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Cellular damage and physiological alterations in the soft scale insect *Kilifia acuminata* (Signoret) induced by avocado oil (*Persea americana*) and dill (*Anethum graveolens*) oils and their joint action in mango

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Abstract
The soft scale, *Kilifia acuminata* (Signoret) (Hemiptera: Pseudococcidae) is the most pests invading mango trees (*Mangifera indica* L.) all over the world. Here the current study aimed to evaluate the efficacy of two plant oils: avocado (*Persea americana* Mill.) (AO), dill (*Anethum graveolens* L.) (DO) and their joint action (AO+DO treated group) as natural insecticides against *K. acuminata* in mango *(Mangifera indica* L.) using spray application via investigating lethal concentration (LC_{50}) and percentage mortality (%) using (0.2, 0.6, 1 and 2 %) of the two tested plant oils and their joint action at 2 %. The obtained results showed that LC_{50} of AO and DO found 0.32% and 0.30 %, respectively. Also, it found high percent mortality of AO (83.91%), DO (87.96%), and AO+DO- treated group (94.33%) at 2% after 48 h. The insect biochemical analysis and Transmission Electron Microscopy "TEM" micrographs were scrutinized of AO+DO- treated group (2%). In the field assessment AO+DO- treated group reduced *K. acuminata* nymphs than AO or DO. In other word, the mean reduction after 45 days in the open field by AO+DO- treated group was 74.66% followed by 54.25 and 56.77 % caused by AO and DO, respectively. Also, this study showed that alone and binary treatments induced dark areas in the nymph bodies. The biochemical analysis of the treated nymphs revealed that there were significant variations in total protein concentration, and the activities of acidic phosphatase (ACP), phenol oxidase (PhO) enzymes, alanine aminotransferase (ALT), asparate aminotransferase (AST), and α-esterase (α-EST) activities under laboratory and field applications (P<0.001) between the treatments at 2% concentration versus control. The physiological disturbances caused in protein content and certain enzymes activities led to clear cell damage and it was shown via ultra micrographs as shown by TEM. The complete cellular damage caused by AO+DO- treated group. In particular, cellular alterations including degraded cell membrane, fragmented cells, damaged normal cells, shrunken cells, an appearance of vacuoles, fatty bodies'fragmentation and an appearance of cell apoptosis. Finally, this study concluded that AO, DO and their promising joint action can act as natural insecticides induced insecticidal activity against *K. acuminata* and can be used in the biological control program in Integrated Pest Management (IPM) in the field.

Keywords: *Kilifia acuminata*, avocado oil, dill oil, joint action, insecticidal activity, physiological alterations, cellular damage, TEM micrographs

Introduction

Among different popular fruits mango (*Mangifera indica* Linn. Family: Anacardiace) is a very consumed and tasty fruit in all over the world. It is rich in vitamins A and C and has super flavor [38]. In current years, mango production in Africa has been decreased due to different factors such as climate change variability, inadequate management practices and biotic stresses (insect pests and diseases) [42]. Different traditional control practices such as pruning, smoking and area clearing have been recommended [35]. The invasive pest species affect mango and subsequently increased insecticides use which dramatically affect ecosystem. In Egypt, mango trees were attacked with scale insects and mealybugs belonging to four families: Diaspididae, Coccidae, Monophlebidae and Pseudococcidae [4]. Among these pests is *Kilifia acuminata* (Signoret) which came from Mexico and Central America. *K. acuminata* is a serious pest attacking several crops causing agricultural crop loss and decreasing the horticultural area [44, 35].

Studies have demonstrated using of essential oils derived from with great success in controlling insect pests [11, 2, 7]. Avocado oil (*Persea americana* Mill.) is very vital essential oil used in feeding, cosmetic industry, therapeutic activities antibacterial, antiwrinkle, antioxidant, and healing properties belonging to Family: Lauraceae. It has ability to absorption of lipophilic functional compounds. It contains high oleic oil, phytosterol content (mainly β-sitosterol), lutein carotenoid, phenolics, lipids, essential minerals (Mg, K, P), tocopherols (mainly vitamin E) and chlorophylls. The main fatty acids of avocado oil are: linoleic acid (13.5%–15.2%), oleic acid (65.4%–67.7%) and palmitic acid (12.8%–13.4%) [12]. Dill essential oil (*Anethum graveolens* L., Family: Apeaceae) constituents distilled from fresh dry herbs were α-phellandrene, limonene, β-phellandrene and p-cymene, carvone and dill ether [26, 27]. These compounds caused insecticidal actions and affected on physiological mechanisms [17]. Collectivity, our findings coincided on the insecticidal activity of avocado (*P. americana* Mill.) and dill (*A. graveolens* L.) plant oils, alone and their binary treatments against the soft scale; *K. acuminata* (Signoret) (Hemiptera: Pseudococcidae) pest in mango via investigating insect toxicity, biochemical analysis, Ultra structural studies, and field evaluations.

Materials and methods

Materials

The two used commercial oils; avocado (*P. americana* Mill.) and dill (*A. graveolens*) oils were obtained from the Purity company, Egypt.

The tested insect

The soft scale; *K. acuminata* were collected from the infested mango trees growing in the experimental farm of Horticulture Research Station at Al-Kanater El-Khaireya Station (30°11′36″N 31°08′13″E), Qualyobia, Horticulture Research Institute, Agricultural Research Center (ARC). Randomly chosen mango samples were taken from each of the four cardinal directions (East, West, North and South) and were transferred to the laboratory. Where they were kept at a temperature of roughly 25±1°C and 65±1°RH (Relative Humidity). The prefect insect taxonomy was carried out in Scale Insects and Mealybugs Research Department, Plant Protection Research Institute, (ARC). Bioassays were conducted under laboratory conditions in Petri dishes (diameter = 9 cm) that had lids with openings (diameter =6 cm) covered with fine muslin for ventilation. The two disks mango leaves of approximately the same size were placed in the Petri dishes. Nymph classes of *K. acuminata* were tested.

Methods

Laboratory bioassay

Plant oils dilutions

The tested four treatments were evaluated under laboratory conditions of AO and DO suspending on 0.01% Tween in distilled water (0.2, 0.6, 1 and 2%) and untreated as a negative control to investigate LC_{50}.

Experimental design

Infested mango leaves with *K. acuminata* nymphs were applied together with negative control treatment. One mL of each concentration (0.2, 0.6, 1 and 2%) of AO and DO were taken and sprayed on the infested mango leaves. Also, the fourth group was treated by the binary treatment ″AO+DO-treated group″ (2%). Each concentration replicated three times and each replicate contains 20 nymphs.

Spraying technique was conducted using a small volume vessel of pharmaceutical use with a spray vaporisateur. The excess run off solution was removed from the Petri dishes immediately after spraying and the dishes were then covered with the lids bearing the ventilation holes to prevent vapor accumulation.

Estimation of mortality test (LC_{50})

The obtained data of mortality test were resulted from each dose-response trial. These data were subjected to probit analysis. Also, values of LC_{50} and LC_{90} were estimated[16]. Data were statistically analyzed by SPSS 11.0 (SPSS 2011). Counting the live and dead nymphs of *K. acuminata* 24 and 48 h post treatment application by a binocular stereomicroscope was successfully conducted.

All values were calculated using Abbott's formula as given below to obtain percent mortality of different treatments.

% *Corrected Mortality* = % *Mortality* after treatment - % *Mortality* in control/ 100- % *Mortality* in control.

Dead nymphs were identified by gently striking the pest with a small brush and observing any remains of their body. The homogenate was then promptly shifted to fresh tubes under ice bath conditions and centrifuged at 12,000 rpm for 30 min at 4°C. The clear supernatant was taken for storage at −20°C for subsequent enzyme studies. The untreated group was used as a negative control group.

Field experiment

Experimental design and application technique

The field trial was conducted during December,19 to January,30 2024/2025, following a completely randomized design (CRD) with three replications which were selected on each plot. The oils AO, DO along with their binary treatment (AO+DO-treated group), were applied at a concentration of 2%. These were mixed with water and Tween to create a stable emulsion for the treatment using a hand sprayer.

Sampling technique procedure

A ten randomly leaves were collected from each replicate of the cardinal directions from the marked trees. Leaves were taken and kept separately in bags and transferred to the laboratory for inspection. The total numbers of live nymphs on each leaf were counted. Mortality of nymphs was recorded fortnight after spraying for one month and half. The pest mortality percentages were calculated according to [22].

$$\%\text{Reduction} = 1 - (Ta \times Cb) / (Tb \times Ca) \times 100$$

T_a = number of insects after treatment

T_b = number of insects before treatment

C_a = number of insects in control plots after treatment

C_b = number of insects in control plots before treatment

Biochemical analysis

Estimation of aspartate and alanine transaminases activities (AST and ALT)

Aspartate and alanine transaminases (AST and ALT) activities were determined according to [39] of nymphs' homogenate.

Acidic phosphates enzyme (ACP) activity

The acid phosphatase activity was assessed using method of [32] as the amount of phenol product by enzymatic hydrolysis reaction with disodium phenyl phosphate as substrate.

Estimation of enzymatic immune responses and detoxification process

Phenoloxidase (PHO) activity

The phenoloxidase activity of *K. acuminata* nymph's homogenate was estimated according to the scheme of [25] using catechol as the substrate. The reaction mixture was consisted of 0.5 mL phosphate buffer (0.1M, pH=7), 200 µL enzyme solution, and 200 µL catechol solution (2%). The activity was restrained calorimetrically at 405 nm.

α-Esterase (α-EST) enzyme activity

α- naphthol product resulted from of hydrolysis of α-naphthyl acetate (strong blue) and β-naphthyl acetate (strong red as substrate, respectively), were calorimetrically measured at 510 nm [47].

Total protein (TP) concentration

Total protein of nymph homogenate was evaluated calorimetrically following the routine termed by [19]. A violet purple color was made as the intensity of protein after added Biuret reagent, and unhurried at 546 nm.

Ultrastructural studies by transmission electron microscopy (TEM)

Nymphs were collected after 48 h after treatment with AO+DO- treated group at 2% and untreated nymphs for transmission electron microscopy. They were collected and fixed in a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M PBS (pH 7.4) for 24 h at room temperature. Tested samples were rinsed three times for 15 min each time with 0.1 M PBS, then dehydrated in a progressive ethanol gradient of 50, 60, 70, 80, 90, 95 and 100% for 15 min each time. Tissues were placed in LR white resin as follows: 2:1 ethanol: resin for 4 h, 1:1 ethanol: resin for 4 h, 1:2 ethanol: resin for 4 h, and 100% resin overnight. Samples were then embedded in LR white resin and polymerised at 40 °C for 48 h followed by ultrathin sectioning, and sections (~90 nm) were stained with 4% aqueous uranyl acetate for 10 min and imaged using a transmission electron microscopy (TEM) [31].

Data analysis

The mortality percentages of *K. acuminata* were determined and corrected using [1]. Then, they were statistically analyzed according to [16] to estimate LC_{50}, LC_{90} and slope values. Also, toxicity index was computed for different treated groups by comparing them with the most effective one using Sun's equation. The obtained data from the biochemical analysis in laboratory and data of field trial were calculated as mean±SE using the statistical software package SPSS that was used a one-way ANOVA trial to create a comparison between the biochemical indicators followed by Tukey's post hoc assessment IBM (2011) [24].

Results

The efficiency of AO, DO and AO+DO- treated group on *K. acuminata*

Toxicity bioassay

The results of laboratory treatments of AO and DO against *K. acuminata* nymphs shown in Fig.1 (A and B) and Table 1. The obtained data revealed that there was a significant toxicological effect between the two plant oils against the insect through spraying technique. The mortality test was measured using Abbotts' equation through the relation between the percentage response (%) and concentration of the oils (%).

The LC_{50} AO and DO indicated a higher toxic effect after 48h (Fig. 1). LC_{50} test of AO found 0.32 % (arrow) for LC_{50}, 4.04 %for LC_{90}, and 0.09 %for LC_{25}. LC_{50} test of DO found 0.30 % (arrow) for LC_{50}, 0.36 % for LC_{90}, and 0.081% for LC_{25}. The toxicity index of DO induce 92.83%. The toxic impact of DO is more than AO

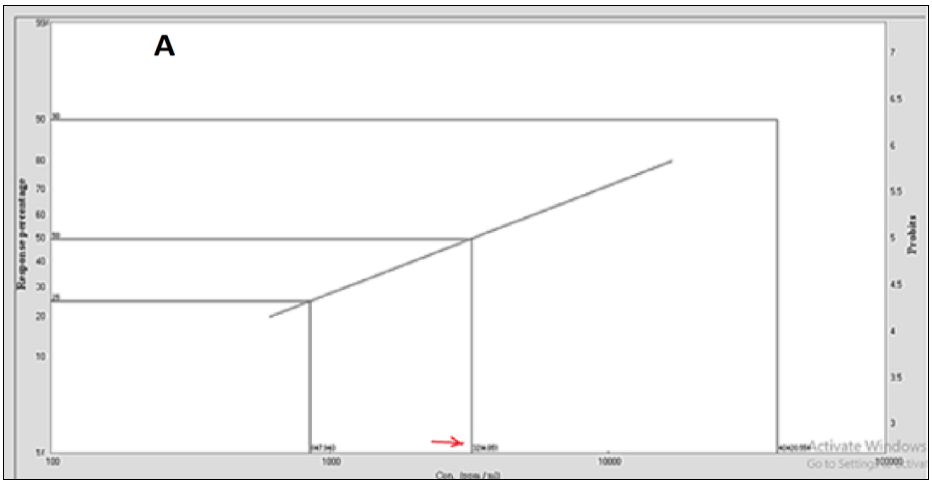


Fig 1: (A). Toxicity bioassay of the effect of AO against *K. acuminata* nymphs on mango leaves using spraying method: LC_{50} and LC_{90}

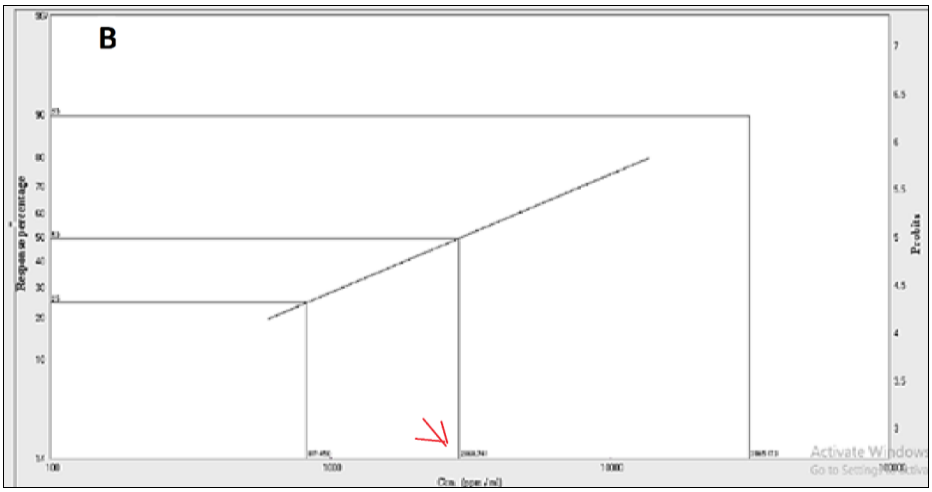


Fig 1: (B). Toxicity bioassay of the effect of DO against *K. acuminata* nymphs on mango leaves using spraying method: LC_{50} and LC_{90}

Table 1 and Fig.2 revealed that the LC_{50} of DO (0.30%) is lower than AO (0.32%), thus the mortality percentage of DO at high conc. (2%) (87.96 %) compared to AO (83.91%). The percent mortality of binary group of AOs+DO- treated group at 2% (94.33 %) was higher than AO (83.91%) and DO (87.96 %) at 2%.

Table 1: Toxicity of AO and DO against *K. acuminata* was shown as mortality percentage, LC_{50} and LC_{90}.

Tested compounds conc. (%)	Mortality percentage (%)	LC25 (%) confidence limits at 95%	LC50(%) confidence limits at 95%	LC90 (%) confidence limits at 95%	Slope±SE	$\chi 2$	Toxicity index
AO 0.2 0.6 1 2	43.49 55.47 73.86 83.91	0.085	0.32	4.04	1.17±0.11	8.51	--
DO 0.2 0.6 1 2	45.83 57.99 70.93 87.96	0.08	0.30	3.6	1.19±0.10	11.77	92.83

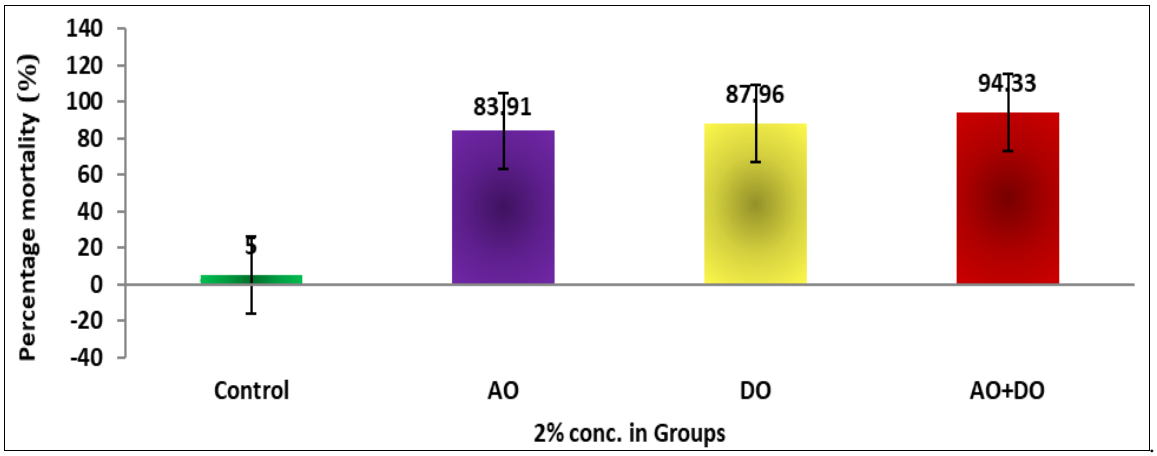


Fig 2: The comparison of the percent mortality of AO and DO and AO+DO- treated group at 2%. Bars indicate the standard error (SE)

Malformations in *K. acuminata* nymphs induced by AO, DO and AO+DO- treated group as shown by a binocular stereomicroscope

Toxicity effects of AO and DO on nymphs *K. acuminata* (Fig. 3). (4A): Control insects: with normal morphology and a body surface completely covered by the waxy layer, which play an important role in protecting the pest especially from desiccation and parasites contamination. (4B): Treated nymph's with AO showing malformations in body such as: a change in body color that becomes darker with small amount of the waxy secretions on the body surface at different concentrations. (4C): Treated nymph's with DO showing malformations in body such as: a change in body color that becomes darker with large amount of the waxy secretions on the body surface and severe desiccation after exposure to the DO at different concentrations. (4D): Treated nymphs with the joint action AO+DO- treated group (2%) showing a large change in body color darker, a large amount of the waxy secretions on the body surface, severe desiccation, and scatter of dead cells on mango leaves. The toxic effect of the binary treatment was higher than alone treatments. Thus, the insecticidal activity of the AO+DO-treated group (2%) caused high efficiency in controlling this pest.

Fig.3. Microscopic photographs of *K. acuminata* nymphs showing malformations induced by AO, DO and AO+DO- treated group (2%): (A): Control insects (B) Treated with AO (C) Treated with DO, (D) and Treated with AO+ DO showing a large change in body color darker, severe desiccation, and scatter of dead cells on mango leaves at 2%

The efficiency of AO, DO and AO+DO- treated group on *K. acuminata* in mango field

The treatments AO, DO and AO+DO-treated group at 2% showed a significant difference in *K. acuminata* nymph populations 14, 30 and 45 days after treatments (Table 2).

Replicates treated with AO+DO-treated group after 14 days recorded the highest reduction percent of *K. acuminata* as 86.14 % followed by AO which gave 70.02 %. On the other hand, the lowest reduction in *K. acuminata* was recorded by DO (65.99 %). Also, after 30 days, AO+DO-treated group was still the most effective in reducing nymph population as 59.56 % [LSD=1.75]. While, it was followed by DO which reduced nymph population as 50 % and AO had the lowest reduction percent as 24.72%. The nymph population was dramatically affected with AO+DO- treated group after 45 days in mango field versus control or check (Table 2). In other word, AO+DO- treated group reduced nymph population as 78.28 % followed statistically by AO 68.01 %. However, DO recorded the lowest reduction percent on nymph population as 54.33% [LSD= 1.38] (*P*< 0.01*)*. The mean reduction of the tested groups after three times revealed that AO+DO- treated group reduced nymph population as 74.66 % and significantly differed from the AO and DO groups. In other word, AO+DO- treated group reduced insect population with high percent as compared with AO or DO till the end of the experiment. The joint action of two the tested plant oils was superior in reducing insect population than each one alone.

Table 2: Mean ± SE of insect nymphs of *K. acuminata* in mango field after three intervals post-application. AO, DO and AO+DO-treated group versus control over December,19 to January,30 2024/2025

Treatments	Pre-count	Mean ± SE and reduction % after application (days)						Mean of reduction %
		14		30		45		
		Mean. No.	R.%	Mean. No.	R.%	Mean. No.	R.%	
Control	61.25	85.25±0.48^{a}		48±1.22^{a}		64.75±0.63^{a}		
AO	104.25	43.50±0.65^{c}	70.02^{b}	37.75±0.5^{b}	24.72^{c}	34.25±0.5^{d}	68.01^{b}	54.25^{b}
DO	79.75	61.5±0.87^{b}	65.99 ^{C}	31.25±0.85^{c}	50.00^{b}	56.25±0.85^{b}	54.33^{c}	56.77^{b}
AO+DO-treated group	177.5	35.25±0.63^{d}	86.14^{a}	38.5±0.65^{b}	59.56 ^{a}	40.75±0.48^{c}	78.28^{a}	74.66 ^{a}
F		1570.2**	3773.68**	51.54**	1253.37**	611.74**	903.92**	19.26**
Sig		0.00	0.00	0.00	0.00	0.00	0.00	0.00

Means coupled by the same letter in a column do not differ significantly. **= Very highly significant.

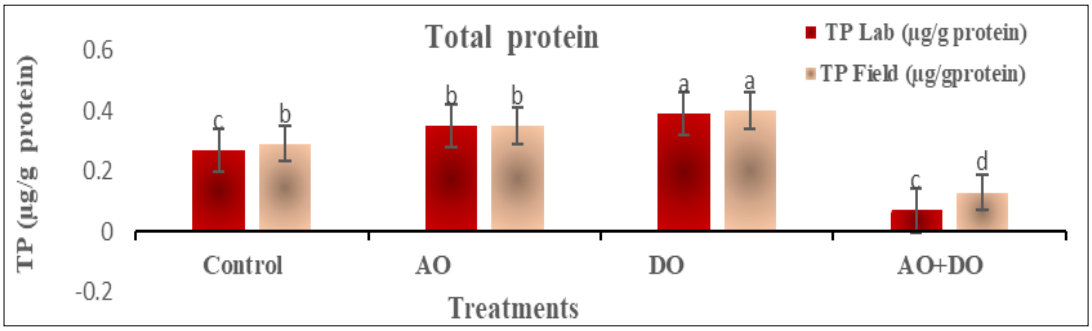
The biochemical alterations in *K. acuminata* nymphs treated with AO, DO and AO+DO- treated group under laboratory and field conditions

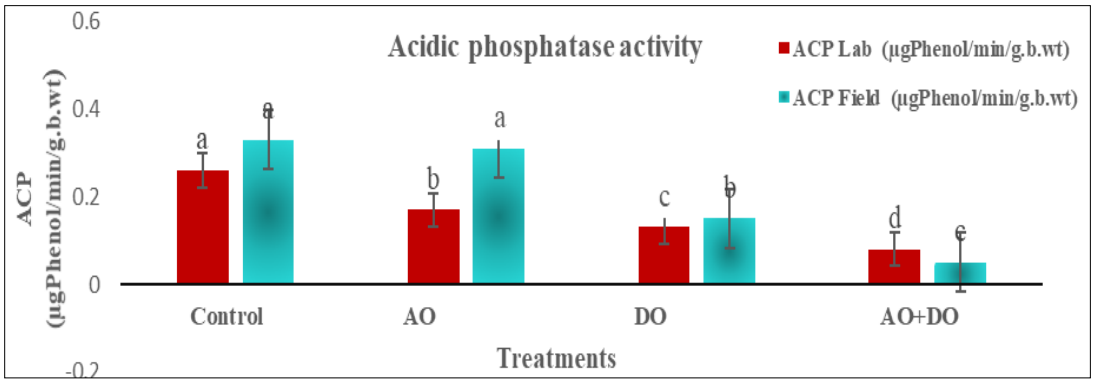
The reduction in the pest population in laboratory and field assessments as well as insect malformation observations led to do biochemical analysis to clear the physiological disturbance in nymphs caused by the treated oils. The highest concentration 2% of the three tested treatments which gave highest reduction in the laboratory and field studies. So, the treated insects of the tested plant oils and their binary treatment at 2% were subjected to the biochemical analysis. One-way ANOVA was conducted to examine a difference in in total protein content and enzymes activities among treatments. The obtained results showed that the total protein (TP) concentration induced a significant increase by AO and DO oils (0.35±0.02, 0.39±0.03 µg/g protein, respectively), however, TP concentration induced a significant decrease by AO+DO-treated group (0.07±0.04 µg/g protein) versus the control (0.27±0.012 µg/g protein) under laboratory conditions. TP concentration induced a significant increase by AO and DO groups (0.35±0.2 and 0.40±0.01 µg/g protein, respectively), while TP concentration induced a significant decrease by AO+DO- treated group (0.13±0.01 µg/g protein) versus the control group (0.29±0.01 µg/g protein) under field conditions. The acidic phosphatase (ACP) enzyme activity significantly declined in the insects after treatment with DO and AO+DO- treated groups under laboratory conditions (0.08±0.007 and 0.03±0.01 µg Phenol/min/g.bwt, respectively) compared to the control group (0.27±0.017 µg Phenol/min/g.bwt,), however, it wasn't significantly differed with AO versus control. The same result of ACP activity was obtained under field conditions, ACP declined in

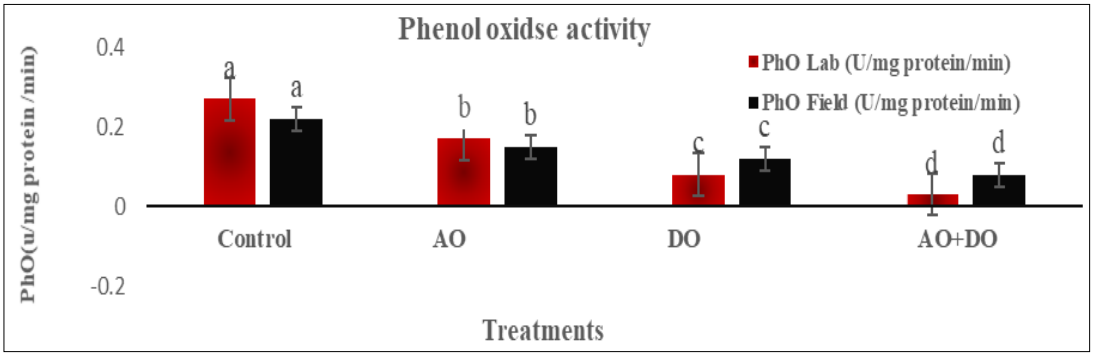
insects in plots treated with DO or AO+DO- treated group (0.15±0.014 and 0.05±0.007 µgPhenol/min/g.bwt, respectively) versus control (0.33±0.029 µgPhenol/min/g.bwt). However, it didn't differ with AO treatment verus control (Fig.4).

The detoxification phenol oxidase (PhO) activity significantly dropped in insects after treatment with AO, DO and AO+DO- treated groups in the laboratory (0.17±0.018, 0.08±0.007 and 0.03±0.01 U/mg protein/min ,respectively) compared to the control group (0.27±0.017 U/mg protein/min) and field (0.15±0.012, 0.12±0.011 and 0.08±0.009 U/mg protein/min,respectively) compared to the control group (0.22±0.013 U/mg protein/min). Data in Fig. 4 revealed that, the alanine aminotransferase (ALT) enzyme activity significantly decreased in insects after treatment with AO, DO and AO+DO- treated groups under laboratory conditions (0.04±0.007, 0.04±0.004 and 0.03± 0.011U/mg protein, respectively) compared to the control group (0.06±0.006 U/mg protein) and field conditions (0.03±0.009, 0.04±0.006 and 0.02±0.006 U/mg protein,respectively) compared to the control group (0.05±0.007 U/mg protein).

The asparate aminotransferase (AST) activity significantly dropped in *K. acuminata* nymphs after treatment with AO, DO and AO+DO- treated groups under laboratory conditions (0.06±0.006, 0.05±0.005 and 0.03±0.004 U/mg protein/min,respectively) compared to the control group (0.08±0.007 U/mg protein/min) and under field conditions it was (0.15±0.012, 0.12±0.011and 0.08±0.009 U/mg protein/min) compared to the control group (0.07±0.005 U/mg protein/min). The decrease in binary treatment was more than the alone one. The detoxicifiaction enzyme α-esterase (α-EST) activity significantly dropped in *K. acuminata* after treatment with AO, DO and AO+DO - treated groups under laboratory conditions (0.51±0.008, 0.48±0.01and 0.03±0.01 µl α-naphthol/ mgprotein/min,respectively) compared to the control group (0.53±0.04 µl α-naphthol/ mgprotein/min) and field conditions (0.49±0.012, 0.47±0.12 and 0.08±0.01 µlα-naphthol/mg protein/min,respectively) compared to the control group (0.52±0.013 µlα-naphthol/mg protein/min). Generally, the decrease in binary group was more than the alone one. Moreover, the effect of plant oils on insect nymphs in the laboratory was more than the field.







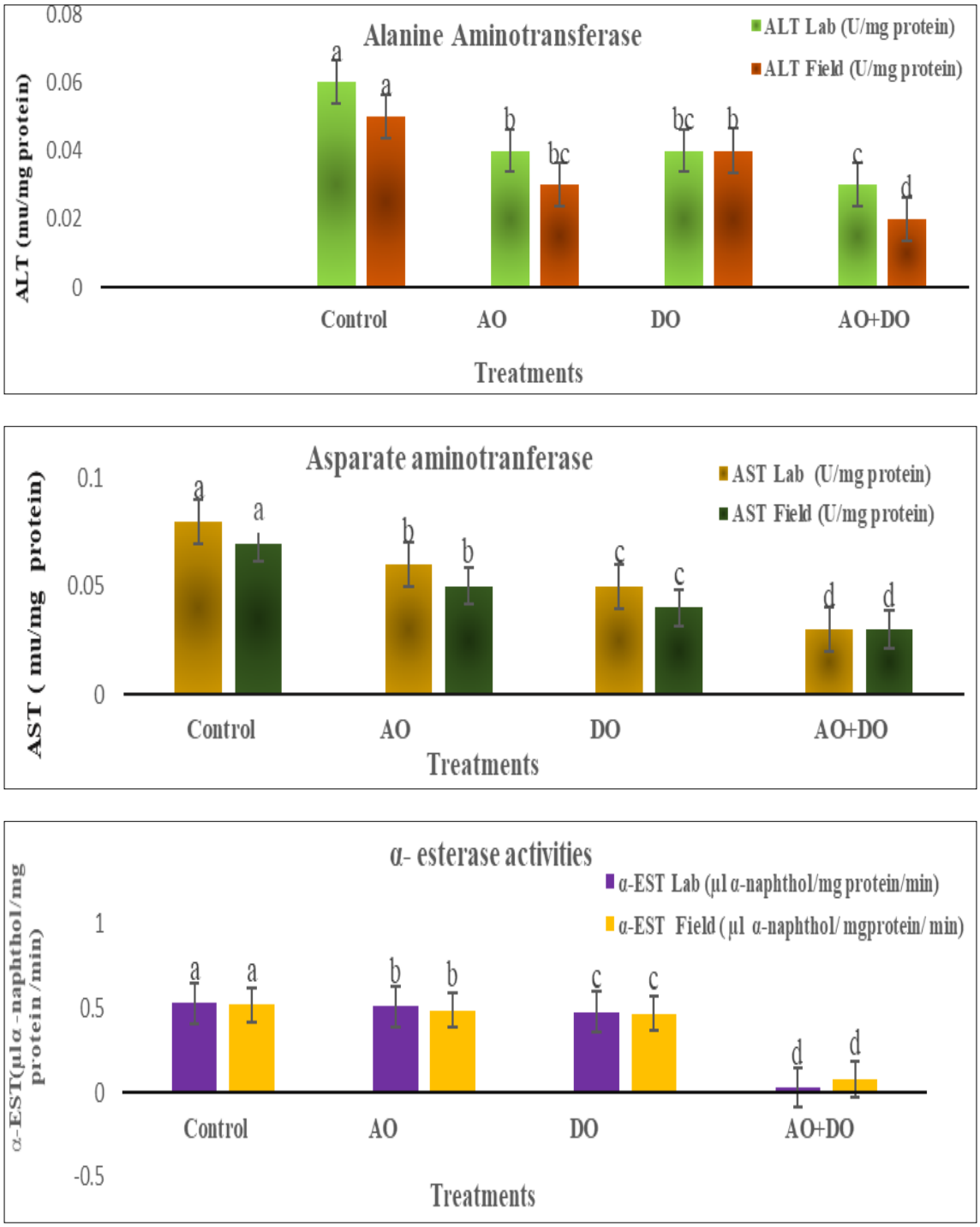


Fig 4: The biochemical parameters of the effect of AO, DO and binary group (AO+DO) on of *K. acuminata* nymphs: Total protein concentration (µg/g protein) and the activities of acidic phosphatase (ACP) (µgPhenol/min/g.b.wt.) , phenol oxidase (PhO) (U/mg protein/min) enzymes, alanine aminotransferase (ALT) (mU/mg protein), asparate aminotransferase (AST) (mU/mg protein), and α-esterase (α-EST) activities under laboratory and field conditions against *K. acuminate* nymphs at 2%. Bars topped with different letters are significantly different. Bars indicate the standard error (SE)

Cellular damage of *K. acuminata* findings as shown by the transmission electron microscopy (TEM) induced by AO + DO -treated group (2%)

Ultra-microphotographs of the Transmission Electron Microscopy (TEM) (x 5000) showing the cellular damage induced by AO + DO -treated group (2%) (Fig.5).

The toxicity effect of the joint action or the binary treatment was superior and these ultra micrographs justified the obtained results in reducing nymph population as follows: (5A) control nymphs: micrographs showing normal thickness of cell membrane, normal nucleus without damage or shrinkage, clear fatty bodies without fragmentation and normal body cavity in general. (5 B) treated nymphs with AO+DO- treated group (2%) different malformations were shown such as: degrading the thickness of the cell membrane, fragmentation of cells, damage in the normal nucleus, the cell size was shrunken and vacuoles were appeared. As well as, fatty bodies became fragmented and cell apoptosis were appeared

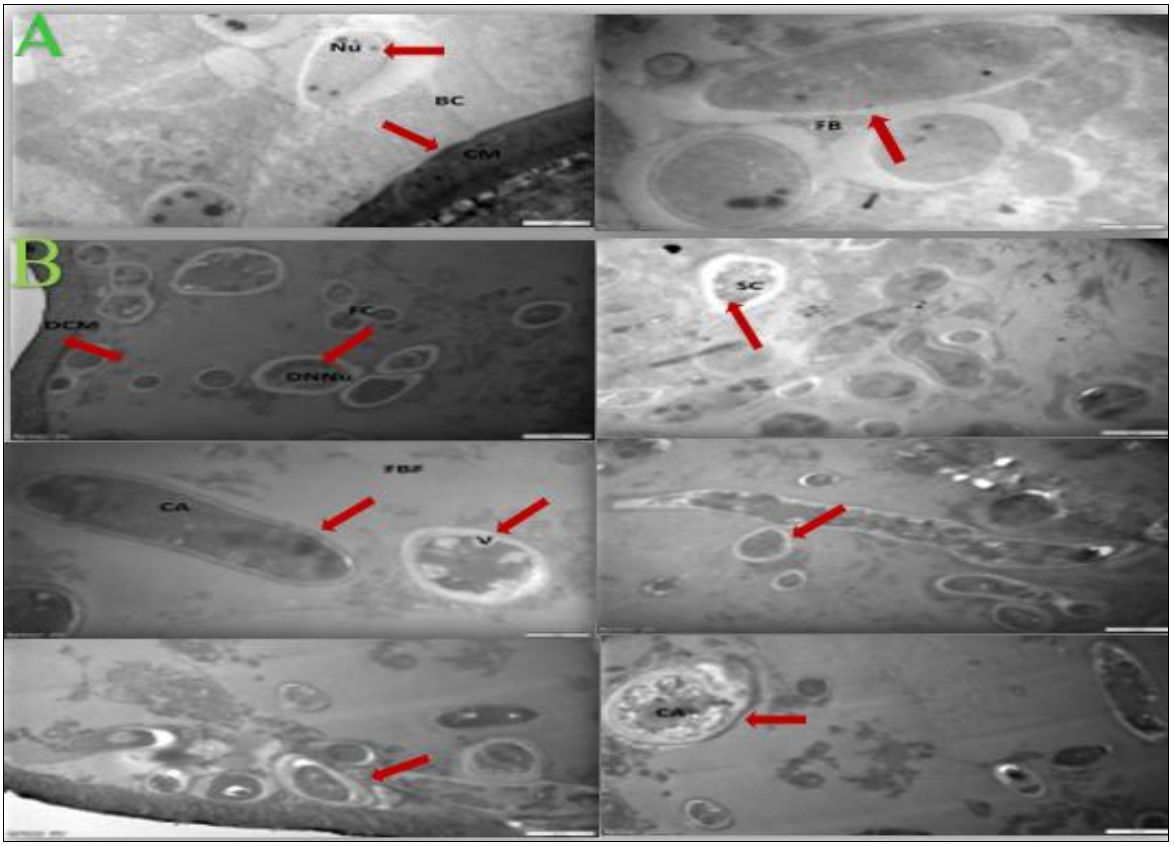


Fig 5: Ultra-microphotographs of the Transmission Electron Microscopy (TEM) showing the impact of AO + DO group (2%) against *K. acuminata*. A) Normal group: *K. acuminata* consisting of cell membrane of the body (CM), body cavity (BC), nucleus (Nu), and fatty bodies (FB). B) Different malformations of *K. acuminata* induced by AO + DO treatment: degraded cell membrane (DCM), Fragmented cells (FC), damaged normal cell nucleus (DNNu), shrunken cells (SC), an appearance of vacuoles (V), fatty bodies fragmentation (FBF), an appearance of cell apoptosis (CA) (×5000)

Discussion

Mango is the most economically important trees and be attacked with different destructive pests the most important one is the Coccidae, *K. acuminata*. Excessive use of chemical insecticides has excluded natural enemies. So, we stressed in this work on the studying alternatives to chemicals in order to involve them in pest management strategies. Plant essential Oils (EOs) are volatile mixtures of terpenoids and related aromatic compounds, which are secondary plant metabolites. They can be used to manage insect pests as repellent, insecticidal, oviposition and feeding deterrents, growth regulators and insect toxicity. The insecticidal activity of the essential oils was different relying on their chemical constituents, which the majority is terpenoids. These compounds expressed neurotoxic activity by inhibition of acetylcholinesterase (AChE) **[15]** and **[34].** The hydrophobic nature of terpenoids led to interact with the lipids and insects' membranes, being cell membrane disruption just one possible consequence of these interactions [5, 37]. Plant essential oils have main components which being blended and resulted in a new formulation (binary mixture) having synergistic properties or additive upon toxicity [36]. Several mechanisms caused by the insecticidal and biological activities of the variable and complex mixtures of essential oils playing on many physiological processes [14]. Our findings coincided with studying the insecticidal activity of AO, DO and their joint action (AO + DO) on *K. acuminate* in mango via laboratory and field studies. In other word, the toxicity bioassay in the laboratory indicated that the percent mortality of binary group of AOs + DO -treated group (2%) was superior to AO or DO. Moreover, the percent mortality of DO was higher than AO at 2 %. The field application is confirmed the toxic impact of oils as the data were obtained in the laboratory. The binary treatment AO + DO -treated group (2%) recorded the highest reduction percentages in nymph population at 14, 30 and 45 days after spraying and consequently the mean reduction was the same. The binary treatment indicated the co-toxicity between phytochemicals of AO and DO. These results were in the same line with [23] showed that limonene has better effect as an emulsion when mixing the tested oil with an emulsifier solution. The resulted mix had variability of the EO insecticidal effect on the target pest. However, in our study the reduction percentages in the field study were reduced in all treatments after 30 days then re-increased after 45 days. Thus, could be due to the change occurred in the weather factors or predation of natural enemies which affected the insect population in the control plots as well as in treated ones or may be due to other unknown factors in the field. In a field study variation in hemipteran insect nymph populations between two successive seasons in all treatments including check or control treatment [3]. AO has many different bioactive components the most important of which was linoleic acid [46]. GC/MS technique of dill essential oil and the obtained results indicated that 45 compounds were identified including α-phellandrene, camphor, dihydrocarvone, trans-isolimonene, carvone and dillapiole were the major compounds **[45]**and this oil gave promising results in control *T. castaneum*. In the same line [49], used GC/MS analysis of the fractions from *A. graveolens* essential oil (dill) and found various terpenes components.

Evaluation bioactive components of Anethum extract by the HPLC apparatus was used by [20]. They found that aromatic and phenolics were the main constituents. As a result of the morphological observations via the change in the body color (minilization) and severe desiccation in treated nymphs with oils were explained by physiological analysis. The disturbance in the total protein concentration and the activities of certain enzymes. The biochemical analysis of nymphs which were taken under the laboratory and field conditions indicated that TP had a significant decrease in *K. acuminata* induced by AO + DO -treated group than AO and DO at 2%. Generally, the tested treatments exhibited great inhibition of ACP, PhO, ALT, AST and α-EST activities in insect nymphs at 2%. The decrease in AO + DO -treated group is more than the alone one. The effect in the laboratory conditions is more than in the field ones. This inhibition in total protein subsequently affected reproduction and development. Also, enzymes inhibition affected growth and all physiological processes in *K. acuminata.* Our findings in the same trend of some authors which indicated that importance of proteins called enzymes which catalyze a diversity of biological routes and generally proteins are the primary biological constituents of the development. Moreover, among the various digestive enzymes in insects' alimentary canals the activities of acid phosphatase and aminotransaminse play a critical role in food digestion in insects [40, 28, 43, 41]. Phenoloxidases are operating insect development and immunity for the early immune defense [50]. Reduction of phenoloxidase enzymes in larvae lead to deteriorate the immune system. Moreover, esterases are the main enzymes for the detoxification process in the pests, the activity of α-esterase is varied after the toxic impact in the pests as result as resistance of their body to the toxic compounds [18, 33]. EOs which in hydrophobic properties and insecticidal activity, were successfully studied for their potential impact on the activity of the key enzymes; AST and ALT as well as detoxification enzymes [6]. Plant-Derived Essential Oils induced an insecticidal activity in mealybugs and induce a change in the physiological parameters: ALT, AST, ACP, and PhO enzymes [30]. Our results were also in accordance with [11, 9, 10] showed that essential oil induce physiological and biochemical alternations in mealybugs and can be used as insecticidal activities. The binary combinations of essential oils induced a high impact in the protection from pests and this were in harmony with our results [21].

Additionally, in this study cellular changes as shown by the Transmission Electron Microscopy (TEM) micrographs confirmed the laboratory and field studies as well as the insect malformations and biochemical examinations about the toxic effect of the combined treatment AO + DO -treated group (2%) which gave the highest reduction in *K. acuminata* nymphs in this study. Ultra-microphotographs of TEM showing the cellular damage induced by AO + DO -treated group (2%). Different malformations such as cell membrane became thinner, cell fragmentation, malformed nucleus, presence of vacuoles, fragmented fatty bodies, cell apoptosis were appeared and generally the cell size were shrunken versus normal cells in the control nymphs. These data came in line with **13** found that clove essential oil affects the reproductive system histochemistry of *Spodoptera frugiperda*. The toxicity of different EOs on *Drosophila suzukii* adults was studied. Obtained results were in the same trend of our findings as EOs not had only insecticidal activity but also made histological and structural alterationsn [14]. Besides [29], recorded histological alterations in the midgut of second and fourth larval instars of *Spodoptera littoralis* induced by using essential oils against the insect larvae. The tested essential oils caused basement membrane separation, degeneration, and loss of some epithelial cell lining.

Collectivity, our results showed that the joint action of the two tested oils AO + DO -treated group (2%) could be the potential alternatives to chemicals in controlling *K. acuminata* in mango fields as it was superior in control *K. acuminata* than alone treatments AO or DO. Moreover, the cellular damage caused by the binary treatment may clear site of actions of these oils and suggest the potential target sites in cells of *K. acuminata* nymphs induced by AO + DO -treated group (2%). Further studies are needed to give confirmation of the binary treatment of AO + DO -treated group (2%) as an effective alternative control tool against *K. acuminata*, to more elucidate its molecular action sites and assessment its toxicity to non-target organisms.

Conclusions

One can conclude that AO + DO -treated group (2%) can be involved as one of the IPM program of *K. acuminata* considering promising alternative to synthetic insecticides.

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Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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