Bioassay Evaluation of Olive Leaf Nano extracts in Controlling the *Myzus persicae* Sulzer under Laboratory Conditions

Mee'ad Hamza Zughair[1*], Asaad Mudhi Sakab[2], Farah Hadi Abdul-Hussein[3]

[1,2,3] Babylon, Iraq, Al-Mussaib Technical College, Al-Mussaib Technical College

***Corresponding author's email:** mee3ad@yahoo.com

Email addresses of co-authors: asaad.sakab@atu.edu.iq , farah.hussein.tcm@atu.edu.iq

Abstract

This study sort of evaluated the insecticidal activity of aqueous and alcoholic nano-formulated extracts taken from olive (*O. europaea* L.) leaves, against the green peach aphid (*Myzus persicae* Sulzer) under lab conditions in Babylon Province Iraq. For the GC-MS analysis of the leaf extract, ten bioactive compounds were identified from different chemical classes, with the most abundant noted as α-terpinyl acetate, aromandendrene, and isospathulenol. Nymphs and adults were exposed to four concentrations, namely 0 25 50 and 100% of both the aqueous and alcoholic nano extracts, then mortality was recorded after 1 3 and 5 days. In the case of the aqueous extract, the highest nymphal mortality reached 73.3% at the 50% level after five-day s, while the control stayed at 13.3%. For adults, mortality was 66.7% at 50–100% concentrations, in contrast to 26.7% in the control. Meanwhile, the alcoholic extract looked more powerful, and it caused complete nymphal mortality (100%) at 50% after five-day s, compared with 33.3% in the control. For adults, alcoholic preparation had 93.3% mortality at the same concentration, versus 26.7% in the control. Mortality increased significantly with concentration as well as with exposure time ($P \leq 0.05$) across all treatments, though the comparison among the concentration means themselves were not significant. Nymphs were repeatedly more susceptible than adults, probably because their cuticles are thinner and their detoxification systems are less developed, not fully mature yet, you know. Based on these findings, the olive leaf nanoextract, particularly the alcoholic preparation, showed strong aphicidal efficacy against *M. Persicae* and it seems worth looking more deeply into integrated pest management programs.

Keywords: Green peach aphid, *Myzus persicae*, *Olea europaea*, Nano-extract, GC-MS.

Introduction

The green peach aphid, *Myzus persicae* (Sulzer), is one of the more destructive and widespread insect pests in world farming, and it can lead to severe losses in vegetables, field crops, and fruit trees [3]. Its danger comes from both the direct kind of harm and the indirect kind that it brings to the host plants.

The direct injury happens because the insect feeds on sap that it draws from leaves, buds, and young stems. This action depletes plant nutrients, and it basically weakens the overall growth, [5]. Common signs include leaf yellowing, curling, and distortion, plus slower shoot growth. In heavy infestations, the plants can become stunted [13].

The indirect damage is often even worse, because *M. persicae* is known to transmit more than one hundred plant viruses. So, it ends up ranked among the most important viral transmitters in agriculture [34]. Two of the economically important viruses it spreads are Potato Leafroll Virus (PLRV) and Plum

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Pox Virus (PPV). Both can sharply lower crop yield and quality, and in the most severe situations they may even result in complete losses [26]. Also, the feeding process produces honeydew, which supports sooty-mould development, reduces photosynthetic performance, and then lowers how market-ready the crop is.

In recent times, botanical nanoformulations have become one of the most promising approaches for boosting plant-based insecticide performance. The idea is that when these formulations improve stability, solubility, and bioavailability, and also enable controlled release, they can raise the overall effectiveness of pest-control programs. This includes programs aimed at aphids [5,32,23]. In general, these formulations are nanoscale delivery systems made from plant materials, or plant extracts, that carry insecticidal properties by themselves. They can be made through different routes, especially biogenic/green synthesis, chemical synthesis, polymeric-nanoparticle fabrication, and nanoemulsion technology [9,33,31].

A number of carrier matrices can be applied when building these systems. Examples are biodegradable polymers, isolated plant active compounds, metal oxides, and essential oils. All of them have shown improved formulation stability and biological efficacy in earlier studies [33,9].

Here, this work examines the insecticidal effects of water and alcohol nano-formulated extracts from olive, *Olea europaea* L. leaves, against the green peach aphid, *Myzus persicae* Sulzer, under laboratory settings. Also determine which bioactive compounds are behind this activity using GC-MS analysis.

Materials and Methods

Insect collection and rearing

Colonies of the green peach aphid were collected from eggplant and cucumber crops grown in plastic greenhouses and open fields in Babylon Province, Iraq. Samples were transported in transparent nylon bags to the postgraduate laboratory of Al-Mussaib Technical College, where the species was confirmed under a dissecting microscope. Pure, continuous colonies were established following the rearing method of [21] and maintained on potted wheat plants to sustain the colony and provide insects for the bioassays.

Plant material

Olive (*O. europaea* L.) leaves were collected from the gardens of Al-Mussaib Technical College, washed to remove dust, air-dried in the shade, ground into powder, and stored in a glass container in a refrigerator until be used.

Preparation of the aqueous olive leaf extract

The hot-water extract was done, roughly, based on the procedure from [2]. Twenty grams of leaf powder were combined with 200 mL distilled water inside a 500-mL flask, then mixed using a magnetic stirrer for 30 min at 40 °C. After that, the flask was covered and left resting for about 24 h, and afterward it was filtered; the filtrate was then centrifuged at 4000 rpm and finally dried in an oven set at 45 °C to get the crude, dry extract. Next, 2 g of this dried extract were dissolved into 100 mL of distilled water to form a 2% solution (20 mg/mL) as the stock, after which the working concentrations 25, 50, and 100 mg/mL were prepared. For comparison, distilled water was used as the control, at 0%.

Preparation of the Alcoholic Extract from Olive Leaves

The method described by Harborne (1984) was followed for the preparation of the

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alcoholic extracts. Briefly, 10 g of the dried powdered olive leaves were weighed and placed in a Soxhlet extractor, and 200 mL of ethanol was added. Extraction was carried out for 24 h at a temperature of 45 °C. The resulting extract, containing the crude plant-extracted compounds, was concentrated using a rotary evaporator. The extraction procedure was repeated several times to obtain a sufficient quantity of the extract. Subsequently, the extract was dried in an electric oven at 40–45 °C. The dried extract was then transferred into tightly sealed glass containers of known weight and stored in a refrigerator until use.

To evaluate the effect of the crude alcoholic extract of olive leaves, 8 g of the dried extract were dissolved in 5 mL of 99% ethanol and 3 mL of the emulsifying agent Tween 20. The volume was then made up to 100 mL with distilled water. Thus, the stock solution had a concentration of 8%, equivalent to 80 mg/mL. From the stock solution, concentrations of 25, 50, and 100 mg/mL were prepared. The control treatment consisted of 5 mL of ethanol and 3 mL of Tween 20, with the volume made up to 100 mL using distilled

GC-MS identification of bioactive compounds

The olive leaf extract was analyzed using gas chromatography mass spectrometry (Shimadzu GCMS-QP2010 Plus) at the Ministry of Science and Technology, to identify constituent compounds were inside it. The compounds that showed up were then taken as identified, because the retention times and mass spectra were matched with the instrument's mass spectral library, so basically the fingerprints lined up.

Preparation of AgNO3 solution

Analytical grade silver nitrate (AgNO3) was obtained from Al-Manar Scientific Office, Babylon. A 50 mM stock solution was prepared, using a molar mass of 169.87 g mol^{-1} (Ag = 107.87, N = 14.00, O3 = 48.00), so 8.4935 g of AgNO3 were dissolved in 1000 mL of deionized water. The mixture was heated at 60 °C for 10 min and then it was cooled, before being used again.

Plant extract as a reducing agent

Ten grams of fresh olive leaves were rinsed several times using tap water, and then distilled water, cut into little pieces, and put into 100 mL of distilled water. After that, the blend was warmed on a magnetic hot-plate stirrer at 45 °C for about 20 min with steady mixing, until a dark solution showed up , then it was filtered so it could get a pale-yellow extract, which served as the reducing agent [30].

Green synthesis of silver nanoparticles

Silver nanoparticles were synthesized by slowly pouring 100 mL of the plant extract stock into 900 mL of deionized water; after that 50 mL of this mixture was taken out and combined with 50 mL of the olive leaf extract, then it was warmed at 50 °C for around 20 min, while stirring steadily on a magnetic hot plate. Throughout, the hue shift of the liquid was watched, since it reflects bioreduction as well as nanoparticle generation. The final nano extract was later kept in dark glass containers until it was needed [24].

Bioassay and statistical analysis

Nymphs and adults of *M. persicae* had been exposed to four concentrations (0, 25, 50, and 100%) of the aqueous as well as alcoholic olive-leaf nano extract, and then mortality was recorded at 1 3, and 5 days. The data were handled with analysis of variance under a factorial setup concentration × exposure period, and the average values were separated

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using the least significant difference (LSD) test, with $P \leq 0.05$.

Results and Discussion

GC-MS identification of active compounds in the olive leaf extract

GC–MS analysis of the olive leaf extract identified ten compounds coming from different chemical classes (Figure 1), though the most abundant was α-terpinyl acetate (16.91% peak area). Then aromandendrene turned out at 18.43% and isospathulenol was higher 20.49% plus another isomer peak at 6.25%. Along with these, there were also styrene, (-)-globulol, ledene oxide-(II), another isospathulenol isomer, and two decahydronaphthalenemethanol isomers, all of them sorted out mainly by retention time together with relative peak area.

The compound profiles of plant extracts really shift depending on plant species, the tissue, and even the extraction technique. For instance, HPLC based analysis of *Cardaria draba* provided a much more fatty-acid driven pattern [14] . That kind of outcome sort of highlights that both the plant itself and the analytical method strongly steer which compounds end up being recovered from a given extract.

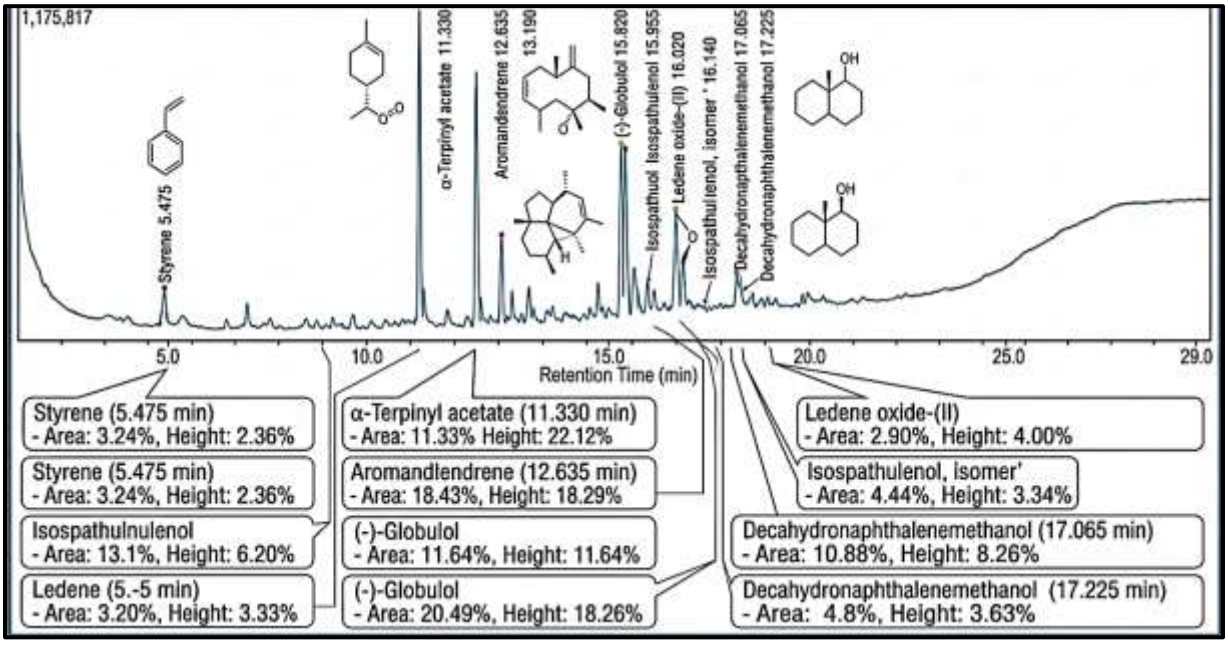
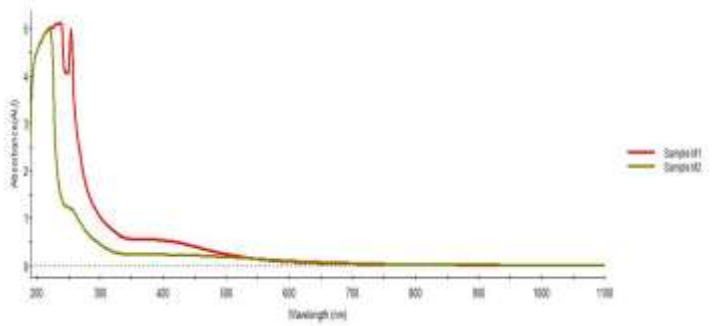


Figure 1. GC-MS chromatogram and peak analysis of bioactive compounds identified in the olive leaf extract.

Ultraviolet–Visible Spectrophotometric (UV–Vis) Analysis

The results of the ultraviolet– visible (UV–Vis) absorption spectral analysis, measured using a spectrophotometer over the wavelength range of 300–700 nm, revealed an increase in the absorption intensity of both the plant - derived nanoparticles extracted from the leaves of *O. europaea* L, as shown in Figure (1-1), The maximum absorption peaks were observed at wavelengths of 440 and 435 nm, respectively. The highest absorption wavelength was observed at these respective peaks. UV–Vis spectrophotometric analysis is considered one of the most widely used techniques for the structural characterization of nanoparticles (Alloush, 2020)



(1- 1) Ultraviolet–Visible (UV–Vis) Spectrophotometric Analysis of Plant-Synthesized Ag-NPs Using *O. europaea* L

Table 1. Mortality (%) of *M. persicae* nymphs treated with the aqueous olive leaf nano extract

Concentration (%)	1 day	3 days	5 days	Concentration mean
100	13.3	53.3	66.7	44.4
50	0.0	26.7	73.3	33.3
25	0.0	33.3	33.3	22.2
0 (control)	13.3	13.3	13.3	13.3
Period mean	6.7	31.7	46.7	-

LSD (0.05): concentration = 10.26; period = 8.88; interaction = 17.76.

Effect of the aqueous olive leaf nano extract on *M. persicae* nymphs

Nymphal mortality rates increased clearly with the extract concentration along with the time exposed ($P \leq 0.05$) (Table 1). The greatest mortality, 73.3%, showed up at 50% concentration after five-day s, and it sat next to 13.3% in the control over that same period. Meanwhile the 100% and 25% concentrations caused 66.7% and 33.3% mortality. The mean values for concentration did not really separate in a meaningful way, but the exposure period did make a difference. The

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five-day exposure had the top average mortality (46.7%), which was significantly higher than the one-day mean (6.7%) and the three-day mean (31.7%).

Table 2. Mortality (%) of *M. persicae* adults treated with the aqueous olive leaf nano-extract

Concentration (%)	1 day	3 days	5 days	Concentration mean
100	13.3	60.0	66.7	48.9
50	6.7	26.7	66.7	33.3
25	0.0	26.7	26.7	17.8
0 (control)	13.3	13.3	26.7	17.7
Period mean	8.3	31.6	46.7	-

LSD (0.05): concentration = 20.25; period = 17.54; interaction = 35.08.

Effect of the aqueous olive leaf nano extract on *M. persicae* adults

Adult mortality was also quite influenced by the aqueous nano extract ($P \leq 0.05$) (Table 2) . The 50% and 100% concentrations, for whatever reason, showed the most pronounced five-day mortality (66.7%), which was much more than the control at 26.7%. As happened with the nymphs, the concentration averages were statistically in line with each other, but the length of exposure over five-day s produced the biggest mean mortality (46.7%) which was also significantly higher than the one-day and three-day values (8.3% and 31.6%).

Table 3. Mortality (%) of *M. persicae* nymphs treated with the alcoholic olive leaf nano-extract

Concentration (%)	1 day	3 days	5 days	Concentration mean
100	20.0	86.7	93.3	66.7
50	0.0	80.0	100.0	60.0
25	0.0	73.3	80.0	51.1
0 (control)	26.7	26.7	33.3	28.9
Period mean	11.6	66.6	76.6	-

LSD (0.05): concentration = 21.20; period = 18.36; interaction = 36.73.

Effect of the alcoholic olive leaf nano extract on *M. persicae* nymphs

The alcohol based nano extract seemed more effective than the aqueous based formulation (Table 3). In fact, full nymphal mortality, 100%, showed up at the 50% level after five-days, but the control only reached 33.3%, while the 100% and 25% levels produced 93.3% and 80.0% mortality in that same period. Also, the average mortality went up again in a significant way the longer the nymphs were exposed, ending at 76.6% after five-days, compared to 11.6% and 66.6% at one-day and three days.

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Table 4. Mortality (%) of *M. persicae* adults treated with the alcoholic olive leaf nano-extract

Concentration (%)	1 day	3 days	5 days	Concentration mean
100	13.3	60.0	73.3	48.9
50	6.7	40.0	93.3	46.7
25	0.0	60.0	60.0	40.0
0 (control)	6.7	20.0	26.7	17.8
Period mean	6.6	45.0	63.3	-

LSD (0.05): concentration = 16.38; period = 14.18; interaction = 28.37.

Effect of the alcoholic olive leaf nano extract on *M. persicae* adults

Adult mortality kept to roughly the same pathway (Table 4): the 50% concentration ended up giving the highest five-day mortality, 93.3%, compared with 26.7% in the control group. Meanwhile 100% and 25% had 73.3% and 60.0% mortality respectively, even though it does not look as neat. Also, the five-day mean mortality was 63.3% and it clearly surpassed the one-day and three-day means, those were 6.6% and 45.0%.

Discussion

Across both extract types and life stages, mortality rose steadily with concentration and even more with exposure duration, suggesting a time-based mode of action, or so it seems. Nymphs were pretty much more susceptible than adults, most likely because their cuticle is thinner and less developed, and their detoxification mechanisms are still immature, so penetration of the active compounds happens easier [29,12].

The increase in mortality observed in the control treatment over the studied exposure periods may be attributed to natural parasitism of aphids by parasitoids. Aphid parasitoids, particularly species belonging to the families Braconidae (Aphidiinae) and Aphelinidae, develop internally within the aphid body, where the parasitoid larva feeds on the host's internal tissues and eventually kills it. Following parasitoid-induced mortality, the aphid body becomes hardened and swollen, forming a characteristic structure known as a "mummy." Therefore, the occurrence of naturally parasitized aphids in the control treatment may have contributed to the increased mortality recorded with increasing exposure time.

Also, the nano extract has a small particle size plus a high surface-area-to-volume ratio, and that probably helps it stick to the insect cuticle and move through the cuticle as well as across cell membranes. Once this happens, cellular physiology gets thrown off, with proteins, enzymes, and nucleic acids getting targeted or harmed. On top of that, reactive oxygen species get induced, so it end up with oxidative stress, mitochondrial injury, and membrane disassembly, and eventually it all turns into cell and insect death [29,7]. As for the bioactive olive leaf parts, mainly phenolics like oleuropein and hydroxytyrosol along with flavonoids and terpenes, they are reported to mess with feeding behavior, growth, and multiple physiological processes in insects. In addition, some other plant secondary metabolites can also block acetylcholinesterase (AChE) activity, so acetylcholine builds up, neurons get hyperactive, motor control drops , paralysis follows , and death comes next [16].

These outcomes match quite well with multiple reports of aphicidal performance of

biosynthesized nanoparticles. The researchers often describe as neurotoxic and physiological disruptions in pests. For example, Chakravarthy et al. [12] observed that inorganic silver and TiO_2 nanoparticles caused noticeable neurotoxic as well as physiological effects in *Spodoptera litura*. Roni et al. [28] mentioned that silver nanoparticles, made from Nerium oleander leaf extract, triggered tremors convulsions paralysis and eventual death in *Anopheles stephensi* larvae, whereas [6] recorded a clear high toxicity of neem (*Azadirachta indica*)-mediated silver nanoparticles against *Aedes aegypti*, and it went up with concentration and exposure length, which in other words, mirrors what is seen in the present results.

Also, the comparative effectiveness of plant-origin nano-formulations on aphids has been noted by Hussain et al. [17], who explained 92% mortality in *M. persicae* using silver-nanoparticle formulations prepared from *Datura stramonium*, *A. indica*, *Nicotiana tabacum*, and *Melia azedarach* at 1000 ppm after 72 h. Bhattacharyya et al. [8] similarly reported full mortality of the rose aphid (*Macrosiphum rosae*) using tomato-leaf-mediated silver nanoparticles at 500 ppm. Likewise, [15] found marked, dose-and-time dependent mortality of *Aphis craccivora* nymphs and adults after treatment with *Clerodendrum inerme* root-exudate extracts rich in phenanthrenol and ethenyl-ester compounds, and [25] showed that ethanolic extracts of *Catharanthus roseus* and *Iresine herbstii* significantly changed the life-history indicators of *Aphis gossypii*, with *C. roseus* showing the stronger action.

So overall these results strengthen the idea, and the evidence trail, that phenolic-, terpenoid-, and essential-oil-rich plant extracts can function as toxic, deterrent, and feeding-altering agents against aphids, and usually with lower environmental risk compared to traditional insecticides [1,11,18,19]. When those extracts are formulated as nanoparticles, they tend to gain better stability and improved cuticular penetration of the active constituents, which means better efficacy while also needing lower doses, and therefore less ecological pressure [10,20]. Olive leaf nano extracts then, especially the alcoholic version, therefore, feel like a promising eco-friendly piece for integrated pest-management campaigns targeting *M. persicae*, and also other sap-sucking insects [22,4].

Conclusion

Olive (*O. europaea* L.) leaf extract, made into aqueous and alcoholic nanoparticle suspensions, came up with a clear insect killing effect against both nymphs ,and also adults of the green peach aphid when tested in the lab. It looked like the alcoholic nano extract performed better than the aqueous one, reaching total nymphal death at 50% concentration after five-day s. Death levels seemed to rise in step with concentration, and even more so, with longer exposure time. Also, nymphs turned out to be more vulnerable than adults. Then GC-MS analysis showed ten bioactive compounds in the extract, which kind of backs up the idea that it really can act insecticidally. Overall, these results suggest that olive leaf nano extracts deserve more work, like field studies, plus checking non-target safety, as a more eco-friendly option inside integrated pest management programs for *M. persicae*.

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