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Biological Effective of organic solvent extracts of *Mirabilis jalapa* Leaves in the Non-cumulative for mortality of Immature stages *Culex quinquefasciatus* Say(Diptera : Culicidae)

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Abstract

The present research evaluated the efficacy of organic solvent extracts (hexane, ethyl acetate, ethyl alcohol) separately using ppm (0,100,250, 500,1500, 1000, 2000) concentrations of *Mirabilis jalapa* leaves in the non-cumulative moetality of immature *stages* of *Culex quinquefaciatus* then estimate LC50 and LC90 by probit analysis. The hexane extract of the leaves *M. jalapa* of plant was the most affected in the non-cumulative mortality from that of ethyl acetate and ethyl alcohol. in the non-cumulative mortality of the immature stage . Where LC50 for egg mortality was ppm (2107.7, 2743.5, 3062.7) for hexane extract, ethyl acetate and ethyl alcohol respectively For the four larval . the first larval stages were the most sensitive of the larval stages of all extracts .The hexan extract was LC50ppm (373.1, 456.2, 758.4, 1941.5) ethyl acetate extract was LC50 ppm (508.1, 673.8, 1369.5, 2745.3) for the four larvae stage**s** respectively. The LC50 for ethyl alcohol extract ppm (511.1, 850.4, 1916.2, 3342.2) . In instar pupa the value of LC50 ppm (1993.9, 2183.5, 3562.9) for hexane extract, ethyl acetate and ethyl alcohol respectively.

KEYWORDS*:*

Mirabilis jalapa. Culex quinquefasciatus. organic solvent extracts. hexane extract, ethyl acetate, ethyl alcohol.

INTRODUCTION

Culex quinquefasciatus is a major vector of several pathogens such as dengue Wrchermesingh and Mendis1980), encephalitis virus, West Nile virus (Rutledge et al. 2003) and Vires Zika (Vorou , 2016). The use of chemical pesticides has shown resistance to insect (Shono and Scoot,2oo3). Therefore, it was necessary to find new pesticides with low toxicity and slow decomposition unlike chemical pesticides, pesticides of plant origin (Adel and Abdul, 1979) It is these plants plants *Mirabilis jalapa* to the belong to family Nictaginaceae or the Rose family at four o'clock because most of their species bloom after four o'clock in the afternoon (Levin *et al*. (2001). It contains many active compounds Trigonellne alkaloid and Jalapa alkaloid Due to the lack of studies on the effect of the above plant on some life aspects of *C. quinquefasciatus* mosquitoes and their use as an alternative to chemical .pesticides, we conducted the present research .

Materials and methods of work

Collect and diagnose plant samples

Sufficient leaves were collected from the plant in November 2018 from private nurseries in Qadisiyah governorate. The plant was identified by Dr. Suhaila Al-Lami, Al-Qadisiyah University, College of Education On it *M. jalapa* plant back to the family Nictaginaceae. The leaves of the plants were taken and cleaned from the dust and washed and dried naturally in the shade and at room temperature. The leaves were then grinded by an electric grinder and kept in dark plastic containers until use

Preparation of the permanent farm Cx.qinquefasciatus

Collected immature instar (eggs and larvae) from one of the places of drainage water was placed in plastic basins filled with chlorine-free water and added rats diet and The insect was diagnosed by Dr. Ghaida Abbas, Qadisiyah University, College of Veterinary Medicine This insect was Cx.qinquefasciatus. For the purpose of obtaining a pure permanent farm, the pupa was transported in a plastic cage with a tulle clip and plastic plates containing cotton saturated with a 10% sugar solution to feed the modern insect and to obtain eggs boats followed. According to the method (Mehdi and Mohsen 1989), The female mosquitoes were fed three days after dawning on the blood of their pigeons. Then the boats were transferred to the eggs of new water contain food larvae were followed up until the emergence of full and Taking into account replacement of water every three days

Preparation of plant extracts

Preparation of organic solvent extracts of *Mirabilis jalapa* leaves

Three different polar organic solvents were selected to obtain the organic extracts hexane, ethyl acetate and ethyl alcohol. According to the method(Harborn 1984)for Prepar of organic solvent extracts 10 g of leaves plant powder were taken and placed in Soxholet Extractor for 24 hours per organic solvent at 40-45 ° C The extracted material was then concentrated using a rotary evaporator at a temperature of 40-45 C, then the sample was transferred to glass plates and placed in the electric oven and at a temperature of 45 C to evaporate the solvent. The sample was taken and stored in the refrigerator until use.

Preparation of organic solvent extract

For the purpose of determining the bio-effectiveness of organic solvent extracts, a stock solution was prepared. 1 g of dry extract was dissolved by 5ml of the solvent and the volume was completed to 100ml distilled water From this solution the concentrations were prepared which were ppm (2000,1500,1000,500,250,100) . For the control treatment, she was taking 5 ml of each used flask and completing the volume to 100 ml of distilled water

Determine of biological activity of Organic Solvent Extracts (Hexane, Ethyl Acetate, Ethyl Alcohol) for of *Mirabilis jalapa* Leaves in Non - Cumulative mortality of Immature stage of *Cx. quinquefasciatus*

Determination of biological activity against eggs

To find out the effect of organic solvent extracts (hexane, ethyl acetate, ethyl alcohol) for *M.jalapa* leaves, eggs were taken 24 hours old with five replicates for each concentration in addition to the control treatment by one repeater for each concentration and one egg boat was placed in a 300ml plastic container with 100ml of each of the concentrations ppm (100,250, 500, 1000, 1500, 2000) for the three extracts and spray the eggs superficially and in a quantity of 2ml per repeater by hand sprayer with the same concentration that was put in it and after hatching the eggs were calculated. The losses are corrected according to Abbott's equation

Determination of biological activity against larvae

40 larvae were taken for each repetition from each of the four immature larval instar. 5 plastics containers containing 100ml of each of the concentrations of the extracts mentioned in the previous paragraph.(0.5) g of rats diet were added to each containers in addition to the control treatment by one repeater for each concentration . Where the instar were prepared They were followed up until they alienation to the later instar, and the mortality were recorded at each concentration after 24 hours. The losses are corrected according to Abbott's equation

Determination of biological activity against pupa

Isolated pupa from the permanent insect farm by 40 larvae per repeater and placed in a 300ml container of 5 replicates and followed the same test method as in the previous paragraphs except the addition of bush in addition to the control treatment by one repeater for each concentration. The losses are corrected according to Abbott's equation

Statistical analysis

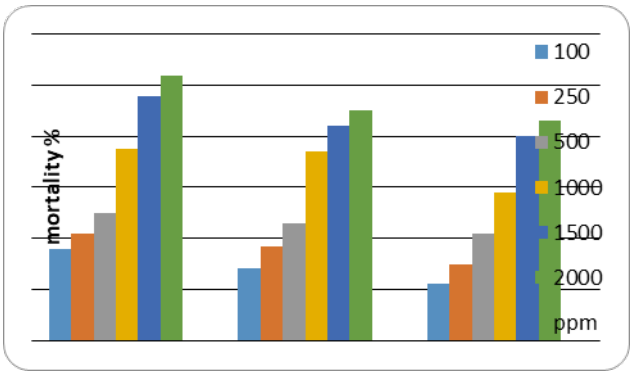
The results of the present study were analyzed statistically according to the method (Qassas, 2014). The percentages of mortality were corrected according to Abbott formula (1925). LC50 and LC90 were calculated using Probit analysis Finney (1971), and K-squared, P value and regression equation for each extract or compound were determined

Results and discussion

Effect of organic solvent extracts of *Mirabilis jalapa* Leaves on the non-cumulative mortality of immature stage of *Cx quinquefasciatus*

The effect in the mortality of eggs

The effect of the concentrations of organic solvent extracts (hexane, ethyl acetate, ethyl alcohol) of *M.jalapa* leaves on the mortality percentage of Cx . quinquefasciatus eggs is shown in Figure(1) Where we observe the most effective of the hexane extract over the extracts of ethyl acetate and ethyl alcohol as well as we note a direct relationship between the concentration of the extract and the percentage of non-cumulative mortality of eggs, where the proportion of mortality increases with the concentration of the extract as the concentration of ppm 2000 gave the highest mortality rate for all extracts using which reached (52,45,43)%of (hexane, ethyl acetate, ethyl alcohol).Table (1) shows that LC50 for hexane extract of ppm (2107.7) and LC90 ppm (43269.9) Ethyl acetat was LC50 ppm (2743.5) and LC90 was ppm (114592.6) then ethyl alcohol LC50 ppm (3062.7) and LC90 ppm (116151.9)



Effect of organic solvent extracts of leaves on percentage mortality non-cumulative of Cx. quinquefasciatus

The low rate of hatching of eggs by the use of hexane solvent may be due to the fact that these extracts prevent gas exchange or hardening of the egg shell and then the death of the embryo and non-hatching of the egg (Adel and Abd, 1979) . Al-Khafaji

(2003) showed that the hexane extract of *P.harmala* was effective in *C.pipiens* eggs Where the ratio was 20.08% at 200 mg / ml concentration. show Twig *et al*(2009) noted that the hexane extract of Schanginia aegptiaca led to the destruction of Cx mosquito eggs. quinquefasciatus - 18.7% Kareem (2016) reported that the hexane extract of the leaves and flowers of the *cinerariaefolium* plant exceeded all its concentrations over its ethyl acetate and ethyl alcohol in the mortality eggs of *Cx. quinquefasciatus*

Table (1) LC90, LC 50 and Bioactivity of organic solvent extracts of leaves *M. jalapa* in mortality of eggs Cx. quinquefasciatus

	M. jalapa		
	hexsan	ethyl acetate	ethyl alcohol
LC50 ppm	2107.7	2743.5	3062.7
Limits 95%	811.8-1552.1	1828.7-5310.3	2017.6-6070.8
LC90 ppm	43269.9	114592.6	116151.9
Limits 95%	117070.5-212032.5	35305.4-945786.5	36102.1-934377.5
X2	3.818	3.004	2.487
P value	0.431	0.557	0.647
Regression equation	Y=-2.89+0.79*X	Y=-2.66+0.77*X	Y=-2.76+0.79*X

Larval stage **The effect in the mortality of**

The effect of the concentrations of organic solvent extracts (hexane, ethyl acetate, ethyl alcohol) on the leaves of *M. jalapa* on the percentage of larval mortality of *Cx. quinquefasciatus* is shown in the figure(2). We note the hexane extract was the most effective from ethyl acetate and ethyl alcohol as well as a direct correlation between the concentration of the extract and the ratio of non-cumulative mortality as the percentage of mortality increases with the concentration of the extract as the concentration of ppm 2000 gave the highest rate mortality for all extracts **.The values of hexane extract were reached (82, 75, 67, 52)%The** ethyl acetate extract was (75,65,57,50) %. In ethyl alcohol extract, the percentage of larval instar was(67,62,55,43)%. From Table (2), the relationship between the values of LC50 and the exposure periods of the extracts is reduced. Affected by the extracts where the resistance increases as the instar of life increases

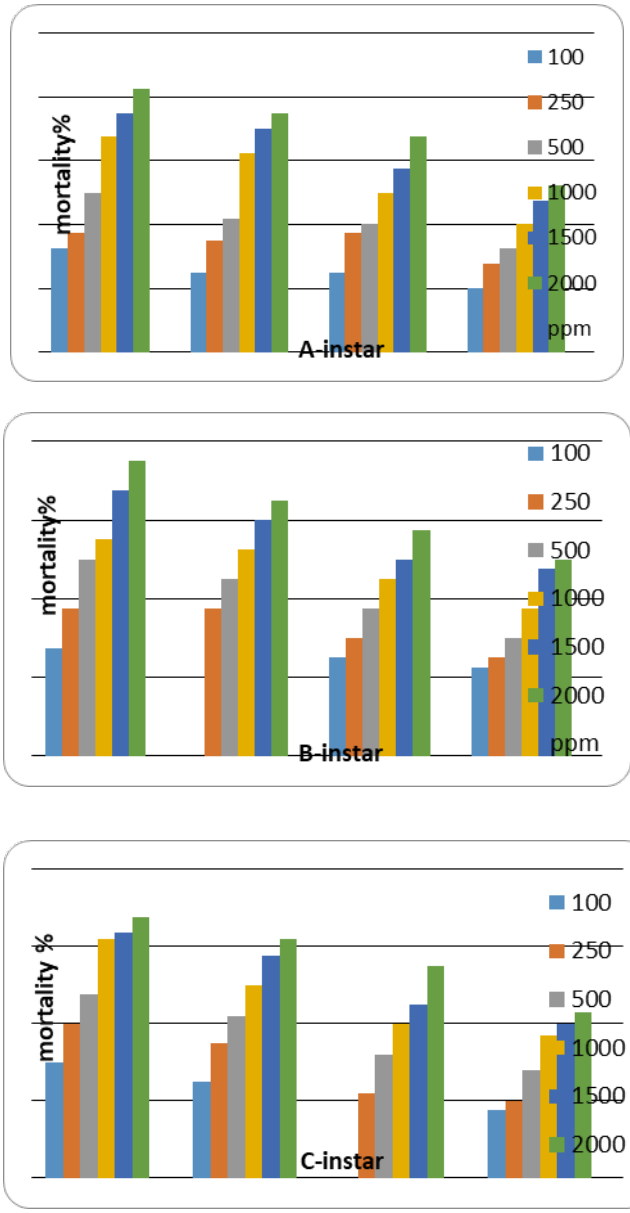


Figure (2) Effect of organic solvent extracts A- hexane B- ethyl acetate C ethyl alcohol of the leaves *M.jalapa* in mortality percentage of Larval stageof *Cx. quinquefasciatus*

It may be because the latter instar have been able to convert the toxic compounds found in the various plant extracts into non-toxic compounds Detoxification by enzymes called Mixed F. oxidation (M.F.O), while the first instar of this can not lack this enzymatic system (Adel and Abdul 1979). Or the newly hatched larvae need large quantities of food for the purpose of growth, which leads to the entry of large quantities of the extract with food into the gut leading to poisoning or may be the reason is that the compounds of these plants inhibitory nutrition, leading to the death of larvae due to lack Nutrition (1969, Frankle). The

results show that the LC50 of hexane extract of *M.jalapa* plant ppm (373.1, 456.2, 758.4, 1941.5)
. The value of LC90 was ppm (3757.1, 49537,30420,14234) and the ethyl acetate of was LC50 ppm (508.1, 673.8, 1369.5, 2745.3) and LC90 was ppm (12139.6, 45472.9, 103066.7, 402742.6) respectively. LC50 for ethyl alcohol extract of *M.jalapa* plant was ppm(511.1, 850.4, 1916.2, 3342.2) The LC90ppm (23801.8, 48223, 108018.9, 386539.8)**.**

Maheswaran *et al*. (2008) reported that the hexane extract of *L.aspera* most effect from chloroform and ethanol for the same plant in mortality *Cx. quinquefasciatus* Bernard *et al*. (2012) indicated that the LC50 value of the hexane and ethyl acetate extract of the roots of *Tithonia diversifolia* in the mortality of the fourth stage larvae of *Aedes aegypti* ppm (800,1700) respectively after 24 hours of treatment. Singh (2018) shown The ethyl acetate extract of *G. lanceolarium* achieved 100% mortality for the first, second and third phases and the fourth mortality 69%

Table (2) LC90, LC 50 and Bioactivity of hexane extracts of *M.jalapa* leaves in mortality of Larval instar of *Cx. quinquefasciatus*

	hexan			
	st1	2nd	3rd	4th
LC50 value	373.1	456.2	758.4	1941.5
Limits 95%	235.6-531.6	295.6-657.2	461.9-1418.8	1044.5 =9557.7
LC90 value	5647.6	7533.8	33421	146918.1
Limits 95%	2884.2-20762.5	3582.1-33393.5	8703-1265199.1	20180.8-12378368.9
X2	1.955	0.893	0.777	0.251
P value	0.744	0.926	0.941	0.993
Regression equation	Y=-2.81+0.09*X	Y=-2.79+1.05*X	Y=-2.25+0.78*X	Y=-2.23+0.68*X

Table (3) LC90, LC 50 and Bioactivity of ethyl alcohol extracts of *M.jalapa* leaves in mortality of Larval instar of *Cx. quinquefasciatus*

	Ethyl Acetate			
	1 st	2 nd	3rd	th4
LC50 ppm	508.1	673.8	1369.5	2745.3
Limits 95%	316.7-774	373.2-1340.6	760.6-5207.2	1261.8-42213
LC90 ppm	12139.6	45472.9	103066.7	402742.6
Limits 95%	4788-96281.6	10014.8-6608573.7	17667.1-138323709.4	31401.9=2.798E+10
X2	0.981	0.264	0.384	0.752
P value	0.913	0.992	0.984	0.945
Regression equation	Y=-2.52+0.93*X	Y=-1.95+0.69*X	Y=-2.02+0.65*X	Y=-2.01+0.58*X

Table (4) LC90, LC 50 and Bioactivity of ethyl alcohol extracts of *M.jalapa* leaves in mortality of Larval instar of *Cx. quinquefasciatus*

	ethyl alcohol			
	1st	2nd	3 rd	4 th
LC50 ppm	511.1	850.4	1916.2	3342.2
Limits 95%	281.8-858.9	510.5-1759.9	1074-7432.9	1492.8-55965.4
LC90 ppm	23801.8	48223	108018.9	386539.8
Limits 95%	6777-704254.3	10260-3201457.1	17536-2264152	32168.1-1.173E+10
X2	0.352	0.131	0.872	0.189
P v	0.986	0.998	0.929	0.996
Regression equation	Y=-2.08+0.77*X	Y=-2.17+0.74*X	Y=-2.4+0.73*X	Y=-2.19+0.62*X

The effect in the mortality of pupa instar

The effect of concentrations of organic solvent extracts (hexane, ethyl acetate, ethyl alcohol) on leaves of leaves *M.jalapa* on the percentage of pupa mortality of mosquitoes is shown in Figure 3 *Cx. quinquefasciatus.* We note the hexane extract most effect that from ethyl acetate and ethyl alcohol extract. We also note that there is a direct correlation between the concentration of the extract and the percentage of non- cumulative mortality as the mortality rate increases with the concentration of the extract as the concentration of ppm 2000 gave the highest mortality rate

(45, 43, 35)%As shown in Figure (3). As shown in Table (5), LC50 for hexane extract ppm (1993.9), LC90 was ppm(117477.6), for ethyl acetate extract was LC50 ppm (2183.5) and LC90 was ppm (170883.2). (3562.9) and LC90 was ppm (182642.3)in ethyl alcohol extrct

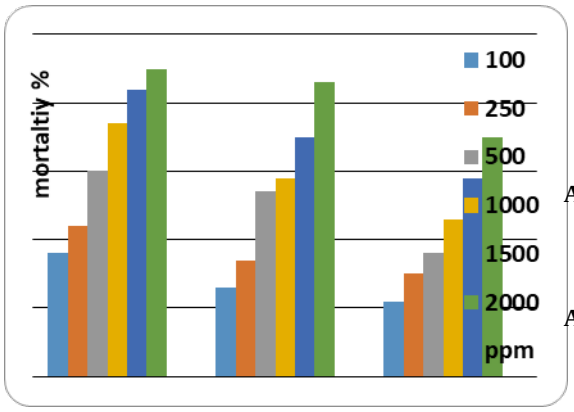


Figure (3) Effect of organic solvent extracts of the leaves of the *M.jalapa* In mortality percentage of pupa. of *Cx. quinquefasciatus*

Table (5) LC90, LC 50 and Bioactivity of organic solvent extracts of leaves *M.jalapa* in mortality of pupa of *Cx. quinquefasciatus*

	M.jalapa		
	hexan	ethyl acetate	ethyl alcohol
LC50 value	1993.9	2183.5	3562.9
Limits 95%	837.3-5636.3	1126.9-13889.7	1314-16052
LC90 ppm	117477.6	170883.2	182642.3
Limits 95%	17434-715593014	22860.2-392748036	22712.8-148087599
X2	0.239	0.416	0.179
P value	0.993	0.981	0.996
Regression equation	Y=-2.14+0.67*X	Y=-2.19+0.66*X	Y=-2.37+0.7*X

The death of pupa and the non-emergence of adults is the effect of extracts used as insect growth regulators and their effect on adult exit hormone (Makkar et al 2007). Sivagnaname and Kalyansundraam (2004) showed that the hexane extract of the leaves of *Atlanita monophlla* was effective in *An.stephensi* larvae. Ali (2007) pointed out that the treatment of the mosquito virgins mentioned by the alcohol extract of the leaves of Duranta ssp gave a mortality rate that was between (66.66 -93.33)% in concentrations of ppm (800-10). Ramar and Ignacimuthu (2015) indicated that the hexane extract of *Corton sparciflorus* leaves was more effective than the chloroform and ethyl acetate extracts in the mortalityof Larvale . *Cx. quinquefasciatus*

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