol	0.37
35.868	1619	1629	Junenol	20.73
35.944	1621	1622	β -Gurjurene	4.17
36.002	1628	1637	τ -Muurolol	4.70
36.073	1633	1672	Epizonarene	2.69
36.349	1636	1649	α -Cadinol	15.54
36.563	1638	1676	1,2,3,4-Tetrahydro-1,6-dimethyl-4-(1-methylethyl)-1-naphthalenol	0.62
37.392	1640	1687	Schyobunol	1.09
37.735	1650	1694	Eudesma-4(15),7-dien-1 β -ol	0.41
38.030	1658	1752	(+)- γ -Costol	0.22
38.440	1687	1730	10-Hydroxyoplopanone	0.46
41.463	1696	1810	14-Hydroxy- δ -cadinene	1.07
42.340	1698	1867	Platambin	0.36
44.225	1710	1983	β -Cyclocostunolide	0.42
Sesquiterpenes				91.27
Oxygenated sesquiterpene hydrocarbons				51.84
Non-oxygenated sesquiterpene hydrocarbons				39.43
Non-terpene hydrocarbons				0.88
Total composition%				92.15

Even though there are no reports on the essential oil of the aerial part of *P. ovalis* using the hydrodistillation approach, previous studies on the essential oils of some *Pluchea* plant species using other extraction techniques displayed good agreement with the results obtained in this study regarding the dominance of sesquiterpenes. For example, some earlier research findings on GC/MS analysis of EO of pet-ether extract of the aerial part of *P. ovalis* reported about 15 chemical constituents which account for 61% of the total composition. The same study demonstrated that sesquiterpenes are the dominant chemical components with 52.85%, while oxygenated hydrocarbons represented 40.88% and selinene was a major component (33.40%) of the EO of *P. ovalis* [32].

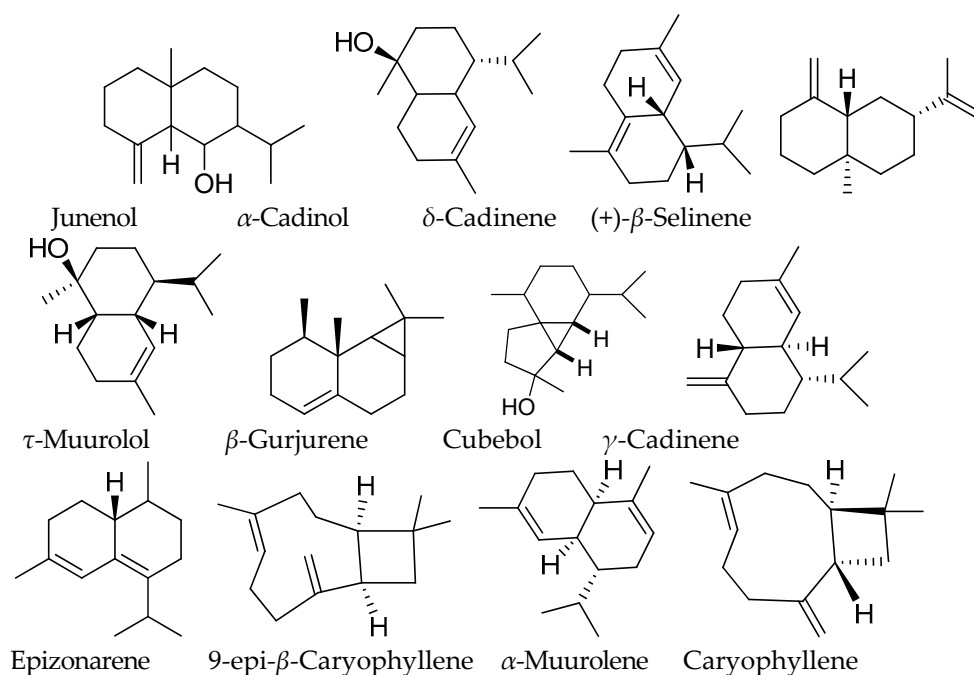


Figure 2. Structures of the first 12 main constituents of essential oil of aerial part of *P. ovalis*.

Sesquiterpenes were also reported as dominant chemical constituents of EO of leaves of *P. dioscoridis* using the hydrodistillation technique, where farnesol and α-madeleine were major components with a percentage composition of 16.5% and 18.84%, respectively [25,42]. Eudesmane-type sesquiterpene was also reported from EOs of *P. dioscoridis* as one of the significant chemical constituents [43,44]. From the stem and callus powder of *P. lanceolata*, hexadecanoic acid (19.23%) and 2-(vinylxy) ethanol (16.46%) were reported as major components for their EOs [45].

3.3. Green Synthesis of AgNPs

The optimized reaction conditions as pH of AgNO₃ solution (11), the volume of AgNO₃ solution (80 mL), the concentration of AgNO₃ solution (3×10^{-3} M), the volume of POEO solution (30 mL), time of reaction (30 min), and temperature of reaction (80 °C) were used for the green synthesis of AgNPs (POEO-AgNPs).

3.4. Characterization of POEO-AgNPs

Major FTIR peaks absorptions of POEO are observed at 3454 cm⁻¹, 2922 cm⁻¹, 2860 cm⁻¹, 1700 cm⁻¹, 1639 cm⁻¹, 1449 cm⁻¹, 1374 cm⁻¹, and 855 cm⁻¹ (Figure 3). FTIR peak at 3454 cm⁻¹ represents alcoholic -O-H bond stretching. The *sp*³C-H symmetric and asymmetric bond stretching are absorbed in 2922 cm⁻¹ and 2860 cm⁻¹, respectively. Peak absorption at 1700 cm⁻¹ and 1639 cm⁻¹ correspond to the bond stretching of the olefin C=C functional group. Peaks observed at 1449 cm⁻¹ and 1374 cm⁻¹ indicate the presence of aromatic C-H in-plane bending and a peak at 855 cm⁻¹ demonstrates aromatic C-H out-of-plane bending. FTIR peaks obtained for functional groups of chemical constituents of POEO are in significant agreement with some previous studies [34,46–48]. FTIR analysis is also used to identify the bond formation possibility between Ag metal surface and chemical constituents of EO in green AgNPs [37]. FTIR spectra indicate the disappearance of major peaks in the constituents of POEO and the emergence of two dominant spectra at 3295 cm⁻¹ and 1632 cm⁻¹ for POEO-AgNPs assigned to O-H and olefinic C=C bond stretching which is in line with the previous report by Akintayo et al. [34]. Therefore, oxygenated and olefinic groups are more probably used as reducing, stabilizing, and capping sites in POEO-AgNPs.

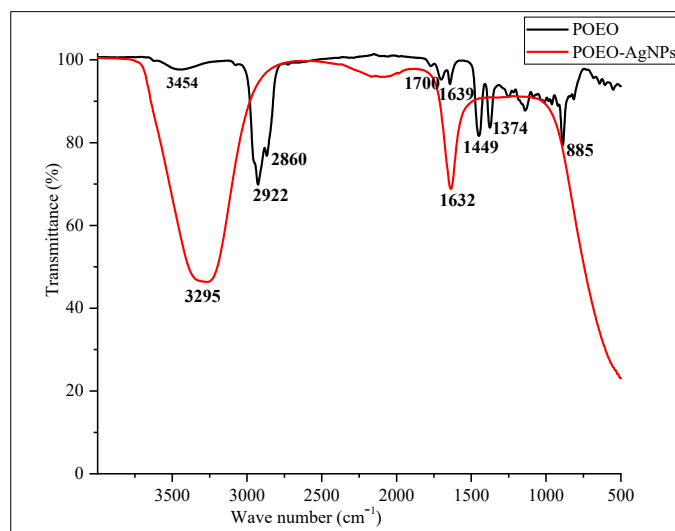


Figure 3. The combined FTIR spectra of essential oil of *P. ovalis* (POEO) and its silver nanoparticles (POEO-AgNPs).

UV–Vis spectroscopic analysis also confirms the successful formation of POEO-AgNPs (Figure 4). The characteristic UV–Vis spectra of AgNPs were absorbed in 428 nm due to the characteristic electron excitation phenomena on the surface of POEO-AgNPs which are responsible for the appearance of the brown solution. Hence, the obtained results are in good agreement with the previously reported studies [36,49–51].

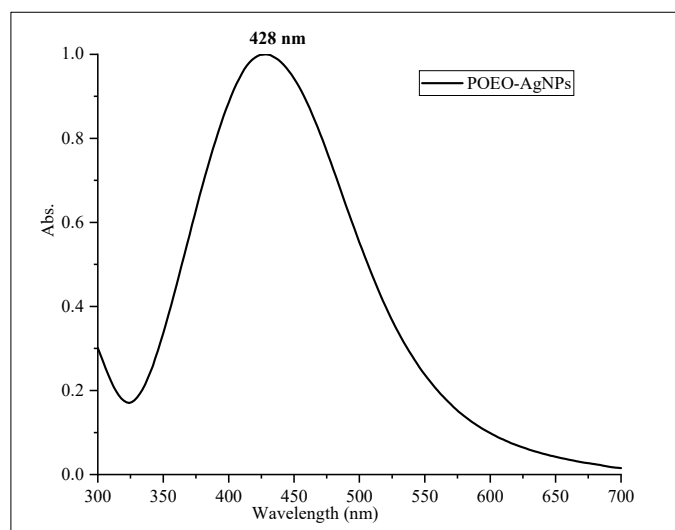


Figure 4. UV–Vis spectra of essential oil of *P. ovalis* mediated silver nanoparticles (POEO-AgNPs) at the optimized reaction conditions.

The average size, shape, and surface morphology of POEO-AgNPs were determined by using SEM image analysis (Figure 5). SEM image displays a predominately spherical shape of POEO-AgNPs with an average size of 80.44 nm, which was determined using ImageJ software. The EDX profile depicts the presence of characteristic peaks of Ag element at 3.0 keV. These results confirmed that the formation of POEO-AgNPs agrees with some literature reports on the biogenic synthesis of AgNPs in some previous studies [38,52].

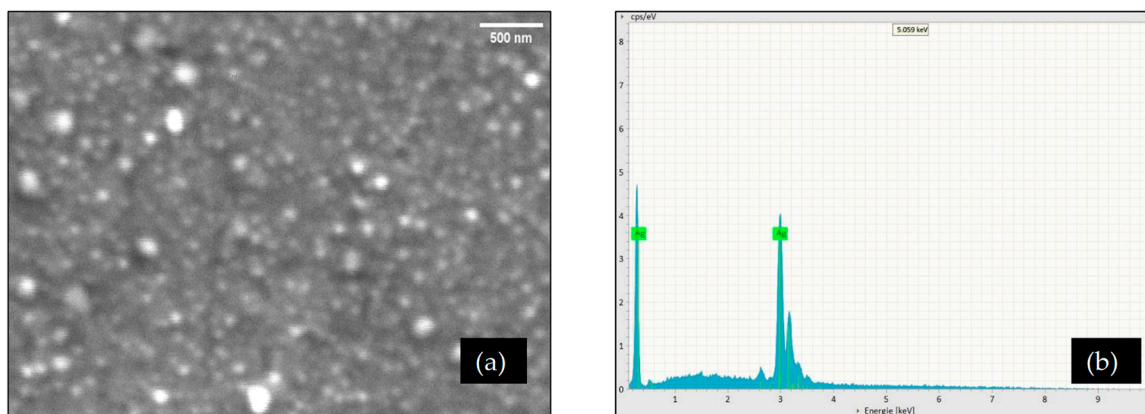


Figure 5. (a) SEM image; (b) EDX of essential oil of *P. ovalis* mediated silver nanoparticles (POEO-AgNPs).

The crystal property of POEO-AgNPs is determined using the analysis of XRD peaks (Figure 6). XRD peaks observed at 2θ values of 38.2° , 44.1° , 64.6° , and 77.8° correspond to (111), (200), (220), and (311) plane indices, respectively, which indicate the face-centered cubic structure of POEO-AgNPs. The determined XRD results are in line with some previously reported results with JCPDS Card No. 65-2871 on other plants' extract-assisted AgNPs by Mahajan et al. [39].

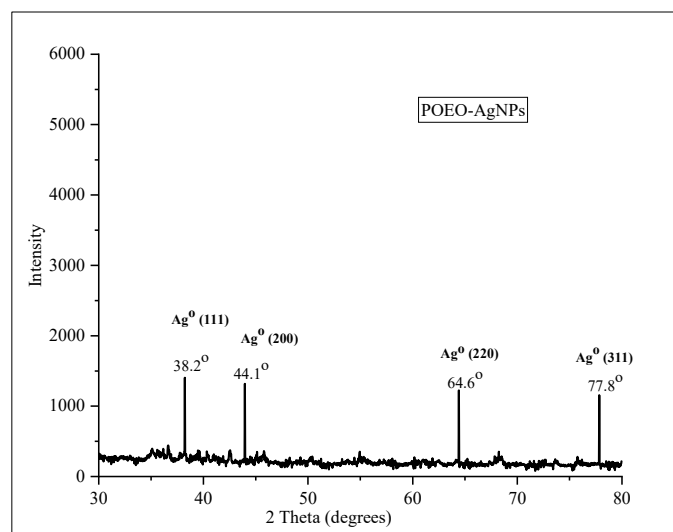


Figure 6. XRD peaks of essential oil of *P. ovalis*-mediated silver nanoparticles (POEO-AgNPs).

In this study, the average particle size, zeta potential, and polydispersity index of POEO-AgNPs are obtained as 132 nm, -64.7 mV, and 0.457, respectively (Figure 7). Particle size and surface potential are important parameters to confirm the stability of synthesized nanoparticles [40]. According to some literature reports, significant electrostatic repulsion to develop stability for the synthesized AgNPs was reported for surface potential values greater than ± 30 mV [53]. Hence, the known literature reports highly support the obtained surface potential result (-64.7 mV) for POEO-AgNPs.

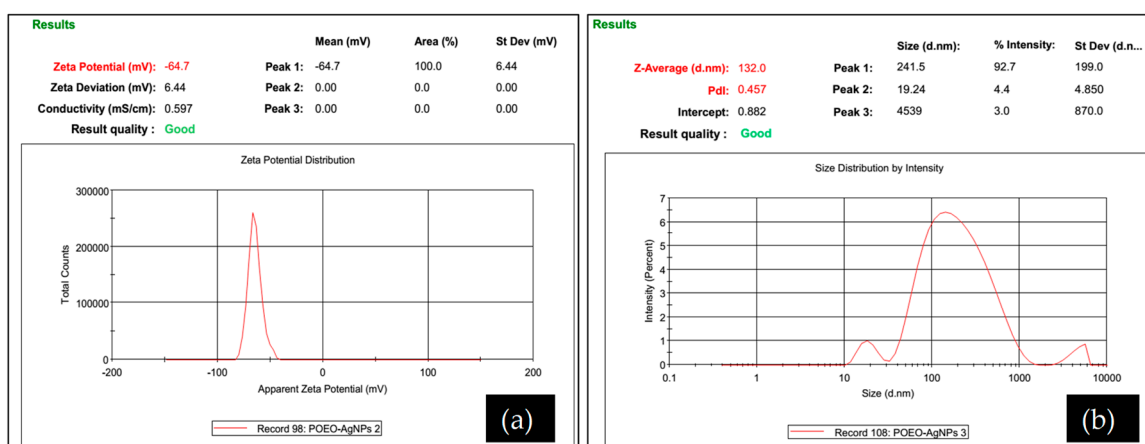


Figure 7. (a) Zeta size; (b) surface potential of essential oil of *P. ovalis* mediated silver nanoparticles (POEO-AgNPs).

The samples of POEO-AgNPs were stored for 6 months at 4 °C and room temperature for their stability tests. Monitoring with UV–Vis spectroscopy, POEO-AgNPs displayed good physicochemical stability at both temperature conditions for a longer period, as shown in Figure 8. Therefore, the potential physicochemical stability of synthesized green AgNPs may be an important attribute to their efficient applications for a longer period at different temperature conditions.

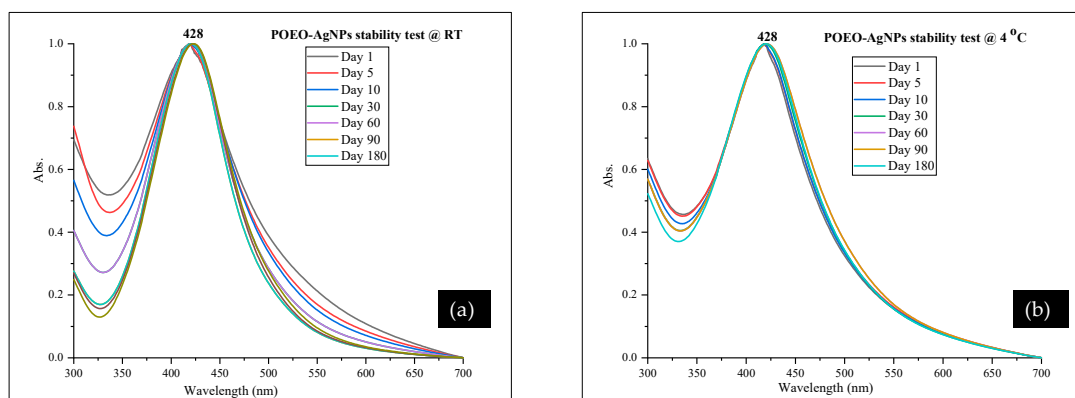


Figure 8. Physicochemical stability tests of essential oil of *P. ovalis* mediated silver nanoparticles (POEO-AgNPs) for 6 months: (a) at room temperature, (b) below 4 °C.

3.5. Larvicidal Activity Tests of POEO and Its AgNPs Solutions against Fall Armyworm Larvae

Larvicidal activity tests of POEO and POEO-AgNPs solutions were carried out at different concentrations and their effects against 2nd instar fall armyworm were evaluated after an exposure time of 72 h (Figure 9). The POEO solution test at 500, 250, and 125 µg/mL showed a mortality percentage of 63.30%, 56.70%, and 46.70%, respectively (Table 2). Tukey HSD analysis showed that the concentrations of POEO solution indicate non-significant ($p > 0.05$) larvicidal activity. However, more larvicidal effects were observed as the concentration of essential oil increased. For the test POEO solution, the maximum larvicidal activity was obtained at the concentration of 500 µg/mL with a mortality percentage of 63.30%. The larvicidal effect of POEO solution is higher at a stronger concentration due to the increase in the amount of sesquiterpene constituents. The significant larvicidal activity of essential plant extracts is mainly due to the biological activity of main chemical constituents [54]. Full strength (100%), 50%, and 25% of POEO-AgNPs solution were also applied for the larvicidal activity evaluation and the results indicate non-significant differences ($p > 0.05$), and their mortality percentages were determined as 53.30%, 43.30%, and

30.00%, respectively (Table 2). At full strength (100%) of POEO-AgNPs solution, the maximum larvicidal effect is observed because of the presence of more concentrated bioactive components in the specific solution. This result is consistent with the previously reported research findings on some other plant extracts used for mosquito larvae control [55]. Tukey HSD analysis exhibits significantly different larvicidal activity ($p < 0.05$) for POEO solution and higher mortality percentage than its AgNPs at all concentrations of test solutions against 2nd instar larvae of fall armyworm insect. The larvicidal activity evaluation of the POEO-AgNPs solution generally shows a lower mortality percentage as compared to the POEO solution (Table 2). The reason for the reduction of larvicide effect may be the lower accessibility of functional groups of chemical constituents, which are responsible for bioactivity due to their use as capping and stabilizing agents in the POEO-AgNPs formation and ineffectiveness of synthesized AgNPs due to its size tuning nature [56].

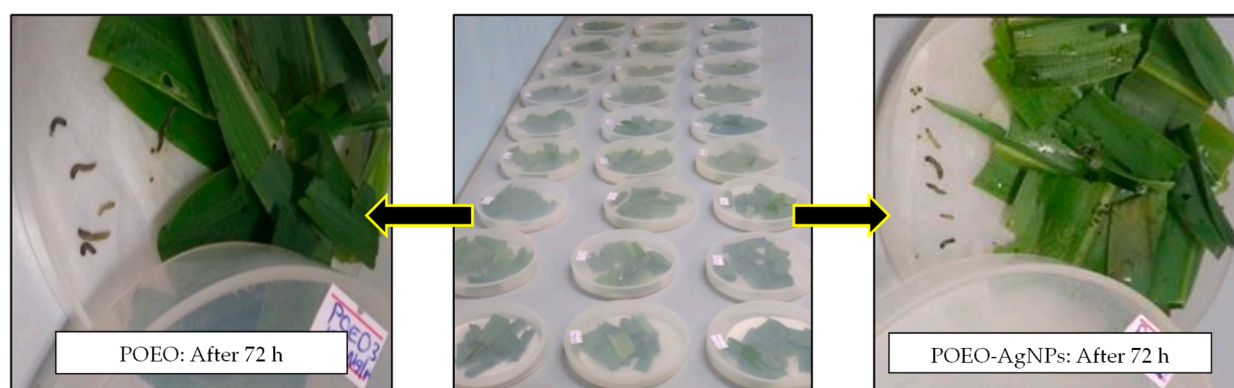


Figure 9. Some photographic documentation of POEO and POEO-AgNPs solutions activities against 2nd instar larvae of fall armyworm.

Table 2. The effects of various concentrations of POEO and its AgNPs on fall armyworm, *S. frugiperda*, larvae mortality rate, and lethal concentrations.

Test Sample	Concentration	Larvae *				
		Total	Dead (Mean + SD)	Mortality%	LC50	LC90
POEO solution ($\mu\text{g/mL}$)	500	10	6.33 + 1.15 ^a	63.30	154.88	11,749.00
	250	10	5.67 + 0.58 ^a	56.70		
	125	10	4.67 + 1.53 ^a	46.70		
POEO-AgNPs solution	100%	10	5.33 + 1.15 ^b	53.30	69.18	1318.26
	50%	10	4.33 + 0.58 ^b	43.30		
	25%	10	3.00 + 0.00 ^b	30.0		
Dursban (+ve control)	-	10	9.67 + 0.58	96.70		
Tween-80 (-ve control)	-	10	0.00 + 0.00	0.00		
DH ₂ O (-ve control)	-	10	0.00 + 0.00	0.00		

* Dead larvae numbers are expressed as the mean of three replicates $\pm$ SD. Positive control = Dursban solution, negative controls = distilled H₂O and Tween-80 solution, full strength of POEO-AgNPs solution = 100% conc. A one-way analysis of variance (ANOVA) was performed with IBM SPSS 26 and means with different letters show significantly different values.

Probit analysis showed that test concentrations of POEO solution were found to possess larvicidal effects against 2nd instar larvae of fall armyworm with the lethal concentrations at 50% and 90% (i.e., LC₅₀ and LC₉₀) values of 154.88 and 11,749.00 $\mu\text{g/mL}$, respectively, at 72 h. Similarly, LC₅₀ and LC₉₀ values of concentrations of POEO-AgNPs solution were determined as 69.18 and 1318.26%, respectively, (Table 2). However, larvicidal effects of some genus *Pluchea* were reported in the earlier literature reviews, in this study,

the synthesis of NPs and larvicidal activity examination using EO and AgNPs of *P. ovalis* are reported for the first time. *P. dioscoridis* exhibited significant larvicidal activity against *Culex pipiens* with $LC_{50} = 71.86$ ppm [25]. Some studies support the actions of POEO and its AgNPs mechanisms for larval death. The lethal effects of POEO and POEO-AgNPs against larvae of fall armyworm may be due to their enzymatic activity inhibition effects [17]. EOs of plants and their chemical constituents may also disrupt the endocrinological balance of the organisms. Chemical constituents of EOs are reported to damage larvae respiration, feeding systems, and the flying ability of adult insects [12]. There are also some proposed possible mechanisms of action of AgNPs on the organism; the first possibility is the higher surface areas of AgNPs create contact with the organism and allow the attachment of NPs to the cell membrane and easily penetrate the organism and cause disruption of their contents [57]. The second possibility is after the penetration of AgNPs into the mitochondria of microorganisms, they release Ag^+ ions which cause the death of cells of the organism [58].

4. Conclusions

In this study, essential oil from the aerial part of *P. ovalis* (POEO) was isolated using the hydrodistillation technique. GC/MS analysis shows POEO contains sesquiterpenes (91.27%), the phytochemicals known for their potential larvicides, as the mainly dominant chemical component with junenol (20.73%) as the major compound of POEO. The next leading chemical components are α -cadinol (15.54%), δ -cadinene (12.93%), (+)- β -selinene (5.86%), τ -muurolol (4.70%), β -gurjurene (4.17%), cubebol (3.00%), γ -cadinene (2.99%), and epizonarene (2.69%). The chemical constituents of POEO are used as the potential bio-reducing, capping, and stabilizing agents in the synthesis of AgNPs. The successful formation of POEO-AgNPs was confirmed by the characteristic UV–Vis spectra absorption at 428 nm, EDX peak appearance at 3.0 keV, and their crystalline nature determined from XRD peaks and the assigned lattice indices. The physical, chemical, and biological properties of the synthesized AgNPs were determined from their average size, surface potentials, functionality, and significant storage physicochemical stability at different temperature conditions by using zeta nanoanalyzer, FTIR, and UV–Vis analyses. The significant larvicidal activity of POEO solution (500, 250, and 125 μ g/mL) and POEO-AgNPs solutions (0.083 g/mL) at full strength (100%), 50%, and 25% against 2nd instar larvae of fall armyworm was observed in the assays. Larvicidal tests of POEO and POEO-AgNPs solutions show LC_{50} and LC_{90} at 154.88 and 11,749.00 μ g/mL and 69.18 and 1318.26%, respectively. This research report is the first finding on the essential oil of the aerial part of *P. ovalis*, its AgNPs, and their larvicidal activity from the ecology of Ethiopia as a potential green control technique for crop insects such as fall armyworm. Hence, in the future, the efficacy of POEO and POEO-AgNPs suggests that extracts of the plant species and their sources can be exploited as less toxic, sustainable, biocompatible, cost-effective, and eco-friendly larvicides as an alternative crop pest management.

Author Contributions: Conceptualization, Y.H.G.; formal analysis, Y.H.G.; funding acquisition, P.K.; investigation, Y.H.G.; methodology, Y.H.G.; supervision, M.G.T. and R.K.B.; validation, F.B.T., A.B., M.G.T., E.M.E., S.A.F., K.S.C., I.Š. and P.K.; visualization, F.B.T. and B.A.; writing—original draft, Y.H.G.; writing—review and editing, F.B.T., A.B., M.G.T., E.M.E., S.A.F., B.A., K.S.C., I.Š. and P.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the members of the Sustainable Agro-Environment International Research Group (SAEIRG/2022/01).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data or materials regarding this research are available from the corresponding author on reasonable request.

Acknowledgments: The authors want to thank the Ambo Agriculture Research center for allowing us to use their laboratory resources. We also acknowledge Habtamu, the Entomology research team, and the Virology research team, Ambo Research Center, for their genuine assistance during larvicidal activity experiments.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Siddhartha, K.; Chinniah, C.; Shanthi, M. In vitro bioassay of certain botanical oils for their efficacy against maize fall armyworm (J.E. Smith) *Spodoptera frugiperda* (Noctuidae: Lepidoptera). *J. Entomol. Zool. Stud.* **2019**, *7*, 606–609.
- Tamboa, J.A.; Day, R.K.; Lamontagne-Godwin, J. Tackling fall armyworm (*Spodoptera frugiperda*) outbreak in Africa: An analysis of farmers' control actions. *Int. J. Pest Manag.* **2020**, *66*, 298–310. [\[CrossRef\]](#)
- Sagar, G.C.; Aastha, B.; Laxman, K. An introduction of fall armyworm (*Spodoptera frugiperda*) with management strategies: A review paper. *Nippon J. Environ. Sci.* **2020**, *1*, 1010. [\[CrossRef\]](#)
- Ahmed, K.S.; Idrees, A.; Majeed, M.Z.; Majeed, M.I.; Shehzad, M.Z.; Ullah, M.I.; Afzal, A.; Li, J. Synergized toxicity of promising plant extracts and synthetic chemicals against fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in Pakistan. *Agronomy* **2022**, *12*, 1289. [\[CrossRef\]](#)
- Phambala, K.; Tembo, Y.; Kasambala, T.; Kabambe, V.H.; Stevenson, P.C.; Belmain, S.C. Bioactivity of common pesticidal plants on fall armyworm larvae (*Spodoptera frugiperda*). *Plants* **2020**, *9*, 112. [\[CrossRef\]](#)
- Minh-Trong, T.; Linh-Phuong, N.; Dinh-Truong, N.; Le Cam-Huong, T.; Chi-Hien, D.; Kim, C.T.T.; Thanh-Danh, N. A novel approach using plant embryos for green synthesis of silver nanoparticles as antibacterial and catalytic agent. *Res. Chem. Intermed.* **2021**, *47*, 4613–4633. [\[CrossRef\]](#)
- Aouadi, G.; Soltani, A.; Grami, L.K.; Abada, M.B.; Haouel, S.; Boushih, E.; Chaanbi, M.; Elkahoui, S.; Hajlaoui, M.R.; Jemaa, J.M.B.; et al. Chemical investigations on Algerian *Mentha rotundifolia* and *Myrtus communis* essential oils and assessment of their insecticidal and antifungal activities. *Int. J. Agric. Biol.* **2021**, *26*, 667–680. [\[CrossRef\]](#)
- Isman, M.B. Plant essential oils for pest and disease management. *J. Crop Prot.* **2000**, *19*, 603–608. [\[CrossRef\]](#)
- Kerdudo, A.; Gonnot, V.; Ellong, E.N.; Boyer, L.; Chandre, F.; Adenet, S.; Rochefort, K.; Michel, T.; Fernandez, X. Composition and bioactivity of *Pluchea carolinensis* (Jack.) G. essential oil from Martinique. *Ind. Crops Prod.* **2016**, *89*, 295–302. [\[CrossRef\]](#)
- Martins, E.; Poncelet, D.; Rodrigues, R.C.; Renard, D. Oil encapsulation techniques using alginate as encapsulating agent: Applications and drawbacks. *J. Microencapsul.* **2017**, *34*, 754–771. [\[CrossRef\]](#)
- Dias, C.N.; Alves, L.P.L.; Rodrigues, K.A.F.; Brito, M.C.A.; Rosa, C.S.; Amaral, F.M.M.; Monteiro, O.S.; Andrade, E.H.A.; Maia, J.G.S.; Moraes, D.F.C. Chemical composition and larvicidal activity of essential oils extracted from Brazilian legal Amazon plants against *Aedes aegypti* L. (Diptera: Culicidae). *Evid.-Based Complement. Altern. Med.* **2015**, 490765. [\[CrossRef\]](#)
- Chantawee, A.; Soonwera, M. Efficacies of four plant essential oils as larvicide, pupicide and oviposition deterrent agents against dengue fever mosquito, *Aedes aegypti* Linn. (Diptera: Culicidae). *Asian Pac. J. Trop. Biomed.* **2018**, *8*, 217–225. [\[CrossRef\]](#)
- Alves, A.; Filho, P.; Pessoa, G.C.; Yamaguchi, L.F.; Kato, M.J. Larvicidal activity of essential oils from Piper Species against strains of *Aedes aegypti* (Diptera: Culicidae) resistant to Pyrethroids. *Front. Plant Sci.* **2021**, *12*, 685864. [\[CrossRef\]](#)
- Gupta, M.; Gupta, D. Essential oils: As potential larvicides. *J. Drug Deliv. Ther.* **2022**, *12*, 193–201. [\[CrossRef\]](#)
- Kale, S.K.; Parishwad, G.V.; Husainy, A.S.; Patil, A.S. Emerging agriculture applications of silver nanoparticles. *ES Food Agrofor.* **2021**, *3*, 17–22. [\[CrossRef\]](#)
- Montes-Garcia, V.; Squillaci, M.A.; Diez-Castellnou, M.; Ong, Q.K.; Stellacci, F.; Samori, P. Chemical sensing with Au and Ag nanoparticles. *Chem. Soc. Rev.* **2021**, *50*, 1269–1304. [\[CrossRef\]](#)
- Shehabeldine, A.M.; Elbahnasawy, M.A.; Hasaballah, A.I. Green photosynthesis of silver nanoparticles using *Echinochloa stagnina* extract with reference to their antibacterial, cytotoxic, and larvicidal activities. *Bionanoscience* **2021**, *11*, 526–538. [\[CrossRef\]](#)
- Allam, R.O.; Badawy, A.M.; Ali, M.A. Green synthesized silver nanoparticles for controlling subterranean termites, *Psammotermes hypostoma* (Desn.). *SVU-Int. J. Agric. Sci.* **2022**, *4*, 135–143. [\[CrossRef\]](#)
- Poudel, D.K.; Niraula, P.; Aryal, H.; Budhathoki, B.; Phuyal, S.; Marahatha, R.; Subedi, K. Plant-mediated green synthesis of AgNPs and their possible applications: A critical review. *J. Nanotechnol.* **2022**, 2779237. [\[CrossRef\]](#)
- Sahayaraj, K.; Madasamy, M.; Radhika, S.A. Insecticidal activity of bio-silver and gold nanoparticles against *Pericallia ricini* Fab. (Lepidoptera: Archidae). *J. Biopestic.* **2016**, *9*, 63–72.
- Rehman, H.; Majeed, B.; Farooqi, M.A.; Rasul, A.; Sagheer, M.; Ali, Q.; Akhtar, Z.R. Green synthesis of silver nitrate nanoparticles from *Camelina sativa* (L.) and its effect to control insect pests of stored grains. *Int. J. Trop. Insect Sci.* **2021**, *41*, 3031–3039. [\[CrossRef\]](#)
- Benelli, C.G.; Losic, D. Nanoparticles for pest control: Current status and future perspectives. *J. Pest Sci.* **2017**, *91*, 1–15. [\[CrossRef\]](#)
- Huq, M.A.; Ashrafudoulla, M.; Rahman, M.M.; Balusamy, S.R.; Akter, S. Green synthesis and potential antibacterial applications of bioactive silver nanoparticles: A review. *Polymers* **2022**, *14*, 742. [\[CrossRef\]](#) [\[PubMed\]](#)
- Perera, W.; Kevers, C.; Michiels, J.A.; Spengler, S.I.; Dommes, J. Variation in phenolic constituents and antioxidant capacities of plant organs of three Cuban species of *Pluchea* Cass. (Asteraceae) under ex vitro and in vitro growth conditions. *J. Med. Plant Res.* **2013**, *7*, 2527–2536. [\[CrossRef\]](#)

25. Hussain, H.; Al-Harrasi, A.; Abbas, G.; Ur Rehman, N.; Mabood, F.; Ahmed, I.; Saleem, M.; van Ree, T.; Green, I.R.; Anwarg, S.; et al. The genus *Pluchea*: Phytochemistry, traditional uses, and biological activities. *Chem. Biodivers.* **2013**, *10*, 1944–1971. [\[CrossRef\]](#)
26. Srivastava, P.; Shanker, K. *Pluchea lanceolata* (Rasana): Chemical and biological potential of Rasayana herb used in traditional system of medicine. *Fitoterapia* **2012**, *83*, 1371–1385. [\[CrossRef\]](#)
27. Mandeel, Q.; Taha, A. Assessment of in vitro antifungal activities of various extracts of indigenous Bahraini medicinal plants. *Pharm. Biol.* **2005**, *43*, 340–348. [\[CrossRef\]](#)
28. Saba, N.; Khatoun, R.; Ahmad, V.U.; Khan, S.S. A new eudesmane sesquiterpene from *Pluchea arguta*. *Nat. Prod. Comm.* **2011**, *6*, 1–2. [\[CrossRef\]](#)
29. Ruan, J.; Li, Z.; Yan, J.; Huang, P.; Yu, H.; Han, L.; Zhang, Y.; Wang, Y. Bioactive constituents from the aerial parts of *Pluchea indica* less. *Molecules* **2018**, *23*, 2104. [\[CrossRef\]](#)
30. Goyal, S.K. Biological studies of the plants from genus *Pluchea*. *Ann. Biol. Res.* **2011**, *2*, 25–34.
31. Shahin, S.M.; Jaleel, A.; Alyafei, M.A. The essential oil-bearing plants in the United Arab Emirates (UAE): An overview. *Molecules* **2021**, *26*, 6486. [\[CrossRef\]](#)
32. Alghamdi, S.B. Identification of volatile constituents of Albaha *Pluchea ovalis*. *Int. Mod. Pharm. Res.* **2020**, *4*, 60–69.
33. Magalhães, L.A.M.; Lima, M.P.; Marques, M.O.M.; Facanali, R.; Pinto, A.C.S.; Tadei, W.P. Chemical composition and larvicidal activity against *Aedes aegypti* larvae of essential oils from four *Guarea* species. *Molecules* **2010**, *15*, 5734–5741. [\[CrossRef\]](#)
34. Akintayo, G.O.; Lateef, A.; Azeez, M.A.; Asafa, T.B.; Oladipo, I.C.; Badmus, J.A.; Ojo, S.A.; Elegbede, J.A.; Gueguim-Kana, E.B.; Beukes, L.S.; et al. Synthesis, bioactivities, and cytogenotoxicity of animal fur-mediated silver nanoparticles. *IOP Conf. Ser. Mater. Sci. Eng.* **2020**, *805*, 1–24. [\[CrossRef\]](#)
35. Paula, A.; Melo, Z.D.; Vinicius, M.; Brisola, D.O.; Sganzerla, W.G. Antibacterial activity, morphology, and physicochemical stability of biosynthesized silver nanoparticles using thyme (*Thymus vulgaris*) essential oil. *Mater. Res. Express* **2020**, *7*, 015087. [\[CrossRef\]](#)
36. Singh, D.; Rathod, V.; Ninganagouda, S.; Hiremath, J.; Singh, A.K.; Mathew, J. Optimization and characterization of silver nanoparticle by endophytic fungi *Penicillium* sp. isolated from *Curcuma longa* (Turmeric) and application studies against MDR *E. coli* and *S. aureus*. *Bioinorg. Chem. Appl.* **2014**, 408021. [\[CrossRef\]](#)
37. Maciel, M.V.B.; Almeida, A.R.; Machado, M.H.; Elias, W.C.; da Rosa, C.G.; Teixeira, G.L.; Noronha, C.M.; Bertoldi, F.C.; Nunes, M.R.; de Armas, R.D.; et al. Green synthesis, characteristics, and antimicrobial activity of silver nanoparticles mediated by essential oils as reducing agents. *Biocatal. Agric. Biotechnol.* **2020**, *28*, 101746. [\[CrossRef\]](#)
38. Alsubki, R.; Tabassum, H.; Abudawood, M.; Rabaan, A.A.; Alsobaie, S.F.; Ansar, S. Green synthesis, characterization, enhanced functionality, and biological evaluation of silver nanoparticles based on *Coriander sativum*. *Saudi J. Biol. Sci.* **2021**, *28*, 2102–2108. [\[CrossRef\]](#)
39. Mahajan, P.G.; Dige, N.C.; Vanjare, B.D.; Phull, A.R.; Kim, S.J.; Lee, K.H. Gallotannin mediated silver colloidal nanoparticles as multifunctional nano platform: Rapid colorimetric and turn-on fluorescent sensor for Hg²⁺, catalytic and in vitro anticancer activities. *J. Lumin.* **2019**, *206*, 624–633. [\[CrossRef\]](#)
40. Ramdath, S.; Mellem, J.; Mbatha, L.S. Anticancer and antimicrobial activity evaluation of Cowpea-Porous-Starch-Formulated silver nanoparticles. *J. Nanotechnol.* **2021**, 5525690. [\[CrossRef\]](#)
41. Barman, K.; Chowdhury, D.; Baruah, P.K. Bio-synthesized silver nanoparticles using *Zingiber officinale* rhizome extract as efficient catalyst for the degradation of environmental pollutants. *Inorg. Nano-Met. Chem.* **2020**, *50*, 57–65. [\[CrossRef\]](#)
42. Elgamal, A.M.; Ahmed, R.F.; Abd-Elgawad, A.M.; Gendy, A.E.; Elshamy, A.I.; Nassar, M.I. Chemical profiles, anti-cancer, and anti-aging activities of essential oils of *Pluchea dioscoridis* (L.) DC. and *Erigeron bonariensis* L. *Plants* **2021**, *10*, 667. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Kibrom, G.; Dibaba, A.; Nigist, A. Cuathemone sesquiterpenes and flavones from *Laggera tomentosa* endemic to Ethiopia. *Bull. Chem. Soc. Ethiop.* **2010**, *24*, 267–271.
44. Gao, C.X.; Lin, F.X.; Luo, Y.X.; Zhou, X.M. Eudesmane-type sesquiterpenes and aporphine-type alkaloids from *Fissistigma maclurei*. *Biochem. Syst. Ecol.* **2021**, *97*, 104298. [\[CrossRef\]](#)
45. Kavi, G.; Raghunandan, S.N.; Deepika, A. GC-MS analysis and identification of daidzein by high performance thin layer chromatography (HPTLC) of *Pluchea lanceolata*: A bone healing plant of semi-arid land. *J. Pharm. Res.* **2012**, *5*, 257–260.
46. Karthik, R.; Chen, S.M.; Elangovan, A.; Muthukrishnan, P.; Shanmugam, R.; Lou, B.S. Phyto mediated biogenic synthesis of gold nanoparticles using *Cerasus serrulata* and its utility in detecting hydrazine, microbial activity, and DFT studies. *J. Colloid Interface Sci.* **2016**, *468*, 163–175. [\[CrossRef\]](#)
47. Keijok, W.J.; Pereira, R.H.A.; Alvarez, L.A.C.; Prado, A.R.; da Silva, A.R.; Ribeiro, J.; de Oliveira, J.P.; Guimarães, M.C. Controlled biosynthesis of gold nanoparticles with *Coffea arabica* using factorial design. *Sci. Rep.* **2019**, *9*, 16019. [\[CrossRef\]](#)
48. Khan, S.; Singh, S.; Gaikwad, S.; Nawani, N.; Junnarkar, M.; Pawar, S.V. Optimization of process parameters for the synthesis of silver nanoparticles from Piper betle leaf aqueous extract, and evaluation of their antiphytofungic activity. *Environ. Sci. Pollut. Res.* **2020**, *27*, 27221–27233. [\[CrossRef\]](#)
49. Chokshi, K.; Pancha, I.; Ghosh, T.; Paliwal, C.; Maurya, R.; Ghosh, A.; Mishra, S. Green synthesis, characterization, and antioxidant potential of silver nanoparticles biosynthesized from de-oiled biomass of thermotolerant oleaginous microalgae: *Acutodesmus dimorphus*. *RSC Adv.* **2016**, *6*, 72269–72274. [\[CrossRef\]](#)

50. Suwannakul, S.; Wacharanad, S.; Chaibenjawong, P. Rapid green synthesis of silver nanoparticles and evaluation of their properties for oral disease therapy. *Songklanakarin J. Sci. Technol.* **2018**, *40*, 831–839. [\[CrossRef\]](#)
51. Khodadadi, S.; Mahdinezhad, N.; Fazeli-Nasab, B.; Heidari, M.J.; Fakheri, B.; Miri, A. Investigating the possibility of green synthesis of silver nanoparticles using *Vaccinium Arctostaphylos* extract and evaluating its antibacterial properties. *Biomed. Res. Int.* **2021**, 5572252. [\[CrossRef\]](#)
52. Vinayagam, R.; Varadavenkatesan, T.; Selvaraj, R. Evaluation of the anticoagulant and catalytic activities of the *Bridelia retusa* fruit extract-functionalized silver nanoparticles. *J. Clust. Sci.* **2017**, *28*, 2919–2932. [\[CrossRef\]](#)
53. Mikac, L.; Ivanda, M.; Gotić, M.; Mihelj, T.; Horvat, L. Synthesis and characterization of silver colloidal nanoparticles with different coatings for SERS application. *J. Nanoparticle Res.* **2014**, *16*, 1–13. [\[CrossRef\]](#)
54. Sutthanont, N.; Attrapadung, S.; Nuchprayoon, S. Larvicidal activity of synthesized silver nanoparticles from *Curcuma zedoaria* essential oil against *Culex quinquefasciatus*. *Insects* **2019**, *10*, 27. [\[CrossRef\]](#)
55. Mondal, N.K.; Chowdhury, A.; Dey, U.; Mukhopadhyay, P.; Chatterjee, S.; Das, K.; Datta, J.K. Green synthesis of silver nanoparticles and its application for mosquito control. *Asian Pac. J. Trop. Dis.* **2014**, *4*, 204–210. [\[CrossRef\]](#)
56. Rónavári, A.; Kovács, D.; Igaz, N.; Vágvölgyi, C.; Boros, I.M.; Kónya, Z.; Pfeiffer, I.; Kiricsi, M. Biological activity of green-synthesized silver nanoparticles depends on the applied natural extracts: A comprehensive study. *Int. J. Nanomed.* **2017**, *12*, 871–883. [\[CrossRef\]](#)
57. Perveen, R.; Shujaat, S.; Naz, M.; Qureshi, M.Z.; Nawa, S.; Shahzad, K.; Ikram, M. Green synthesis of antimicrobial silver nanoparticles with *Brassicaceae* seeds. *Mater. Res. Express* **2021**, *8*, 055007. [\[CrossRef\]](#)
58. Durán, N.; Durán, M.; de Jesus, M.B.; Seabra, A.B.; Fávaro, W.J.; Nakazato, G. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. *Nanomed. Nanomed-Nanotechnol.* **2016**, *12*, 789–799. [\[CrossRef\]](#)