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The effect of extracts of some organic solvents of some plants on the nymphal stages of the Periplaneta americana

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Abstract

The study was conducted to identify the effect caused by some organic solvent extracts (hexane, ethyl acetate, ethyl alcohol) of the hexane extract was superior to its counterparts of ethyl acetate and ethyl alcohol extracts. The concentration of 200 ppm gave the highest percentage of *P.americana* nymph mortality for all tested plants within 72 hours. The values of LC_{50} and the exposure periods of the extracts were as the value of LC_{50} decreases as the exposure period increases. For the extracts, it is noted that the early nymph is the most sensitive stage if it recorded the highest values of death rates and all solvents used. The reason for the death of the early nymph at a higher rate than the rest of the stages in all treatments may be explained by reasons including the thinness of the cuticle surrounding the early nymph at the beginning of its formation, or that the newly hatched nymph is they need large amounts of food for their growth, which causes large amounts of the extract and their food to enter the digestive canal, leading to poisoning. Or the reason may be that the compounds in these plants inhibit nutrition, which leads to the death of the nymphs due to lack of nutrition.

As for the advanced nymphal stages of the *P.americana*, the superiority of the cochineal plant and all organic extracts and all concentrations was recorded over the thistle plant, which was also superior to the *Haloxylon Bunge*. It was noted that the hexane extract was superior to the extract of ethyl acetate and ethyl alcohol, and the rate of death was recorded as it increased with an increase in the concentration of the extract, so the concentration of ppm 200 gave the highest percentage of mortality for the tested plants, and showed the values of LC_{50} and the periods of exposure to the extracts, as the value of LC_{50} decreases as the duration of exposure to the extracts increases. We note that the advanced nymph stage is the stage least affected by the extracts, so resistance increases as the age of the stage increases. The reason may be due to the ability of the advanced nymph stages to by converting toxic compounds found in various plant extracts into non-toxic compounds.

Keywords: *Periplaneta americana, Xanthium strumarium*, *Achillea* L. *Haloxylon Bunge*,

Introduction

Periplaneta americana are among the common household insects that live in human environments in contact with their food and tools, as they can be seen in any place used or inhabited by humans, especially places where food is stored or eaten. There are about 4,000 species of cockroaches spread globally, which are classified into a number of families. Which goes back to (Rank *et al*., 2010). The importance of the *P.americana* stems from its strong relationship with the human environment, it is one of the most harmful insects to humans, as it causes food

contamination through the waste it leaves and the bacteria that cause food poisoning, as well as the bacteria and fungi that it transmits, which are considered dangerous pathogens in the presence of the insect, near waste that is not free of all kinds of microbes, it is at risk of getting stuck to its legs, antennae, and abdomen (Benelli *et al*., 2020).

It can be observed and known about the degree of pollution they cause in food and the environment. It is known that cockroaches feed randomly on garbage and are found in sewage, so they are considered a very effective means of transmitting pathogens. Thus, *P.americana* are mechanical carriers of various pathogens (Gondhalekar *et al*., 2021). In this field, the widespread use and heavy reliance on insecticides to reduce the damage to *P.americana* has led to the development of resistance to these pesticides, especially in the *P.americana*, and this is considered the biggest threat to those working in pest control around the world, the use of plant extracts is considered one of the most effective alternative methods within the framework of the control program, biological efforts to enter the field of using safer pesticides of plant origin as a simple and sustainable method (Soonwera *et al*., 2022). Therefore, the chance of the emergence of insecticides that rely on a single active ingredient against pests to resist such materials is very small, as plants have widespread insecticidal properties and it is clear that they will act as a new weapon in the list as phytochemicals are considered beneficial because of their environmental safety, and are less dangerous to, Synthetic botanical insecticides are simple, inexpensive, have higher acceptability, and can be applied using technologies more suitable for developing countries with low costs (Ahmed *et al*., 2021). In the Iraqi environment, there are various plants that are distinguished by their medicinal importance, some of which

contain toxic compounds such as thistle and nettle. The extracts of these plants (extracts of organic solvents and extracts of crude secondary compounds) are used in biological control operations as an alternative to synthetic chemical pesticides that have toxic effects on human health and the environment, the plant, it also showed a harmful effect on insect development, as it delayed the growth stages of larvae and pupae and prevented the formation of adults, making it a promising candidate for integrated insect control programs (Sonam *et al*., 2015). The *Achillea* is one of the plants that have proven its efficiency in combating many insects, as it causes an increase in the mortality rate and prolongs the molting stages of the larvae and pupae of many insects, *Baccharis dracunculifera* (Silvana *et al*., 2023).

The extract also showed significant larvicidal activity against *Spodoptera frugiperda*, with a dose of 0.2% causing increased mortality and longer larval stages. However, the ethanol extract of *Baccharis dracunculifolia* did not affect the viability of insect eggs (Luis *et al*., 2023). *Haloxylon* is also a desert halophytic plant, and its effect in combating insects has been proven, there are no studies available that specifically indicate the potential effects of plants on combating insects, the plant contains many active substances, including peroxidase, abscisic acid, auxin, gibberellin, proline, and amino acids that may have an effect, effective in controlling insects (Fang & Guanghui, 2022).

Aim of the study :

Due to the scarcity of research on the use of plant extracts, the effectiveness of extracts of secondary and organic compounds of plants (*Haloxylon Bunge*, *Xanthium strumarium*, and *Achillea* L.) in the life of the *P.americana*, was tested for the first time in Iraq to be an alternative to chemical insecticides.

Materials and working methods:

1. Sample collection:

Immature and adult cockroaches of the *P.americana* were collected from a water drainage area in the Al-Hamza district of Al-Diwaniyah city. Samples were also collected from some homes, restaurants, and stores located in the markets in the city of Diwaniyah. Samples were collected during the month of October 2023 until April 2024.

2. Prepare the test animal culture:

They were transported to the laboratory and emptied into glass tubs with a plastic cover. Biscuits and milk powder were added to these tubs to feed the nymphs. Then I covered these tubs with tulle and followed the method (Abdul Ali 2000). For the purpose of obtaining Oothea, the adults were fed the food mentioned above, and the egg sacs were transferred using forceps to a 650 ml plastic container containing water. After the eggs hatched, the nymphs were fed and monitored for the emergence of the adults. Slides of the adults were prepared for the purpose of diagnosis according to the taxonomic keys (Abd Qader, 2000). .

3. Collecting plant samples:

Sufficient samples of the leaves of the *X.Strumarium*, the leaves of the *Haloxylon Bunge* and the leaves of the *Achillea* were collected from Umm

Shawarif Road, west of Ghams district, and the island of Haidariyah and Al-Shabja, southwest of Bahr Al-Najaf, during the month of September and October. After identifying the plants, the leaves of the above-mentioned plants were taken, cleaned of dust, washed, and dried. Naturally in the shade at room temperature, then the leaves were ground after they were completely dry using an electric grinder and stored in plastic containers until use.

4. Preparation of organic solvent extracts of *X.strumarium* and *Haloxylon Bunge* and *Achillea* L. leaves

Three types of organic solvents with different polarities were chosen to obtain organic extracts: (ethyl acetate), which is a medium polar solvent, (ethyl alcohol), which is a polar solvent with a concentration of 96%, and (hexane), which was described as a non-polar solvent (Harbourn, 1984), and the extraction was carried out, with a Soxhlet extractor.

5. Effect of organic solvent extract of *X.strumarium* and *Achillea* L. *Haloxylon Bunge* leaves in the death of early nymph stage of *P.americana*

I took 20 nymphs/replicate of the early nymphs within 24 hours of age, 3 replicates for each concentration, and I used three plastic containers containing 100 ml of each of the concentrations of extracts mentioned in paragraph (1,2,3), in addition to the control treatment containing distilled water with the solvent. Three replicates of each concentration were used in the extraction and (0.5) grams of biscuits were added to each pot for the purpose of feeding the nymph stages and

following up on the subsequent stages. The mortality rates were recorded in each concentration after (24-48-72) hours, and the mortality rate was corrected according to (Abbott, 1925).

6- The effect of organic solvent extracts of *X. strumarium* and *Achillea* L. leaves on the death of advanced nymph stages of *P. americana*.

About 20 advanced nymphs were isolated from the permanent farm of the insect for each replicate, in addition to the control plant, using distilled water with extraction solvent. They were placed in a 300 ml container with three replicates, which were followed according to paragraph (5).

Statistical analysis

The results of the effectiveness of the extracts and secondary compounds at different concentrations and at different times against the different stages of the *P. americana* were statistically analyzed using the statistical program SPSS, version 32, where the percentages of destruction were corrected according to (Abbott, 1925), and a comparison was made between the percentages of destruction of the listed materials. The study used a two-way ANOVA test with the value of the least significant difference (LSD) calculated at a significance level of 5%. The value of LC50 was also calculated for all materials prepared in the study using a probit analysis with the calculation of the chi-square value, the calculated probability value, and the regression equation for each compound. It was used in the study (Rahman, 2015).

Results

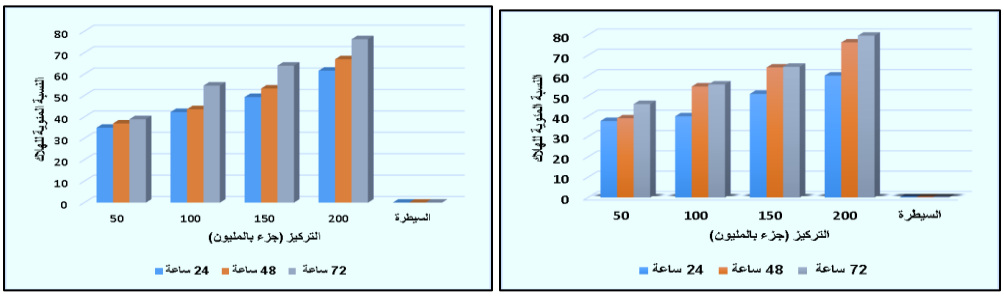
1. The effect on the death of early nymph stages

Fig (1,2,3) show the effect of the concentrations of organic solvent extracts (hexane, ethyl acetate, and ethyl alcohol), respectively, of the leaves of the plants under study on the percentage of death of early nymph stages of *P.americana*. It was recorded that *X.strumarium* was superior in all organic extracts and concentrations to *Achillea* L. It was observed that the hexane extract was superior to the ethyl acetate and ethyl alcohol extracts. We note that there is a direct relationship between the concentration of the extract and the percentage of non-accumulative death, as the percentage of death increases with the increase in the concentration of the extract. The concentration of 200 ppm gave the highest percentage of death for all the tested plants within 72 hours, as the percentage of early nymph death reached (79.66, 76.33%) after 72 hours for the hexane extract. For *X.strumarium* and *Achillea* L., respectively, the percentage of early nymph mortality was (72.66, 73%) after 72 hours for ethyl alcohol extract for two plants, respectively. The percentage of early nymph mortality reached (72.33, 61.34%) after 72 hours for the ethyl acetate extract for the two plants, respectively.

Tables (1,2,3) show the relationship between LC_{50} values and exposure periods to the extracts. The LC_{50} value decreases as the duration of exposure to the extracts increases. We also note that the early nymph is the most sensitive stage if it recorded the highest values of death rates and all the solvents used. The reason for the death of the early nymph may be explained at a higher rate than the rest of the stages in all treatments due to the thinness of the cuticle surrounding the early nymph at the beginning of its formation, or that the newly hatched nymph needs large amounts. A large amount of food for the purpose of their growth, which causes large quantities of the extract with their food to enter the digestive canal,

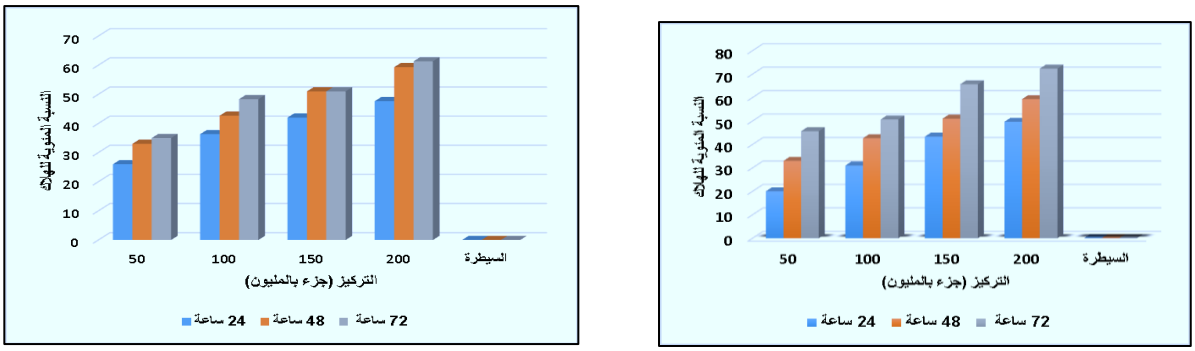
leading to poisoning, or the reason may be that the compounds of these plants prevent nutrition, which leads to the death of the nymphs due to lack of nutrition.

Table (1) shows that the LC_{50} of the early nymph reached (66.76, 79.429%) after 72 hours for the hexane extract of *X.strumarium* and *Achillea* L., respectively. Where Table (2) showed that the LC_{50} of the early nymph reached (71.43, 117.99%) after 72 hours for the ethyl acetate extract for the two plants, respectively. Where Table (3) showed that the LC_{50} of the early nymph reached (78.35, 89.89%) after 72 hours for the extract. Ethyl alcohol of *X.strumarium*, *Achillea* L., and *Haloxylon*, respectively.



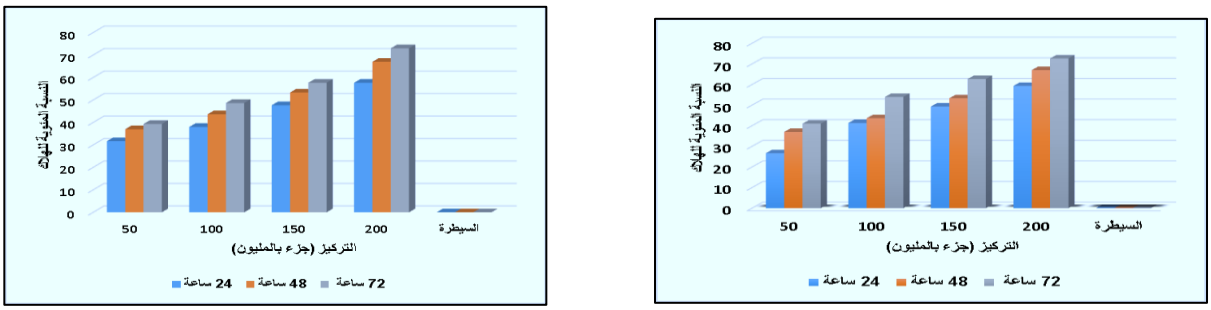
A- *Achillea* L B- *Xanthium strumarium*

Figure (1): The effect of the concentration of hexane extract of the leaves of the plant A. *Achillea* B. *X.strumarium* on the percentage of death of the early nymph stage of the *P.americana*



A- *Achillea* L B- *Xanthium strumarium*

Figure (2): The effect of the concentration of ethyl acetate extract of the leaves of A. *Achillea* B. *X.strumarium* on the percentage of death of the early nymph stage in *P.americana*



A- *Achillea* L B- *Xanthium strumarium*

Figure (3): The effect of the concentration of ethyl alcohol extract of the leaves of A. *Achillea* B. *X.strumarium* on the percentage of death of the early nymph stage in *P.americana*

Table (1): The effect of the concentrations of hexane extract of the (*X.strumarium* and *Achillea* L) on the percentage of LC_{50} values of the early nymph stage of the *P. americana*

IC50	*Achillea* L.			*X.strumarium*		
	24	48	72	24	48	72
IC50 value	130.61	109.48	79.42	132.78	79.42	66.76
Limits 95%	63.1-738.2	42.8-241.2	16.33-121.78	68.6-754.3	16.33-121.78	0.394-107.2
X2	0.308	0.500	0.185	0.421	0.185	0.650
P value	0.857	0.779	0.912	0.810	0.912	0.723
Regression equation	$y = -2.23 + 1.05*X$	$y = -2.45 + 1.2*X$	$y = -3 + 1.58*X$	$y = -1.95 + 0.92*X$	$y = -3 + 1.58*X$	$y = -2.59 + 1.42*X$

Table (2): Effect of concentrations of ethyl acetate extract of (*X.strumarium* and *Achillea* L.) on the percentage of LC_{50} values of the early nymph stage of the *P.americana*

IC50	*Achillea* L.	*X.strumarium*

	24	48	72	24	48	72
IC50 value	235.26	134.1	117.99	204.8	134.11	71.43
Limits 95%	137.1-44513.1	72.3-781.4	61.6-916.22	127.6-44637.6	72.3-781.4	14.8-128.6
X2	0.004	0.073	0.133	0.049	0.073	0.434
P value	0.998	0.964	0.936	0.976	0.964	0.805
Regression equation	$y = -2.27$ +0.96*X	$y = -2.33$ +1.09*X	$y = -2.15$ +1.04*X	$y = -3.26$ +1.41*X	$y = -2.33$ +1.09*X	$y = -2.2$ +1.19*X

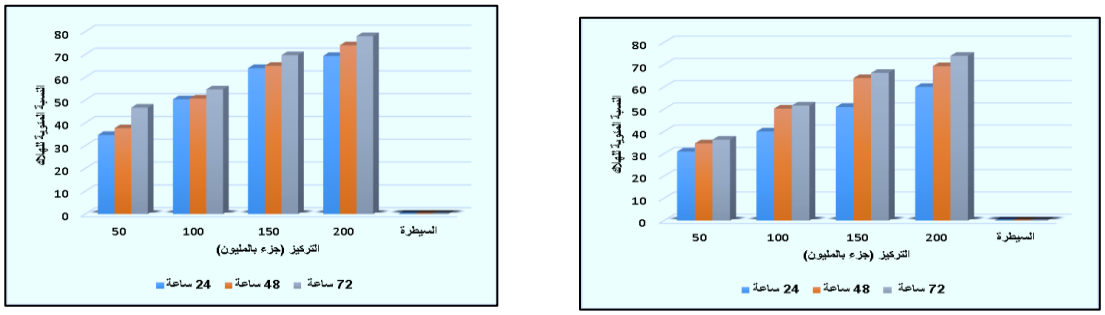
Table (3): The effect of the concentrations of ethyl alcohol extract of (***X.strumarium* and *Achillea* L.**) on the percentage of LC_{50} values of the early nymph stage of the *P.americana*

IC50	*Achillea* L.			*X.strumarium*		
	24	48	72	24	48	72
IC50 value	156.46	114.47	89.89	143.46	109.48	78.35
Limits 95%	94.12-3116.2	3.96-166.6	3.96-166.6	82.91-2932.9	72-1361.1	0.196-132.16
X2	0.247	0.569	0.555	0.042	0.500	0.108
P value	0.884	0.752	0.758	0.979	0.779	0.947
Regression equation	Y=-2.36 +1.08*X	Y=-2.65 +1.36*X	Y=-2.45 +1.19*X	Y=-2.99 +1.38*X	Y=-2.45 +1.2*X	Y=-2.52 +1.33*X

Fig (4,5,6) show the effect of the concentrations of organic solvent extracts (hexane, ethyl acetate, and ethyl alcohol) respectively for the leaves of *X.strumarium* and *Achillea* L. on the percentage of death of advanced nymph stages of *P.americana*, as it was recorded that *Achillea* L. was superior in All organic extracts and all concentrations on *X.strumarium*, which also outperformed *Haloxylon*. It was noted that the hexane extract was superior to the ethyl acetate and ethyl alcohol extracts. We note that there is a direct relationship between the concentration of the extract and the percentage of non-accumulative death, as the

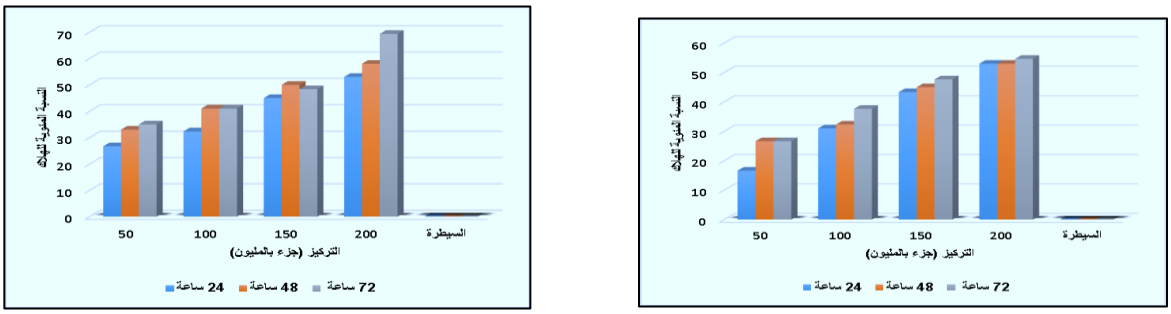
percentage of death increases with the increase in the concentration of the extract. The concentration of 200 ppm gave the highest percentage of death for all the tested plants within 72 hours, as the percentage of death of the advanced nymph reached (74.78%) after 72 hours for the hexane extract. for *X.strumarium* and *Achillea* L., respectively. The percentage of advanced nymph mortality was (69.66, 56.66%) after 72 hours of ethyl alcohol extract for the two plants, respectively. The death rate of the advanced nymph reached (54.66, 69.33%) after 72 hours for the ethyl acetate extract of *X.strumarium*, *Achillea* L., and *Haloxylon*, respectively.

Tables (4,5,6) show the relationship between the LC_{50} values and the exposure periods to the extracts, as the LC_{50} value decreases as the duration of exposure to the extracts increases. We also note that the advanced nymph stage is the stage least affected by the extracts, so the resistance increases as the age of the stage increases. This may be due to the advanced nymph stages being able to convert toxic compounds found in various plant extracts into non-toxic compounds.



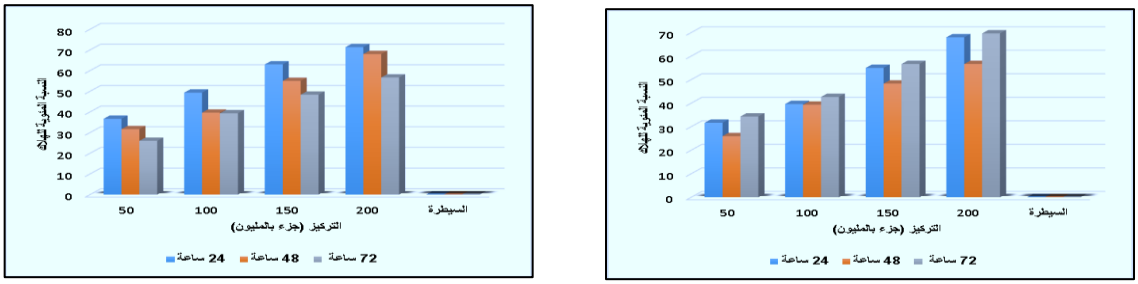
A- Achillea L B- Xanthium strumarium

Figure (4): The effect of the concentration of hexane extract of the leaves of A. *Achillea* B. *X.strumarium* on the percentage of death of the advanced nymph stage of *P.americana*



A- *Achillea* L B- *Xanthium strumarium*

Figure (5): The effect of the concentration of the ethyl acetate extract of the leaves of the A. *Achillea* B. *X.strumarium* on the percentage of death of the advanced nymph stage of the *P.americana*



A- *Achillea* L B- *Xanthium strumarium*

Figure (6): The effect of the concentration of the ethyl alcohol extract of the leaves of the A. *Achillea* B. *X.strumarium* on the percentage of death of the advanced nymph stage of the *P.americana*

Table (4): Effect of hexane extract concentrations of (*X.strumarium* and *Achillea* L.) on the percentage of LC_{50} values of the advanced nymph stage of the *P.americana*

IC50	القيصوم			الحسك		
	24	48	72	24	48	72
IC50 value	92.55	85.06	64.69	138.16	92.55	85.29
Limits 95%	27.37-152.7	22.88-132.06	0.538-103.49	76.1-812.0	27.37-152.7	28.55-129.04
X2	0.044	0.180	0.422	0.150	0.044	0.095
P value	0.978	0.914	0.810	0.928	0.978	0.954
Regression equation	$y = -3.01 + 1.53*X$	$y = -3.06 + 1.58*X$	$y = -2.57 + 1.42*X$	$y = -2.62 + 1.23*X$	$y = -3.01 + 1.53*X$	$y = -3.21 + 1.66*X$

Table (5): Effect of concentrations of ethyl acetate extract of (*X.strumarium* and *Achillea* L.) on the percentage of LC_{50} values of the advanced nymph stage of the *P.americana*

IC50	*Achillea* L.			*X.strumarium*		
	24	48	72	24	48	72
IC50 value	192.62	143.08	119.25	185.6	185.62	166.96
Limits 95%	112.4-45402.3	76.1-712.4	36.75-2068.6	126.1-1094.5	112.4-45402.3	116.8-4211.2
X2	0.267	0.106	1.23	0.019	0.267	0.030
P value	0.875	0.998	0.539	0.990	0.875	0.985
Regression equation	$y = -3.92 + 1.73*X$	$y = -2.25 + 1.05*X$	$y = -2.71 + 1.31*X$	$y = -3.92 + 1.73*X$	$y = -2.66 + 1.16*X$	$y = -2.73 + 1.23*X$

Table (6): The effect of the concentrations of ethyl alcohol extract of (*X.strumarium* and *Achillea* L.) on the percentage of LC_{50} values of the advanced nymphal stage of the *P.americana*

IC50	*Achillea* L.			*X.strumarium*		
	24	48	72	24	48	72
IC50 value	155.76	117.46	90.25	155.76	117.46	107.23
Limits 95%	91.8-24518.1	61.21-275.8	21.71-149.34	91.8-24518.1	61.21-275.8	41.91-227.42
X2	0.135	0.564	0.017	0.564	0.017	0.516
P value	0.935	0.754	0.992	0.754	0.992	0.773
Regression equation	$y = -3.93 + 1.5*X$	$y = -3.175 + 1.1.53*X$	$y = -2.91 + 1.48*X$	$y = -3.17 + 1.53*X$	$y = -2.91 + 1.33*X$	$y = -2.99 + 1.48*X$

Discussions

Based on the results of our study, in which the thistle plant recorded superiority in all organic extracts and concentrations, with the hexane extract superior to the extract of ethyl acetate and ethyl alcohol, and the percentage of death increased with increasing concentration of the extract if the concentration of ppm was 200. The results of our study agreed with (Phayakkaphon *et al*., 2021) who explained in his study the detection of the nymph- and adult-killing activities of *S.collinsiae* root extracts against *P.americana* through oral administration, and the effects of hexane, dichloromethane, ethanol, and raw water extracts on final-

instar nymphs and adults of *P.americana* and after *P.americana* ingestion were tested. For bait containing crude extracts of hexane and dichloromethane, signs of toxicity appeared, such as trembling of the hind legs, trembling of the entire body, inability to move, swelling of the abdomen, and death. After 48 hours, nymphs of *P.americana* that ingested the bait containing the crude extract of dichloromethane had an adjusted mortality rate of 65% - 100% and 20% - 100%, respectively, while none of the nymphs and adults of *P.americana* that ingested the bait containing the crude water extract died (corrected mortality rate of 0%), and upon necropsy The gastrointestinal tracts of those who died from *P.americana*, which ingested the bait containing the crude extract of alkaloid di-dehydrostemofoline and hexane, found that the foregut was distended. TLC analysis showed that the crude extracts of hexane contained the alkaloid di-dehydrostemofoline and unknown fluorescent substances, which could be attributed to the cause of death. *P.americana* to alkaloids and the synergistic effects of other substances in the *S.collinsiae* root extract, the mechanisms of action may involve several pathways involved in nervous system function. Thus, crude extracts of hexane can be developed as alternative active ingredients in a natural insecticide for cockroach control. Our study agreed with Sakulpanich *et al*., (2023) which showed that crude extracts from hexane were highly toxic to *P.americana* and the crude extract from ethanol was less toxic, while the crude extract from water was non-toxic to *P.americana*. Crude hexane extracts killed nymphal, larval and adult stages of *P.americana*, and the alkaloids in *S.collinsiae* root extracts act as neurotoxins and cause irregular symptoms and lethality in *P.americana*. Di-dyhydrostimofolin alkaloid has been reported to possess acetylcholinesterase inhibitory activity; However, other phytochemicals may also be responsible for insecticidal activity, including rotinoids, flavonoids, and other unknown fluorescent substances, which may also

act synergistically to enhance insecticidal properties. Mechanisms of action may involve the phytochemicals present in the root extract. *S.collinsiae* nicotinic acetylcholine receptor regulator, nicotinic acetylcholine receptor regulator-site I, and inhibitors of electron transport in mitochondrial complex I.

The results of our study agreed with Mekhlif & Al-dulaimi, (2012) in studying the effect of alcoholic extracts of three types of plants in the early stages of nymphs and larvae in addition to the pupa *Culex pipines*. Extracts of both *Achillea fragrantissima* when treated at a concentration of 250 ppt caused a 100% killing rate. 100 for two-stage larvae, nymphs and larvae, during the treatment periods of 24 and 48 hours. This is due to the fact that the acetum contains effective secondary metabolic substances, especially alkaloids, and that the ethyl extract preserves the primary substances extracted from the plant, which are more effective on the early stages of insects and can work as promising insecticides against pests.

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