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Synergistic effect of insecticides on the larvae and adults of housefly, *Musca domestica* L.

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Abstract
The present research was undertaken to study the synergistic effect of 3 pyrethroid and 3 organophosphorus insecticides against field strain of the females and the 2nd larval instars of the housefly, *M. domestica* using CDC bottle and dipping application bioassay techniques under laboratory conditions, respectively. It was found that pyrethroid insecticides, Cypermethrin and Cyfluthrin showed the highest co-toxicity factor 14.4, while the lowest co-toxicity factor was 1.5. Whereas, the preliminary toxicity screening of the three organophosphorus insecticides (Diazinon, Propetamphos and Pirimiphos methyl) against *M. domestica* 2nd larval instar showed additive effects with different degrees according to the estimated co-toxicity factor. The mixture of Diazinon and Pirimiphos methyl showed the highest co-toxicity factor 8.10. So it is concluded that mixture of these chemicals has better insecticidal effect against the housefly compare to alone and can be used for the control of housefly.

Keywords: *Musca domestica*, joint action, CDC bottle and dipping bioassay techniques, pyrethroid, organophosphorus insecticides

Introduction
The common housefly, *Musca domestica* Linnaeus (Diptera: Muscidae) is found in all over the world but in warm areas, it is more adaptable [1]. Housefly is distributed globally and is a pest in homes, dairies, food processing, barns, poultry houses, and recreation areas. It can develop a generation within 2 weeks in summer as it has great breeding potential [2]. Housefly lays eggs almost in all kinds of organic material. A good breeding medium is animal or poultry manure. Fermenting vegetation, for instance grass clippings and garbage can also be a good choice for housefly breeding. Housefly experiences total metamorphosis, and comprises different egg, pupal, larval and adult stages [3] Sponging type mouthparts are used by houseflies for feeding. The houseflies sample and eat their food by regurgitation of liquid and dipping it on the food to turn it to liquid as they move between different food sources. Such type of feeding is identified by light colored spots that are referred to as flyspecks [1]. Fecal spots are darker flyspecks that are associated with houseflies. The houseflies are empowered to spread several intestinal diseases due feeding and breeding behaviors in consort with its perseverance for attacking homes [2]. The diseases include dysentery and diarrhea. Mostly, houseflies feed and breed on decomposing matter, waste of human and food, thus, they are considered as mechanical vectors of pathogens (bacteria, protozoa and viruses) to mankind and livestock [4, 5]. Actions such as feeding and milking interfered with houseflies. Apart from this, animal disease transmission is increased by houseflies that not only cause increase medication veterinary service costs but also cause an increased potential human diseases spreads. In addition, its serious insect not only for their annoyance factor but also for being major vectors of several disease- inducing agents [6, 7].

The aim of present work was to study synergistic effect of 3 pyrethroid and 3 organophosphorus insecticides against field strain of the females and the 2nd larval instars of the housefly, *M. domestica* using CDC bottle and dipping application bioassay techniques under laboratory conditions.

Materials and Methods
Colonies of the housefly, *Musca domestica* L.
The colonies of *M. domestica* initiated from adults combined from Slaughterhouse sheep in Jeddah Governorate, consuming a sweeping net. Afterwards, they were shifted into a minor cage (16×16×16 cm) and shifted to the Laboratory of Public Health Pests, Jeddah Amana,

Jeddah Governorate, KSA for tested. This Study was done during May 2015-2016. Respectively.

Insecticides used

Commercial formulations of insecticides were used in this study representing the four main groups of insecticides commonly applied on mosquito control. These insecticides include organophosphorus (Diazinon, Propetamphos and Pirimiphos methyl), synthetic pyrethroide (Deltamethrin, Cypermethrin and Cyfluthrin).

Adult bioassay (CDC bottle bioassay)

For the adult stage of *M. domestica*, assays were adapted according to the WHO technique on the assessment and analysis of insecticides [8]**.** Glass tubes were treated singly with different concentrations of the chosen formulation of compounds. Solvent (acetone ethyl alcohol) were treated in test tubes (control). The treated test tubes were opened in a room kept at 25 ºC, 50-55% relative humidity and constant darkness and the door was kept closed due to no forced ventilation. Twenty females were exposed to the treated surface for the one and 24 hours at the same temperature and relative humidity. Batches of 20 females were introduced into test tubes and allowed to a light and rest on the vertical treated surface. After the exposure period the houseflies were removed and transferred for observation and mortality count after one and 24 hours.

Larval bioassays (dipping technique)

The dipping technique was applied based on the technique explained by [9] with few alterations. Each of the test was executed at 14:10 (light: dark) and kept at 268±2 degree Celsius and 70–80% humidity. The second instar larvae were consumed in the analyses. From all groups, five copies of twenty larvae were consumed at concentration levels. The experiments were performed repeatedly for successive days. From all the groups, the larvae were moderately dipped into insecticide solutions with the use of a dip net; however, such controls were dipped in tap water. Several concentrations insecticides were used plus control. The larvae were shifted to the rearing jars that were comprised of food after dipping for duration of precisely 30 seconds. After the larvae had been dipped, they were reared to determine the consequences of distinct compounds of the life cycle and also the success of development; alternatively, the number of developing flies was logged.

Mixtures toxicity (joint action)

Paired mixtures of different insecticides were newly developed at concentration levels of their corresponding $LC_{25,35,45}$ values. All the mixtures were analyzed in five replicates in consort with controls, and the analysis were conducted based on the procedure described above.

Mortality percentages were examined after duration of 24 hours and the combined (joint) action of the distinct mixtures was presented as Co-toxicity factor based on Sun and Johnson 1960 [10] to distinguish among potentiation, antagonism and additive, by applying the formula given below:

Co - toxicity factor = (O – E) x 100/E; where:

O: is observed % mortality and E: is expected % mortality.

The co-toxicity factor distinguishes the results into three classes, which are a positive factor of ≥ 20 specifies potentiation, a negative factor of ≤ -20 specifies antagonism, and the intermediary values of >-20 to < 20 specify an additive consequence. They were examined again in the context of MD adults to identify the precise projected mortality because the obtained LD25 values were projected mathematically. The projected mortality of the joint pair is given by the summation of the mortalities of single compounds at certain LD25. On the other hand, the observed mortality is the logged mortality achieved 24 hours after exposing to the mixture.

Synergistic/Antagonistic Action

These tests were conducted to identify the synergistic/antagonistic action that occurred due to mixing of a certain quantity of insecticide at the concentration level producing no observed mortality (for instance LD0) with a plant excerpt at its LD50 value. By contrasting mortalities that was achieved with the projected mortality of the mixture (ca. 50 %), the produced synergistic/ antagonistic factor (SF) could point out a clue to the kind of the effect (i.e. SF >1 demonstrates synergism; SF < 1 demonstrate antagonism; SF = 1 demonstrate no obvious effect). All mixtures were examined in four repetitions in consort with an unprocessed control test, based on the procedure described above. In addition, unlike the actual technique, the projected mortality for the mixture was not assumed as a 50 % kill [11]. To attain more accuracy, it was achieved from experimental projections in which mortality of all toxicants (at the LC values) were obtained, added and consumed as the projected mortality. A safety factor of ± 0.05 was assumed while ranking the synergistic/antagonistic results (i.e. no recognizable effect) SF = 1±0.05, synergism: SF >1.05, and antagonism: SF < 0.95

Statistical analysis

Mortality counts were made after 24 hours. The dosage mortality data were exposed to Probit examination according to [12, 13] Mortality percentages were corrected according to Abbott's [14].

Results and Discussion

To examine the combined action of two or greater than two insecticides, the actual toxicity indexes of the components and their mixture are obtained by dosage-mortality curves. The hypothetical toxicity of the similar mixture is equivalent to the addition of toxicity indexes calculated from the percentage of each component multiplied by its respective toxicity index. Consequently, the combined toxicity or Co-toxicity coefficient of a mixture close to 100 specifies likelihood of similar action; independent action generally should give a coefficient smaller than 100, while a coefficient significantly above 100 intensely specifies synergism. Toxicity significantly smaller than that of the strongest toxicant alone specifies antagonism [10].

Joint action studies of different pyrethroid insecticides against the adults of *M. domestica* under laboratory conditions

Results in Table 1 showed that the preliminary toxicity screening of the three pyrethroid insecticides (Deltamethrin, Cyfluthrin and Cypermethrin) against *M. domestica* adult at different concentrations ranged between 0.5-0.001 ppm against field strain under laboratory conditions using CDC bottle bioassay. For the tested insecticides, the $LC_{25,\ 35,45}$ values were of 0.0019, 0.0043 and 0.0091 in case Deltamethrin, while for Cyfluthrin, the $LC_{25,35,45}$ values were 0.0023, 0.0049 and 0.0098, whereas of Cypermethrin were 0.0023, 0.0057 and 0.0125, respectively.

Data in Table 1 show that the slope of field stain of adult stage of *M. domestica* population when using Deltamethrin, Cyfluthrin and Cypermethrin. Slope of field strain in case Deltamethrin, Cyfluthrin and Cypermethrin were 0.8061, 0.8639 and 0.7511, respectively. The mixing of three pyrethroid insecticides (Deltamethrin, Cyfluthrin and Cypermethrin) has resulted in 27 binary mixtures. The mixtures showed additive or antagonism effects with different degrees according to the estimated co-toxicity factor. The results of joint action screening are presented for the first time, in a form of a histogram (Fig. 1, 2, 3). The mixture of Deltamethrin LC_{35}+ Cyfluthrin LC_{25} showed the highest co-toxicity factor (14.44), while the lowest co-toxicity factor (1.25) was entitled to the mixture of Cypermethrin LC_{45} + Cyfluthrin LC_{35}. Nearly similar result was recorded for the mixture of Cypermethrin LC_{45} + Cyfluthrin LC_{45} (1.48). Generally, most of the tested pyrethroids when mixed together were resulted in additive mixtures of co-toxicity factors.

On the other hand, three mixtures of pyrethroid insecticides were resulted in antagonism of co-toxicity factors (Cypermethrin LC_{25} +Deltamethrin LC_{25} (-31.90), Cypermethrin LC_{25} + Cyfluthrin LC_{25} (-32.86) and mixture between Deltamethrin LC_{25}+ Cyfluthrin LC_{25} (-29.52) (Table 2; Fig. 1-3).

The residual toxicity of a pesticide, for a particular smaller period, next to application must achieve greater degree of pest control; particularly for insects of common visiting to the sprayed area such as house flies and further pests of medical importance. Synthetic pyrethroids are generally accepted as neurotoxin that act straightaway on excitable membranes associated with their capability of altering electrical activity in several portions of the nervous system.

Table 1: Toxicity data for the tested insecticides against field strain of the adult stage of *Musca domestica*, as projected after duration of 24 hours exposure times by using CDC bottle bioassay application technique.

Tested Insecticide	LC_{25} (upper – lower)	LC_{35} (upper – lower)	LC_{45} (upper – lower)	Slope
Deltamethrin	0.0019 (0.0002-0.0036)	0.0043 (0.0008-0.0087)	0.0091 (0.002-0.0197)	0.8061
Cyfluthrin	0.0023 (0.0004-0.0044)	0.0049 (0.0012-0.0097)	0.0098 (0.0029-0.0202)	0.8639
Cypermethirn	0.0023 (0.0002-0.0041)	0.0057 (0.0007-0.0113)	0.0125 (0.0021-0.0291)	0.7511

Table 2: Synergistic effect of binary mixtures of insecticides against field strain of the adult stages of *Musca domestica* as projected after duration of 24 hours exposure times by using CDC bottle bioassay application technique.

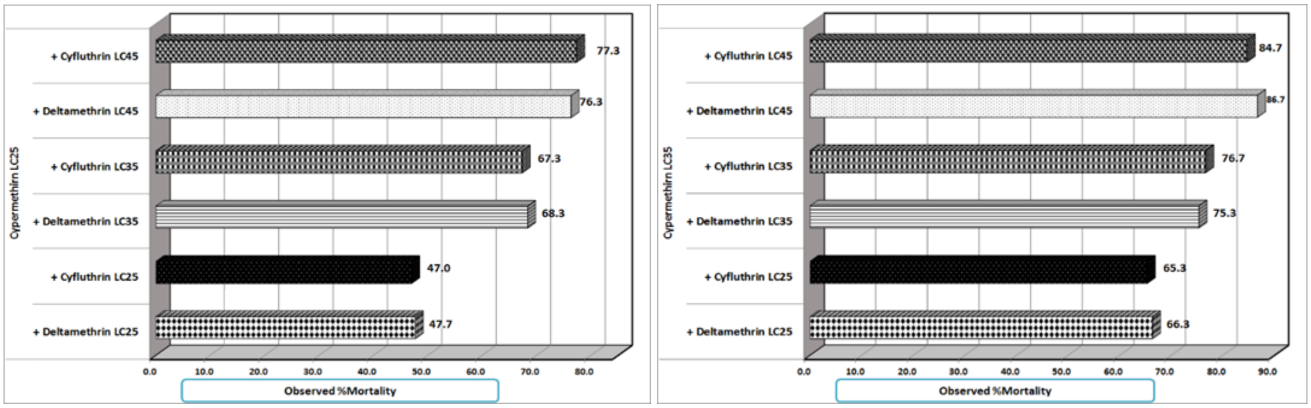
Mixture		Expected % Mortality	Observed % Mortality	Co-toxicity Factor	Joint Action
Cypermethrin LC_{25}	+ Deltamethrin LC_{25}	50	47.67	-31.90	Antagonism
	+ Cyfluthrin LC_{25}	50	47.0	-32.86	Antagonism
	+ Deltamethrin LC_{35}	60	68.33	-2.38	Additive
	+ Cyfluthrin LC_{35}	60	67.33	-3.81	Additive
	+ Deltamethrin LC_{45}	70	76.33	9.04	Additive
	+ Cyfluthrin LC_{45}	70	77.33	10.47	Additive
Cypermethrin LC_{35}	+ Deltamethrin LC_{25}	60	66.33	-5.24	Additive
	+ Cyfluthrin LC_{25}	60	65.33	-6.67	Additive
	+ Deltamethrin LC_{35}	70	75.33	7.61	Additive
	+ Cyfluthrin LC_{35}	70	76.67	9.53	Additive
	+ Deltamethrin LC_{45}	80	86.67	8.34	Additive
	+ Cyfluthrin LC_{45}	80	84.67	5.84	Additive
Cypermethrin LC_{45}	+ Deltamethrin LC_{25}	70	74.67	6.67	Additive
	+ Cyfluthrin LC_{25}	70	72.33	3.33	Additive
	+ Deltamethrin LC_{35}	80	83.33	4.16	Additive
	+ Cyfluthrin LC_{35}	80	81.00	1.25	Additive
	+ Deltamethrin LC_{45}	90	95.75	6.30	Additive
	+ Cyfluthrin LC_{45}	90	91.33	1.48	Additive
Deltamethrin LC_{25}	+ Cyfluthrin LC_{25}	50	49.33	-29.52	Antagonism
	+ Cyfluthrin LC_{35}	60	69.67	-0.47	Additive
	+ Cyfluthrin LC_{45}	70	78.33	11.90	Additive
Deltamethrin LC_{35}	+ Cyfluthrin LC_{25}	60	68.67	14.44	Additive
	+ Cyfluthrin LC_{35}	70	78.67	12.39	Additive
	+ Cyfluthrin LC_{45}	80	87.33	9.16	Additive
Deltamethrin LC_{45}	+ Cyfluthrin LC_{25}	70	79.67	13.81	Additive
	+ Cyfluthrin LC_{35}	80	88.67	10.84	Additive
	+ Cyfluthrin LC_{45}	90	100	11.11	Additive

Expected mortality = summation of mortality (%) from insects exposed to several LC values of each toxicant in a paired combination as examined separately.

Observed % mortality means that of the mixture examined in the similar experimental container at the LC values level of each.

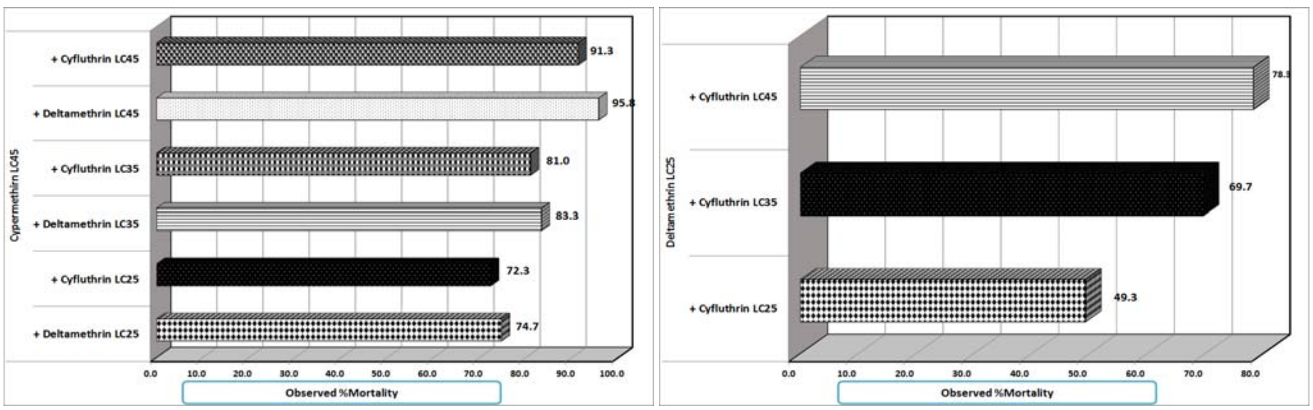
$$\text{Co-toxicity factor} = \frac{\text{Observed \% mortality - Expected \% mortality x 100.}}{\text{Expected \% mortality.}}$$

A positive factor of ≥ 20 specifies potentiation, a negative factor of ≤ -20 specifies antagonism, and the intermediate values of >-20 to < 20 specify an additive effect.



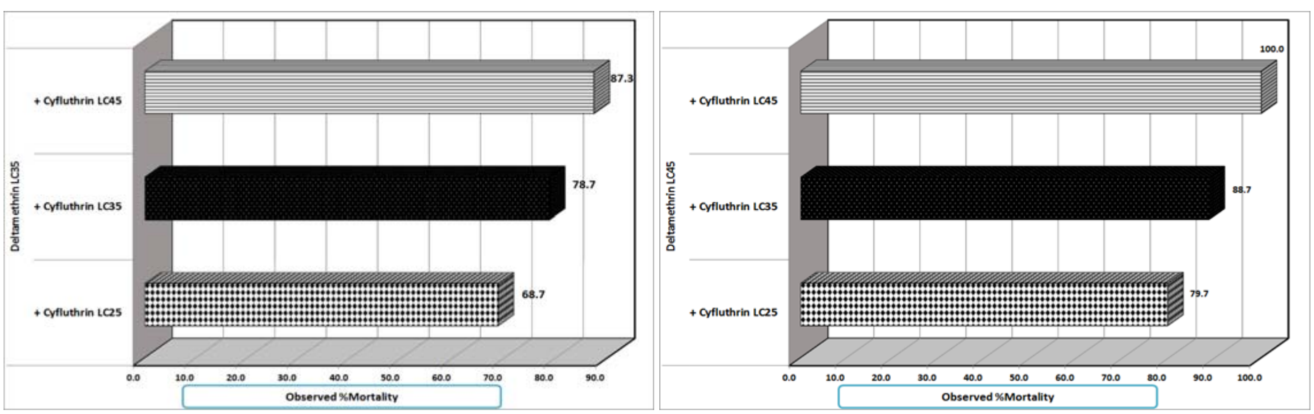
(A) (B)

Fig 1: (a) and (b) shows the synergistic effect of pyrethroid insecticides against field strain of adult stage of *M. domestica* by CDC bottle bioassay method.



(A) (B)

Fig 2: (a) and (b) shows the synergistic effect of pyrethroid insecticides against field strain of adult stage of *M. domestica* by CDC bottle bioassay method.



(A) (B)

Fig 3: (a) and (b) shows the synergistic effect of pyrethroid insecticides against field strain of adult stage of *M. domestica* by CDC bottle bioassay method.

This effect is caused by a sterioselective and structure-related interaction with voltage-dependent sodium channels, the primary target site of the pyrethroids [15].

The obtained results are agree with those obtained by Wickham (2006) [16] it was shown that when combined in pairs of pyrethroids according to the actions of the single isomers of 5-benzyl-3-furylmethyl (±)-*cis,trans*-chrysanthemate to give the (±)-*trans* or (±)-*cis* or (+)-*cis,trans* mixtures on *M. domestica* adults. The obtained mortalities did not vary from those projected by simple additive action obtained by the harmonic mean. On the other hand, the (±)-*cis,trans* mixture exhibited substantial antagonism with a mortality only 60% of that projected. Same assessments by using the separate and joint isomers of bioallethrin [(*R,S*)-3-allyl-2-methyl-4-oxocyclopent-2-enyl (allethronyl) (+)-*trans*-[(1*R*,3*R*)-chrysanthemate] and the respective (+)-*cis*-(1*R*,3*S*)-chrysanthemate specify antagonism obtained to be interrelated with the content of the (*R*)-isomer of the alcoholic moiety.

Joint action studies of different organophosphorus insecticides against the 2nd larval instar of *M. domestica* under laboratory conditions

The preliminary toxicity screening of the three organophosphorus insecticides (Diazinon, Propetamphos and Pirimiphos methyl) against *M. domestica* 2nd larval instar at different concentrations ranged between 0.5-0.001 ppm against field strain under laboratory conditions using dipping technique (Table 3). For the tested insecticides, the LC $_{25, 35,45}$ values were of 0.012, 0.0258 and 0.0515 in case Diazinon, while for Propetamphos, the $LC_{25,35,45}$ values were 0.0207, 0.0403 and 0.0732, whereas of Pirimiphos methyl were 0.0306, 0.0544 and 0.0912, respectively.

Data in Table 4 showed that the slope of field stain of the 2nd larval instar of *M. domestica* population when using Diazinon, Propetamphos and Pirimiphos methyl. Slope of field strain in case Diazinon, Propetamphos and Pirimiphos methyl were 0.7975, 0.8025 and 0.8472, respectively.

The mixing of three organophosphorus insecticides (Diazinon, Propetamphos and Pirimiphos methyl) has resulted in 27 binary mixtures. The mixtures showed additive effects with different degrees according to the estimated co-toxicity factor. The results of joint action screening are presented, for the first time, in a form of a histogram (Fig. 4-6).

The mixture of Diazinon LC_{45}+ Pirimiphos methyl LC_{25}showed the highest co-toxicity factor (8.10), nearly similar result was recorded for the mixture of Diazinon LC_{45} +Pirimiphos methyl LC_{35} (7.08), while the lowest co-toxicity factor (-19.33) was entitled to the mixture of Propetamphos LC_{25} +Diazinon LC_{25}. Nearly similar result was recorded for the mixture of Propetamphos LC_{25} +Pirimiphos methylLC_{25} (-17.33). Generally, most of the tested organophosphorus compounds when mixed together were resulted in additive mixtures of co-toxicity factors.

Zahidul and Khalequzzaman (2002) [17] tested potentiate malathion by other insecticides (dichlorovos, pirimiphosmethyl, phenthoate, monocrotophos, diazinon, cypermethrin, lambda-cyhalothrin, and propoxur). They specified that all tests were conducted by the topical application of the insecticide mixtures on adult housefly, *M. domestica*. The mortality data after duration of 24 hours of application exhibited that almost all insecticides provided increased toxicity of malathion. However, when it was tested jointly with lambda-cyhalothrin and cypermethrin the potentiation effects were maximum. Organophosphorus insecticides are toxic to insect and mammals by virtue of their capability to deactivate the enzyme acetylcholinesterase that is a category of enzymes that catalyzes the hydrolysis of the neuro-transmitting agent acetylcholine (Ach); which leads to poisoning [18].

Table 3: Toxicity data for the tested insecticides against field strain of the 2nd larval instars of *Musca domestica*, as estimated after 24 h exposure times by using dipping technique.

Tested Insecticide	LC_{25} (upper – lower)	LC_{35} (upper – lower)	LC_{45} (upper – lower)	Slope
Diazinon 60%	0.012 (0.0011-0.0124)	0.0258 (0.0024-0.0341)	0.0515 (0.0062-0.0611)	0.7975
Propetamphos 20%	0.0207 (0.0017-0.0247)	0.0403 (0.0055-0.0575)	0.0732 (0.0151-0.1272)	0.8025
Pirimiphos methyl 50%	0.0306 (0.0034-0.00365)	0.0544 (0.0091-0.078)	0.0912 (0.0215-0.1584)	0.8472

Table 4: Joint action of binary mixtures of organophosphorus insecticides against field strain of the 2nd larval instars of *Musca domestica* as projected after duration of 24 hours exposure times by dipping bioassay technique.

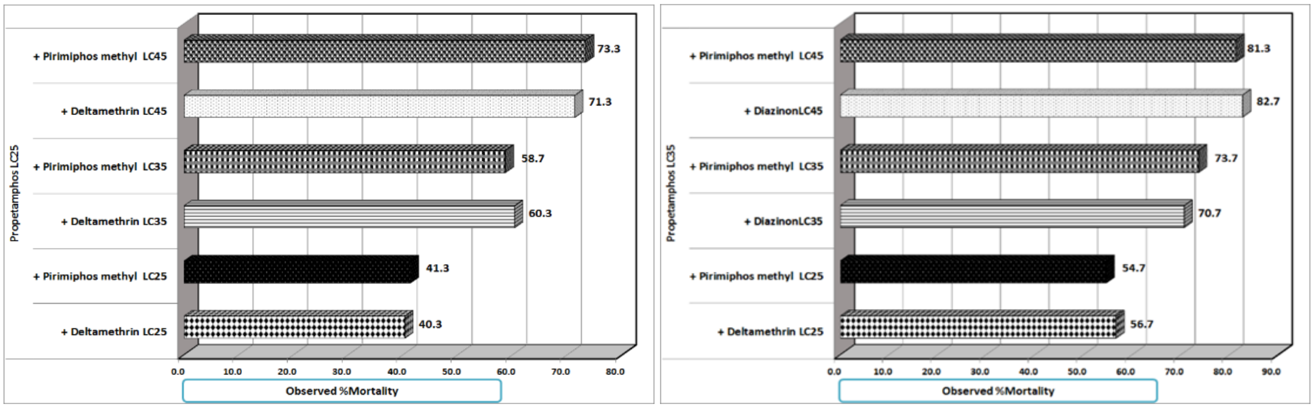
Mixture		Expected% Mortality	Observed % Mortality	Co-toxicity Factor	Joint Action
Propetamphos LC_{25}	+ Diazinon LC_{25}	50	40.33	-19.33	Additive
	+ Pirimiphos methyl LC_{25}	50	41.33	-17.33	Additive
	+ Diazinon LC_{35}	60	60.33	0.56	Additive
	+ Pirimiphos methyl LC_{35}	60	58.67	-2.22	Additive
	+ Diazinon LC_{45}	70	71.33	1.90	Additive
	+ Pirimiphos methyl LC_{45}	70	73.33	4.76	Additive
Propetamphos LC_{35}	+ Diazinon LC_{25}	60	56.67	-5.56	Additive
	+ Pirimiphos methyl LC_{25}	60	54.67	-8.89	Additive
	+ DiazinonLC_{35}	70	70.67	0.95	Additive
	+ Pirimiphos methyl LC_{35}	70	73.67	5.24	Additive
	+ DiazinonLC_{45}	80	82.67	3.33	Additive
	+ Pirimiphos methyl LC_{45}	80	81.33	1.67	Additive
Propetamphos LC_{45}	+ DiazinonLC_{25}	70	69.00	-1.43	Additive
	+ Pirimiphos methyl LC_{25}	70	68.67	-1.90	Additive
	+ DiazinonLC_{35}	80	78.67	1.67	Additive
	+ Pirimiphos methyl LC_{35}	80	75.33	-5.83	Additive
	+ DiazinonLC_{45}	90	91.67	1.85	Additive
	+ Pirimiphos methyl LC_{45}	90	91.33	1.48	Additive
Diazinon LC_{25}	+ Pirimiphos methyl LC_{25}	50	44.67	-10.67	Additive
	+ Pirimiphos methyl LC_{35}	60	61.67	2.78	Additive
	+ Pirimiphos methyl LC_{45}	70	74.67	6.67	Additive
DiazinonL C_{35}	+ Pirimiphos methyl LC_{25}	60	62.67	4.44	Additive
	+ Pirimiphos methyl LC_{35}	70	74.33	6.19	Additive
	+ Pirimiphos methyl LC_{45}	80	83.67	4.58	Additive
DiazinonLC_{45}	+ Pirimiphos methyl LC_{25}	70	75.67	8.10	Additive
	+ Pirimiphos methyl LC_{35}	80	85.33	6.67	Additive
	+ Pirimiphos methyl LC_{45}	90	94.33	4.81	Additive

Expected mortality = summation of mortality (%) from insects exposed to several LC values of each toxicant in a paired combination as tested separately.

Observed % mortality refers to that of the mixture tested in the similar experimental container at the LC values level of each.

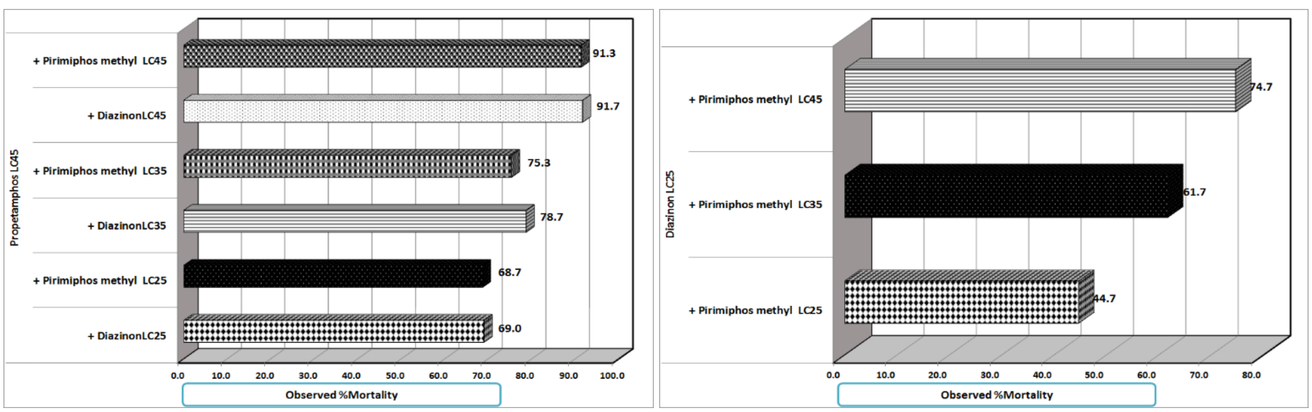
$$\text{Co-toxicity factor} = \frac{\text{Observed \% mortality - Expected \% mortality x 100.}}{\text{Expected \% mortality.}}$$

A positive factor of ≥ 20 indicates potentiation, a negative factor of ≤ -20 indicates antagonism, and the intermediate values of >-20 to < 20 indicate an additive effect.



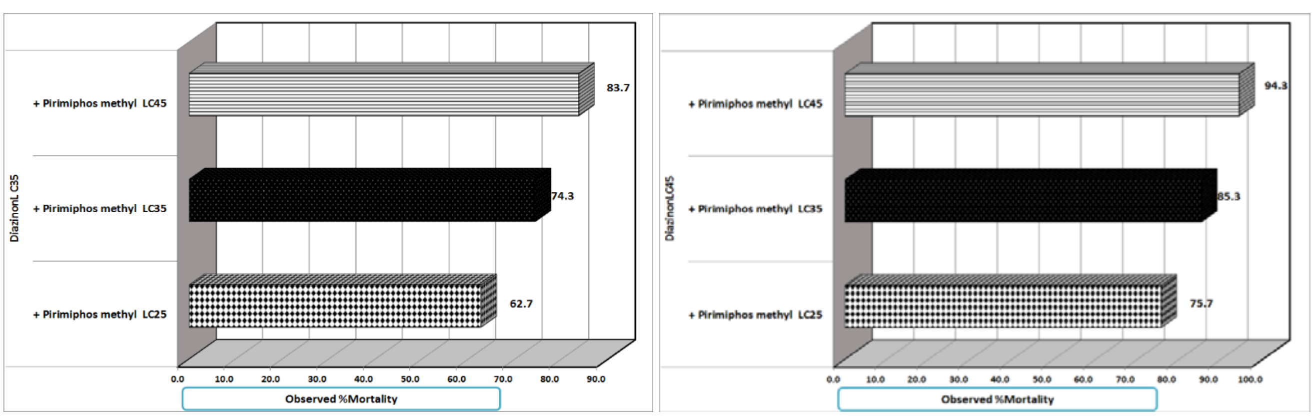
(A) (B)

Fig 4: (a) and (b) shows the synergistic effect of organophosphorus insecticides against field strain of the 2nd larval instars of *M. domestica* by dipping bioassay technique.



(A) (B)

Fig 5: (a) and (b) shows the synergistic effect of organophosphorus insecticides against field strain of the 2nd larval instars of *M. domestica* by dipping bioassay technique.



(A) (B)

Fig 6: (a) and (b) shows the synergistic effect of organophosphorus insecticides against field strain of the 2nd larval instars of *M. domestica* by dipping bioassay technique.

Mixtures comprised of organophosphate and pyrethroid insecticides have been established very beneficial in improving the toxicity of insecticides in several resistant insect pests globally. This type of potentiation or synergism is described by the prevention of esterases or monooxygenases activity [19]. Insecticide mixtures, rotation and/or fine scale mosaics have been suggested as significant tools for resistance management in several insect pests [20]. (Hemingway and Ranson, 2000). Control measure against *M. domestica* in the short-term is the use of conventional insecticides [21].

Most research on the synergistic, antagonistic and additive toxic effects of binary mixtures including phytochemicals have been performed on agricultural pests rather than pests of medical significance [11]. Recognizing these synergist compounds within mixtures may result in the growth of smaller quantities in the mixture to obtain acceptable levels of efficacy. Definitely, joint-action may well extend the effectiveness of synthetic insecticides that will finally be unusable because of resistance [22]. Synergistic action with conventional chemical pesticides identified in the current research could be further exploited for integrated pest management (IPM) programs.

4. Conclusion
Our study revealed that CDC bottle bioassay as the fastest way to evaluate the resistance of housefly towards insecticides. The synergetic effect of insecticides will provide a better effect in reducing the flies population and the magnitude of epidemiology. Our study will also assist the concern authorities to select the most prospective insecticides for successful fly control. These authorities include: Ministry of Health, Ministry of Agriculture, and Ministry of Municipalities.

5. Acknowledgement
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6. References
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