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Research Article

Toxicity of Traditional, Novel and Bio-insecticides and Their Mixtures Against House Fly *Musca domestica* in Relation to Some Biochemical Activities

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Abstract

Background and Objective: Problems caused by organosynthetic insecticides on the environment and non-target organisms has stimulated the use of natural products as an alternative pest control strategy, these natural products have a lower persistence in the environment and therefore, are considered environmentally and toxicologically safer than several of the currently used organosynthetic pesticides. This study was conducted in order to reduce the use of pesticides by mixing chemical insecticides with plant extracts, vegetable oils or microbial insecticides which considered safer to human and environment. **Materials and Methods:** The experiments were conducted in Toxicology Laboratory, Faculty of Agriculture, Menoufia University. The insecticides applied as sugar bait method. The data was statistically analyzed using (one-way ANOVA) one way direction by F-test at LSD 5% probability. **Results:** Most of tested mixtures showed high synergistic effect on house fly adults. The five selected mixtures which recorded the highest synergistic effect achieved significant decrease in total proteins, lipids and carbohydrates, also in α and β-esterase and AChE activity. **Conclusion:** The quantity of pesticides can be reduced, reduced the environmental pollution, costs and achieve safety control of house fly by using mixtures of chemical insecticides with plant oils, plant extracts and microbial insecticides. It could be recommended that, using the promising mixtures which achieved higher synergistic effect as a component in integrated management programs and integrated resistance management strategies of *M. domestica*.

Key words: Safety control, mixtures, mode of action, *Musca domestica*, microbial insecticides

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The house fly, *Musca domestica* L. (*M. domestica*), is one of the main pests of dairies and public health transmitting many diseases to animals and humans[1]. Many insecticides related to traditional and novel groups have been used to manage this insect worldwide. However, it developed resistance to the most of insecticides used, therefore, the important tools suggested to face the resistance to different insecticides is rotation and mixture[2]. The mixture contain organophosphate, pyrethroid or carbamate insecticides increase the toxicity of insecticides, as well as decrease the resistance of insect pests such as *Bemisia tabaci*[3], *Culex quinquefasciatus*[4] and *Musca domestica*[5]. The inhibition of esterases enzymes was suggested as mechanism of action for this type of potentiation or synergism[6,7] or mono-oxygenases activity[8]. Mixing insecticides related to different groups usually have different mode of actions and became very effective in resistance management programs[9]. Due to the high cost and environmental effects of chemical pesticides, the scientists searched for the saver and cheapest alternatives such as botanical products[10]. The residual/surface/aerosols applications increased the development of insecticidal resistance in house fly, besides that, the sprays contaminate food and water, so that, poisoning baits become good choice in house fly control[11].

From the previous view, this article was conducted to control house. fly by using mixtures of some bio-compounds with chemical insecticides to reduce environmental pollution and development of resistance in house fly to insecticides and estimate the toxicity, co-toxicity coefficient of traditional, novel and bio-pesticides and their mixtures against common house fly, *Musca domestica* in relation to some biochemical activities.

MATERIALS AND METHODS

Tested insect: House fly, *Musca domestica* were reared in the insect rearing room at 25-27°C and 55-60% relative humidity. A standard rearing method by Sawicki[12] was adopted to provide 2nd larval instars used for running bioassay tests.

Tested insecticides: Eight commercial formulations of insecticides were used: Lambada cyhalothrin (Lambada® 5% EC), deltamethrin (Decis®5% EC), methomyl (Lannate® 90% WP), buprofezin (Applude® 25% SC), spinosad (Spintor® 24% EC), abamectin (Vertimec® 1.8% EC), B.t (Protecto® 9.4% WP), chlorantraniliprole (Coragen®, Rynaxypyr®20% SC) and indoxacarb (Steward®, Avaunt®15% SC)[13].

Plants and extraction: Pomegranate (*Punica granatum*) fruits and khaya (*Khaya senegalensis*) leaves were collected from the experimental farm of Faculty of Agriculture, Menoufia University, Shebin Elkom, Egypt.

Fruit rind of pomegranate and khaya leaves were air dried at room temperature (27±2°C) for about 20 days. Dried fruit rind of pomegranate and Khaya leaves were powdered with an electrical blender and sieved to get fine powder. About 100 g of khaya leaf powder was submerged in 300 mL of 70% ethanol and 100 g of fruit rind pomegranate powders was submerged in 300 mL of 70% methanol at room temperature. After 24 h, the supernatants were decants and filtered through Whatman filter paper No.5 and dried in a rotary evaporator at 40°C for 1 h to obtain crude extracts which were kept in brown glass bottles[13].

Plant volatile oils: Jojoba oil (*Simmondsia chinensis*) and Parsley oil (*Petroselinum crispum*) were purchased from Elgomhoria Company for medical pharmaceutics.

House fly rearing: Colonies of *Musca domestica* originated from larvae were collected from poultry farm at El-Behira province in May, 2016 and reared under laboratory conditions to adults. Adult house flies were reared in plastic cages, 40×40×40 cm, which were covered with mesh screen with cloth sleeve opening at front. The newly emerged flies were fed with full fat fresh milk soaked in cotton wool, for 3 days, after emergence to enhance egg production, after that adult flies were given milk sugar solution soaked on cotton wool in petri dishes. After 3 days of fly emergence, glass beakers containing larval food were placed in rearing cages for egg lying. The beakers were removed from cages after 2-3 days when eggs were visible and attached to food along the sides of beakers. The food was changed after 2-4 days upon the numbers of larvae per beaker. The beakers were kept in separate cage for fly emergence. When the pupae were formed, these beakers were kept in another cage for adult emergence.

Toxicity bioassay by "sugar bait" methods: About 5 g of sugar were placed in 250 mL glass beaker and saturated with 1 mL acetone containing the toxicant at definite concentrations and allowed to complete evaporation of acetone by electric air dryer. Control was performed by equal quantities of cane sugar plus acetone free of any toxicant.

Ten adults of *Musca domestica* 0-24 h old were transferred to baited glass beaker and covered with muslin cloth, banded with rubber bands and maintains at room temperature for 24 h. To estimate potency of the different substances, different concentrations (5-6 concentrations) and three replicates were prepared. Mortalities were estimated after 24 h.

Mixtures toxicity: Paired mixtures of lambada-cyhalothrin, deltamethrin, methomyl, indoxacarb and coragen with khaya, pomegranate, jojoba oil, parsley oil, abamectin, spinosad and protecto at 1:1 mixing ratio were freshly prepared. Each mixture was tested in four replicates along with control. Mortality percentages were determined after 24 h and joint action of the different mixtures was expressed as co-toxicity coefficient factor were determined according to Sun and Johnson[14].

Biochemical effect: The mixtures which exhibited higher synergistic effect were selected and tested at LC_{30} values on *M. domestica* adults by sugar bait method, where 20 insects were used for each treatment and replicated three times.

Total protein, lipid and carbohydrate contents, α and β-esterase and acetylcholinesterase activity were determined for each mixture and each component to know the mode of action of tested mixtures.

Preparation of insects for analysis: The insects were prepared as described by Amin[15]. They were homogenized in distilled water (50 mg /1 mL). Homogenates were centrifuged at 8000 rpm for 15 min at 2°C in a refrigerated centrifuge. The deposits were discarded and the supernatants, which was referred as enzyme extract, can be stored at least 1 week without appreciable loss of activity when stored at 5°C.

Acetylcholinesterase determination: AchE (acetylcholinesterase) activity was measured according to the method described by Simpson *et al.*[16], using acetylcholine bromide (AChBr) as substrate.

Nonspecific esterases: Alpha esterases (α-esterases) and beta esterases (β-esterases) were determined according to Van Asperen[17] using α-naphthyl acetate or β-naphthyl acetate as substrates, respectively.

Total proteins: Total proteins were determined by the method of Bradford[18].

Determination of total carbohydrates: Total carbohydrates were estimated in acid extract of sample by the phenol-sulphuric acid reaction of DuBois *et al.*[19]. Total carbohydrates were extracted and prepared for assay according to Crompton and Birt[20].

Determination of total lipids: Total lipids were estimated by the method of Knight *et al.*[21] using phosphovanillin reagent prepared by dissolving of 0.6 g pure vanillin in 10 mL ethanol and completed to 100 mL with distilled water. Then 400 mL concentrated phosphoric acid was added.

Statistical analysis: Adult's mortalities after 24 h were estimated and corrected according to Abbott[22]. Probit analysis according to Finney[23] was performed to estimate toxicity values and slope of regression line for each tested substance. The data of biological aspects was statistically analyzed using one way analysis of variance. (ANOVA) by F-test at 5% probability. The measurements were divided using Duncan's multiple range test.

RESULTS AND DISCUSSION

Toxicity of tested compounds: The LC_{50} of different insecticides related to traditional, novel and bio-insecticides on house fly *Musca domestica* adults were presented in Table 1. The data clearly showed that deltamethrin was the most toxic compound followed by indoxacarb, abamectin, methomyl and spinosad recording LC_{50} values, 0.44, 0.71, 1.45, 3.74 and 10.27 ppm, respectively after 24 h from treatment compared with other tested compounds. On the other side

Table 1: Toxicity of traditional, novel and bio -insecticides on house fly (*Musca domestica*) adults

Treatments	LC_{50} (ppm)	Slope±SE	Confidence limits
Lambada cyhalothrin	27.17	0.727±0.088	14.101-58.664
Deltamethrin	0.44	0.560±0.163	0.019-1.508
Methomyl	3.74	0.757±0.131	1.384-7.267
Indoxacarb	0.71	0.751±0.163	0.144-1.853
Chlorantraniliprole	198.09	0.610±0.175	61.14-2848.228
Abamectin			
24 h	1.45	1.174±0.297	0.292-3.099
48 h	0.61	1.123±0.355	0.022-1.643
72 h	0.12	1.118±0.633	-
Spinosad			
24 h	10.27	0.864±0.131	5.413-17.705
48 h	1.296	1.284±0.338	0.261-2.693
72 h	0.05	0.787±0.410	-
Protecto			
24 h	1113.73	0.263±10.171	-
48 h	78.14	0.647±0.181	15.037-208.716
72 h	6.42	0.502±0.189	0.0008-33.548
Khaya extract	1476.73	1.171±0.183	959.379-2409.310
Pomegranate extract	543.01	0.821±0.180	218.450-1287.706
Parsley oil	686.05	0.616±0.164	219.609-2461.565
Jojoba oil	1135.26	1.052±0.228	572.171-2429.799

Table 2: Toxicity and co-toxicity coefficient of lambada-cyhalothrin and volatile oils, plant extracts and microbial insecticide mixtures on *M. domestica* adults

Mixtures ratio (1:1)	LC_{50} (ppm)	Slope±SE	Confidence limit	Co-toxicity coefficient
Lambada+khaya	5.04	0.392±0101	0.443-26.09	1077.96
Lambada+jojoba oil	9.78	0.527±0.108	2.134-33.376	543.66
Lambada+parsley oil	187.61	0.176±0.098	2.948-2.487 E+12	27.85
Lambada+abamectin	1.012	0.246±0.118	4.39 E-19-22.934	269.88
Lambada+spinosad	96.56	0.438±0.133	14.862-1970.708	5.83
Lambada+protecto	37.35	0.394±0.125	2.460-372.104	142.02
Lambada+pomegranate	28.59	0.394±0.125	2.460-372.104	142.02

Table 3: Toxicity and co-toxicity coefficient of deltamethrin and volatile oils, plant extracts and microbial insecticides mixtures on *M. domestica* adults

Mixtures ratio (1:1)	LC_{50} (ppm)	Slope±SE	Confidence limit	Co-toxicity coefficient
Deltamethrin+khaya	2.29	0.572±0.171	0.025-12.468	38.41
Deltamethrin+jojoba oil	0.67	0.527±0.180	0.0002-5.570	131.29
Deltamethrin+parsley oil	5.86	0.629±0.168	0.310-24.693	15.01
Deltamethrin+abamectin	0.05	0.336±0.134	3.025 E-10-0.994	1350.06
Deltamethrin+spinosad	369.27	0.456±0.145	60.777-33.909	4.28
Deltamethrin+protecto	0.74	1.119±0.296	0.183-2.224	117.84
Deltamethrin+pomegranate	0.33	0.328±0.125	4.067 E-6-4.128	266.45

Table 4: Toxicity and co-toxicity coefficient of methomyl and volatile oils, plant extracts and microbial insecticides mixtures on *M. domestica* adults

Mixtures ratio (1:1)	LC_{50} (ppm)	Slope±SE	Confidence limit	Co-toxicity coefficient
Methomyl+khaya	61.75	0.575±0.156	61.75-8.957	12.05
Methomyl+jojoba oil	2.04	0.627±0.181	0.034-10.276	365.49
Methomyl+parsley oil	45.06	0.513±0.151	4.038-222.230	16.91
Methomyl+abamectin	2.31	00.357±0.122	0.013-20.180	233.30
Methomyl+spinosad	170. 05	0.534±0.150	37.284-2454.075	3.22
Methomyl+protecto	2.54	1.355±0.333	0.878-7.038	293.48
Methomyl+pomegranate	51.12	0.431±0.146	2.278-363.200	14.54

spinosad and abamectin was the most toxic tested compounds after 72 h from treatment recording LC_{50} values 0.05 and 0.12 ppm, respectively.

From the obtained results, it can be concluded that tested traditional, novel, microbial insecticides was highly toxic compared to volatile oils and crude plant extracts.

These results were in agreement with Mansour *et al.*[24], who found that all tested insecticides had the highest toxicity compared with plant extracts as sugar bait against house fly adult stage. In addition, Al-Solami *et al.*[25] found that the toxicity of bioinsecticide spinosad was more effective than vectobac against *Aedes aegypti* larvae by about 11.1 times. Also, Norris *et al.*[26] reported that the most toxic tested essential oil (patchouli oil) was 1,700 times less toxic than the least toxic synthetic pyrethroid, bifenthrin on *Aedes aegypti* as topical application, while on *Anopheles gambia*, the most toxic essential oil (patchouli oil) was -685 times less toxic than the least toxic synthetic pyrethroid.

Toxicity of different mixtures: The toxicity of five insecticides in binary mixtures with six of plant extracts, plant volatile oils and microbial insecticides at mixing ratio (1:1) to *M. domestica* adults were presented in Table from 2-6.

The LC_{50} and co-toxicity coefficient of lambada cyhalothrin mixed with khaya extract, pomegranate extract, parsley oil, gogoba oil, abamectin, spinosad and protecto at (1:1) mixing ratio were showed in Table 2. The obtained data showed that mixtures of lambada cynhalothrin with khaya, jojoba, abamectin, protecto and pomegranate extracts achieved high synergistic effects.

It was cleared that the mixture of deltamethrin with abamectin at (1:1) mixing ratio recorded the highest co-toxicity coefficient value in Table 3 followed by deltamethrin+pomegranate, deltamethrin+jojoba and deltamethrin+protecto. On the other side, the mixture of deltamethrin with khaya extract, parsley oil and spinosad showed high antagonistic effect.

As for methomyl, the data showed that the toxicity of methomyl was increased when it mixed with jojoba oil, protecto and abamectin (Table 4). On the other side, the toxicity was decreased when it was mixed with khaya extract, parsley oil, spinosad and pomegranate extract.

The toxicity and co-toxicity coefficient of indoxacarb mixtures with plant extracts, plant volatile oils and microbial insecticides at (1:1) mixing ratio on *Musca domestica* adults were presented in Table 5. The data showed that nearly all combinations demonstrated antagonistic effect except it is mixture with abamectin and protecto which exhibited synergistic effect.

The mixtures of coragen with jojoba, parsley oils and protecto at (1:1) mixing ratio (Table 6) showed high synergistic effect compared with the mixture of coragen with khaya, abamectin, spinosad and pomegranate which exhibited high antagonistic effect.

Table 5: Toxicity and co-toxicity coefficient of indoxacarb and volatile oils, plant extracts and microbial insecticides mixtures on *M. domestica* adults

Mixtures ratio (1:1)	LC_{50} (ppm)	Slope±SE	Confidence limit	Co-toxicity coefficient
indoxacarb+khaya	2.61	1.115±0.323	0.376-7.768	65.71
indoxacarb+jojoba oil	25.08	0.566±0.155	2.259-149.895	5.66
indoxacarb+parsley oil	2.05	0.365±0.148	1.325E-6-22.230	69.19
indoxacarb+abamectin	0.81	0.404±0.128	0.004-6.248	117.68
indoxacarb+spinosad	5.63	1.034±0.243	1.291-16.445	23.59
indoxacarb+protecto	0.02	0.199±0.121	-	7095.94
indoxacarb+pomegranate	0.63	0.270±0.144	-	3.78

Table 6: Toxicity and co-toxicity coefficient of chlorantraniliprole and volatile oils, plant extracts and microbial insecticides mixtures on *M. domestica* adults

Mixtures	LC_{50} (ppm)	Slope±SE	Confidence limit	Co-toxicity coefficient
Chloran+khaya	1008.84	0.395±0.155	140.756-1.351 E+6	34.63
Chloran+jojoba oil	23.45	0.425±0.146	0.390-149.895	1438.57
Chloran+parsley oil	38.62	0.729±0.170	7.677-124.712	795.97
Chloran+abamectin	54.40	0.477±0.133	8.956-535.187	5.30
Chloran+spinosad	84.66	0.522±0.141	16.710-781.423	23.07
Chloran+protecto	82.90	0.388±0.127	9.933-3024.764	405.76
Chloran+pomegranate	7691.78	0.857±0.392	1822.67-3.891E+12	3.78

Chloran: Chlorantraniliprole

Generally, from previous results it can be suggested that the mixtures of traditional, novel and bio-insecticides which exhibited high synergistic effect can be used to reduce amount of insecticides to decrease environmental pollution and hazardous of pesticides on human and it can be used as a component in integrated *M. domestica* management programs and integrated resistance management strategies.

The obtained results were in agreement with Mesbah *et al.*[27], who found that the combinations between methoxyfenozide, profenofos and spinosad with essential oils (flax or linseed and sesame) gave synergistic effects to 4th instar larvae *of Spodoptera littoralis*. Also, Salama *et al.*[28] found that the mixtures of pyrethroids and microbial insecticide, *Bacillus thuriengensis* achieved synergistic effects against cotton leafworm *Spodoptera littoralis*.

Khan *et al.*[29] found that the mixtures of deltamethrin with emamectin benzoate at (1:1) showed significant increase toxicities compared to alone to *Musca domestica*. Furthermore, Mansour *et al.*[24] reported that all tested plant extracts mixed with methomyl, deltamethrin and chlorpyrifos were resulted potentiating mixtures with co-toxicity factors exceeding 90. Also, Islam and Aktar[30] concluded that the mixtures of plant extracts and synthetic pyrethroids insecticides were more effective than the insecticides or plant extracts alone. Thangam and Kathiresan[31] suggested that synergism may be happen due to phytochemicals inhibiting the insect ability to use detoxifying enzymes against synthetic chemicals. The joint action may well prolonged the efficacy of synthetic insecticides that well eventually be useless due to resistance[32]. Mansour *et al.*[33] found that the combinations of botanical extracts and insecticides induced potentiating effect against house fly larvae. Where mixtures of deltamethrin with different plant extracts exhibited high synergistic effects.

Recently, Abbas *et al.*[34] found that the mixtures can increase the efficacy of product and delay the development of resistance, thus it can be used as a useful tool for pest control and reported that mixture of lambada cyhalothrin with emamectin benzoate at (1:1) ratio showed synergistic effect to house fly (*Musca domestica*). Bhan *et al.*[35] found that the combination of temephos and petroleum ether extract of *Correa reflexa* at 1:1 were more effective than other ratios when tested for their larvicidal potentiality against larvae of *Anopheles stephensi* (*A. stephensi*) and *Culex quinquefasciatus* (*C. quinquefasciatus*), the co-toxicity coefficient for the 1:1 mixture were 178.57, 191.67 and 181.82 and 375, 357.14 and 307.6 against *A. stephensi* and *C. quinquefasciatus* larvae, respectively, after 24, 48 and 72 h of exposure. In addition, Farooq and Freed[36] found that the insecticides acetamiprid, emamectin benzoate, imidacloprid and lufenuron in combination with insect pathogenic fungi showed higher mortality than expected with significant synergistic interactions when tested as a bait against *M. domestica,* which recommend the potential of combined use of entomopathogenic fungi and synthetic insecticides for the control of *M. domestica*. Furthermore, the combination of entomopathogenic fungi and synthetic insecticides can decrease the concentrations of the active ingredient required. Al-Solami *et al.*[25] revealed that the chemical insecticide actellic (pirimiphos-methyl) in combinations with spinosad, dudim and neem extract against the mosquito larvae achieved different levels of potentiation revealed by the inhibition of adult formation.

Table 7: Effect of five mixtures of traditional, novel and bio-insecticides on total protein, lipids and carbohydrate contents of *M. domestica* adults

Treatments	Total proteins (mg g^{-1} b.wt.)	Change (%)	Total lipids (mg g^{-1} b.wt.)	Change (%)	Total carbohydrates (mg g^{-1} b.wt.)	Change (%)
Lambada.+khaya	8.73^{c}	-33.71	8.17^{c}	-25.52	8.97^{d}	-34.52
Lambada.	9.80^{b}	-25.59	9.20^{b}	-16.13	10.07^{c}	-26.50
Khaya	12.10^{a}	-8.12	11.23^{a}	+2.37	12.10^{b}	-11.68
Control	13.70^{a}	-	10.97^{a}	-	13.17^{a}	-
LSD (0.05)	0.56	-	0.81	-	0.64	-
Delta.+abamectin	8.13^{c}	-38.37	7.87^{c}	-28.26	8.03^{c}	-41.39
Delta.	9.67^{b}	-26.58	8.87^{b}	-19.14	9.93^{b}	-27.52
Abamectin	9.83^{b}	-25.36	9.40^{b}	-14.31	9.97^{b}	-27.23
Control	13.70^{a}	-	10.97^{a}	-	13.17^{c}	-
LSD (0.05)	0.60	-	0.99	-	0.56	-
Methomyl+jojoba oil	9.43^{d}	-28.40	8.37^{d}	-23.70	9.63^{d}	-29.71
Methomyl	10.33^{c}	-21.56	9.30^{c}	-15.22	10.30^{c}	-24.82
Jojoba oil	12.36^{b}	-6.15	10.10^{b}	-7.93	12.23^{b}	-10.73
Control	13.70^{a}	-	10.97^{a}	-	13.17^{a}	-
LSD (0.05)	0.78	-	0.77	-	0.57	-
Indoxa.+pomegranate	7.13^{c}	-45.86	5.50^{c}	-49.86	7.13^{d}	-47.96
Indoxa.	8.30^{b}	-36.98	6.73^{b}	-38.65	8.83^{c}	-35.55
Pomegranate	12.93^{a}	-1.82	10.83^{a}	-1.28	11.87^{b}	-13.36
Control	13.70^{a}	-	10.97^{a}	-	13.17^{a}	-
LSD (0.05)	0.92	-	0.87	-	1.07	-
Chloran.+jojoba oil	7.70^{d}	-41.53	5.90^{d}	-46.22	7.83^{d}	-42.85
Chloran.	8.80^{c}	-33.18	7.20^{c}	-34.37	9.60^{c}	-29.93
Jojoba oil	12.36^{b}	-6.15	10.10^{b}	-5.49	12.23^{b}	-10.73
Control	13.70^{a}	-	10.97^{a}	-	13.17	-
LSD (0.05)	0.66	-	0.72	-	0.67	-

The same letters means no significant difference at 5% level, % change: Control-treated/control $\times$ 100. Lambada.: Lambada cyhalothrin, Chloran.: Chlorantraniliprole, Indoxa.: indoxacarb, Delta.: Deltamethrin

Effects on total protein, lipid and carbohydrate contents: The data in in Table 7 clearly showed that all tested mixtures reduced the total protein content in *M. domestica* adults compared with control and its components alone. There were significant differences between tested mixtures and its components and control. The highest decreased in protein content was achieved in indoxacarb+pomegranate mixture, where it was 7.13 mg g^{-1} b.wt. and change (%) was -45.86% followed by chlorantraniliprole+jojoba oil, deltamethrin+ abamectin, lambada cyhalothrin+khaya extract and methomyl+jojoba oil, where total protein contents and change % was (7.7 mg g^{-1} b.wt. and-41.53), (8.13 mg g^{-1} b.wt. and -38.37), (8.73 mg g^{-1} b.wt. and -33.71) and (9.43 mg g^{-1} b.wt. and -28.40), respectively.

As for total lipid contents, the data indicated that all mixtures reduced the total lipid content in the adults of *M. domestica* Table 7. There were significant differences between each mixtures and its components alone and control. The mixtures reduced total lipid content more than its components. The highest reduction in total lipid contents was achieved with indoxacarb+pomegranate (5.5 mg g^{-1} b.wt.) and % change was -47.96 less than control, followed by (chlorantraniliprole+jojoba oil), (deltamethrin+abamectin), (lambada cyhalothrin+khaya) and (methomyl+jojoba oil), where the total lipid contents and % change than control were (5.9 mg g^{-1} b.wt. and -46.22%), (7.87 mg g^{-1} b.wt. and -28.26%), (8.17 mg g^{-1} b.wt. and -25.52%) and (8.37 mg g^{-1} b.wt. and -23.70%), respectively.

The data in Table 7 showed that all tested mixtures decreased the total carbohydrate contents in *Musca domestica* adults. There were significant differences between each mixture and its components alone and control.

The indoxacarb+pomegranate mixture achieved the highest decreased in total carbohydrate contents (7.13 mg g^{-1} b.wt.) and% change as-47.96% less than control, followed by (chlorantraniliprole+jojoba oil),(deltamethrin+abamectin), (lambada cyhalothrin+khaya) and (methomyl+jojoba oil), where the total carbohydrate contents and % change were (7.83 mg g^{-1} b.wt. and -42.85), (8.03 mg g^{-1} b.wt. and -41.39), (8.97 mg g^{-1} b.wt. and -34.52) and (9.63 mg g^{-1} b.wt. and -29.71), respectively.

Effects on non-specific enzymes α and β esterase: The data clearly showed that the all tested mixtures reduced α and β esterase activity compared with control and it is component Table 8.

As for α esterase activity, the indoxacarb+pomegranate mixture achieved the highest reduction in the enzyme activity

Table 8: Effect of five mixtures of traditional, novel and bio-insecticides on α and β-esterase activity on *M. domestica* adults

Tested mixture	α-esterase	Activity ratio	Change (%)	β-esterase	Activity ratio	Change (%)
Lambada.+khaya	557.67[c]	0.86	-13.54	470.00[c]	0.73	-26.91
Lambada.	600.00[b]	0.93	-6.98	546.67[b]	0.85	-14.98
Khaya	665.67[a]	1.03	+3.20	644.00[a]	1.00	+0.16
Control	645.00[a]	-	-	645.00[a]	-	-
LSD (0.05)	33.58	-	-	45.57	-	-
Delta.+abamectin	504.67[c]	0.78	-21.76	316.67[c]	0.49	-50.75
Delta.	576.00[b]	0.89	-10.70	392.00[b]	0.61	-39.04
Abamectin	571.67[b]	0.89	-11.37	386.67[b]	0.60	-39.86
Control	645.00[a]	-	-	643.00[a]	-	-
LSD (0.05)	44.48	-	-	30.30	-	-
Methomyl+jojoba	508.33[d]	0.79	-21.19	534.67[d]	0.83	-16.85
Methomyl	561.33[c]	0.87	-12.97	584.67[c]	0.91	-9.07
Jojoba oil	599.53[b]	0.93	-7.05	616.67[b]	0.96	-4.09
Control	645.00[a]	-	-	643.00[a]	-	-
LSD (0.05)	34.89	-	-	23.44	-	-
Indoxa.+pomegr.	311.00[c]	0.48	-51.78	317.00[b]	0.49	-50.70
Indoxacarb	430.67[b]	0.67	-33.33	432.00[c]	0.67	-32.81
Pomegranate	653.00[a]	1.01	+1.24	673.67[a]	1.05	+4.77
Control	645.00[a]	-	-	643.00[b]	-	-
LSD (0.05)	31.87	-	-	22.58	-	-
Chloran.+jojoba	448.33[d]	0.70	-30.49	380.67[c]	0.59	-40.80
Chloran.	513.33[c]	0.80	-20.41	442.33[b]	0.69	-31.21
Jojoba oil	599.33[b]	0.93	-7.08	616.67[a]	0.96	-4.09
Control	645.00[a]	-	-	643.00[a]	-	-
LSD (0.05)	42.02	-	-	31.01	-	-

The same letters means no significant difference at 5% level. Activity ratio (%): Treated/control×100, Change: Control-treated/control×100. Delta.: Deltamethrin, Indoxa.: Indoxacarb, Chloran.: Chlorantraniliprole, Lambada.: Lambada cyhalothrin

Table 9: Effect of five mixtures of traditional, novel and bio-insecticides on AChE activity on *M. domestica* adults

Treatments	AChE	Activity ratio	Change (%)
Lambada.+khaya	290.00[b]	0.99	-1.14
Lambada.	244.00[c]	0.83	-16.82
Khaya	325.67[a]	1.11	+11.03
Control	293.33[b]	-	-
LSD(0.05)	20.60	-	-
Delta.+abamectin	142.67[d]	0.49	-51.36
Delta.	330.67[a]	1.13	+12.73
Abamectin	212.33[c]	0.73	-27.81
Control	293.33[b]	-	-
LSD(0.05)	20.11	-	-
Methomyl+jojoba oil	128.00[d]	0.44	-56.36
Methomyl	166.00[c]	0.13	-43.41
Jojoba oil	233.33[b]	0.80	-20.45
Control	293.33[a]	-	-
LSD(0.05)	24.34	-	-
Indoxa.+pomegranate	127.33[c]	0.43	-56.59
Indoxa.	151.17[b]	0.51	-48.46
Pomegranate	313.00[a]	1.07	+6.71
Control	293.33[a]	-	-
LSD(0.05)	20.68	-	-
Chloran.+jojoba oil	147.33[d]	0.50	-49.77
Chloran.	176.67[c]	0.60	-39.77
Jojoba oil	233.33[b]	0.80	-20.45
Control	293.33[a]	-	-
LSD(0.05)	28.36	-	-

The same letters means no significant difference at 5% level. Activity ratio (%): Treated/control×100, Change: Control-treated/control×100. Chloran.: Chlorantraniliprole, Lambada.: Lambada cyhalothrin, Indoxa.: Indoxacarb, Delta.: Deltamethrin

(311 mg g^{-1} b.wt.) and % change was -51.78 less than control compared with each components alone, followed by (chlorantraniliprole+jojoba oil, (deltamethrin+abamectin), (methomyl+jojoba oil) and (lambada cyhalothrin+khaya), where the enzyme activity and % change were (448.33 mg g^{-1} b.wt. and -30.49), (504.67 mg g^{-1} b.wt. and -21.76), (508.33 mg g^{-1} b.wt. and -21.19) and (557.67 mg g^{-1} b.wt. and -13.54, respectively.

As for β esterase activity, the data in Table 8 showed that all mixture reduced the enzyme activity, (deltamethrin+abamectin) and (indoxacarb+pomegranate) mixtures achieved the highest reduction, where the enzyme activity was reduced to 316.67 and 317 mg g^{-1} b.wt. and % change than control were -50.75 and -50.70, respectively, compared with control and each components, followed by (chlorantraniliprole+jojoba), (lambada cyhalothrin+khaya) and (methomyl+jojoba oil) where the enzyme activity and % change were (380.67 mg g^{-1} b.wt. and -40.80), (470 mg g^{-1} b.wt. and -26.91) and (534.67 mg g^{-1} b.wt. and -16.85), respectively, compared with control and their components alone.

Acetylcholine esterase activity: The effects of five mixtures of traditional, novel and bio-insecticides (which showed highly synergistic effects) on *M. domestica* adults AChE activity were presented in Table 9. The data showed that there were significant differences in AChE between all tested mixtures and their components alone and control. Lambda cyhalothrin+pomegranate and methomyl+jojoba oil mixtures achieved the highest decreased in AChE activity where it was 127.33 and 128 (mg g^{-1} b.wt.), respectively and % change was -56.59 and -56.36, respectively, followed by deltamethrin+abamectin mixture where the enzyme activity was decreased to 142.67 (mg g^{-1} b.wt.) and % change was -51.36 less than control compared with it is component and control. Followed by chlorantraniliprole+jojoba oil mixture which decreased the enzyme activity to 147.33 (mg g^{-1} b.wt.) and % change was -49.77 less than control compared with it is component. On the other side, lambada cyhalothrin+khaya extract mixture achieved the lowest decrease in AChE activity compared with other tested mixtures, where it was 290 (mg g^{-1} b.wt.) and % change was -1.14 less than control.

The obtained results were in agreement with Fetoh and Asiry[37], who found that camphor extract and chlorpyrifos mixture decreased the protein content more than each component in cotton leafworm larvae, where it was 13.5, 31 and 26% for mixture, camphor extract and chlorpyrifos, respectively, as well as, the activity of α-esterase was significantly declined. Recently, Zahran *et al*.[38] recorded that essential oils of *Artemisia monosperma, Origanum vulgare*, *Silphium terebinthifolus* and *Citrus paradise* were highly inhibiting AChE activity in *Culex pipiens* larvae. Moreover, Djemaoun *et al*.[39] revealed that indoxacarb decreased the ovarian levels of proteins, carbohydrates and lipids after treated with sublethal dose in *Blatella germanica*, these biochemical modifications suggested an interference of indoxacarb with the reproductive process. Shaurub and El-Aziz[40] found that both lambada cyhalothrin significantly reduced total carbohydrates, in addition lambada cyhalothrin significantly decrease lipid content and lipase activity in *Culex pipiens* larvae by acting on secondary target. Kassem *et al*.[41] found that the bio-insecticide neem azal, significantly ($p<0.001$) decreased protein, lipid and carbohydrate contents during early and late third larval stage of *Musca domestica*. El Kady *et al*.[42] found that the two bio-insecticides spinotram and vertimec decreased the AChE activity in *Culex pipiens* and *Anopheles multicolor*. On the other hand, the specific of α and β-esterases in exposed mosquito decreased significantly ($p<0.05$) after 24 h of exposure. Sharma *et al*.[43] concluded that extracts of *Artemicia annua* and *Azadiracta indica* produce significant alterations in the biochemical profiles of anopheline and culicine larvae, furthermore, the impacting factors of carbohydrate on carbohydrates, lipid and protein contents of larvae and specific extraction. Megahed *et al*.[44] found that reduction of AChE activity, total protein and total lipid contents were observed in cotton leafworm, *Spodoptera littoralis* 4th instar larvae treated with emamectin benzoate, spinosad and abamectin. Gamil *et al*.[45] found that there were significant decreased in total carbohydrate and protein contents after treated *M. domestica* with *Curcuma longa* (Turmeric).

CONCLUSION

The most of tested mixtures of chemical insecticides with plant extracts, plant oils and microbial insecticides was highly toxic to house fly and decreased the activity of AChE and esterases enzymes and decreased total protein, lipid and carbohydrates content in house fly adults. It can be recommended to use the promising mixtures which achieved highly toxic effect in integrated house fly programs in order to achieved safety control, reduced environmental pollution and human hazardous.

SIGNIFICANCE STATEMENT

This study showed how safe control of house fly was achieved and reducing the use of insecticides used in control of this insect to avoid its high toxicity to the environment and human and living organisms and also reduce the high cost of control and protection from the development of resistance in this insect to these pesticides through the use of mixtures of chemical pesticides with plant extracts, plant oils and also bio-insecticides.

Therefore, the finding of this study will be used as a baseline in integrated house fly management programs .Also, in integrated resistance management to this insect. This finding of this study will help to develop an alternative safety method to control this insect by using promising mixtures of chemical and bio-insecticides.

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