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TOXIC EFFECT OF SECONDARY METABOLITES OF *CLADOSPORIUM HERBARUM* ON LARVAE OF *CULEX PIPIENS* AND *ANOPHELES STEPHENSI* (DIPTERA: CULICIDAE)

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Abstract

Cladosporium herbarum (Persoon) is one of the fungi accompanying mosquitoes and belongs to the branch of the cystic fungi. The present results showed the effect of crude secondary metabolism in fourth larvae phases of *Cladosporium herbarum*, for both kinds of mosquitoes which came from different incubation period. The high mortality rate of larvae phases was after an incubation period 21 days, whereas LC50 values were (112.5, 129, 148.7, 170.3 ppm) of *Cx. pipiens* and (103.2, 119.4, 138.8, 158.6 ppm) for *An.stephensi*. The results of sensitivity revealed that the first phase of *An. Stephensi* was the most sensitive among other phases of *Cx, pipiens*.

Key words: *secondary metabolites, Cl. Herbarum*, IC*50* .

Introduction

The mosquito (Liston) *Anopheles stephensi* is a major vector of malaria in many of the world, including Iraq, where more than 250 million people, as well as more than one million deaths each year in the world, are infected with malaria (WHO, 2018), while the *Culexpipiens L.* To various dangerous viral pathogens, including *St. Louis* virus (causing encephalitis), West Nile and dengue fever and the nematode Wuchereriabancrofti that causes elephantiasis of the so-called filariasis, which wastes the lives of millions of people, more than 700 million people have been infected with filariasis. About 103 billion people in more than 80 countries face the risk of infection with this disease and since the control of vectors is more comfortable than controlling the pathogen itself, researchers have paid attention to mosquito control from an early age and perhaps chemical control was and still is the most effective in eliminating mosquitoes and reducing their damage. In different parts of the world, many manufactured pesticides were used, but the damage caused by them was not a small thing, as their use led to pollution of the environment and the acquisition of target insects The ability to adapt to toxic substances quickly and to start developing immunity against them (Ishak *et al*., 2017), so researchers had to search for other alternatives, one of which is biological control. Pathogenic fungi are among the important factors for their spread and wide presence in nature, as well as being non It is expensive. It is distinguished by its high specialization to confront a specific pest that is manufactured inside the fungal cell in small quantities. Still, its importance is great as it has toxic effectiveness against insects, so attention has turned to secondary metabolites in insect control to be a safer alternative to manufactured pesticides because they are toxic, inhibitory or inhibitory. In insects, the fungus *Cladosporium herbarum* (Persoon) is one of the fungi accompanying mosquitoes and belongs to the branch of the cystic fungi and its effect on the whitefly *Bemisia* sp. and scale insects (Abdel-Baky *et al*., 2000), given the medicinal importance of my mosquito, *An. stephensi* and *Cx. pipiens L.* the investigation of new means to combat them in life and the fact that previous research that contributed to isolating local types of fungi and using them as a vital factor in the control is almost very few, as well as it, have not previously address the fungus *Cl. Herbarum*. Therefore, the study aimed to isolate the fungus above from the larvae of the naturally infected Culex and Anopheles mosquitoes for the first time in Iraq and isolate its secondary metabolites compounds and

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separate them chemically to determine its effectiveness as a biocide that can be manufactured.

Materials and Methods

Isolation of the fungus *Cl. Herbarum*

The fungus was isolated from naturally infected mosquito larvae, where four ponds chosen to collect the larvae of *Culex* and *Anopheles* mosquitoes. The mosquitoes abound in these ponds because they are rich in organic materials. Ten samples were taken to cover the area of our study. Different locations for each pond from December 2018 until September 2019 were transferred to the laboratory, sterilized and then placed in container Petri dishes on PDA medium and dishes were incubated at 25 ± 2°C for seven days, then fungus was diagnosed microscopically.

Preparing the permanent farm for two *Cx. pipiens* and *An. stephensi*

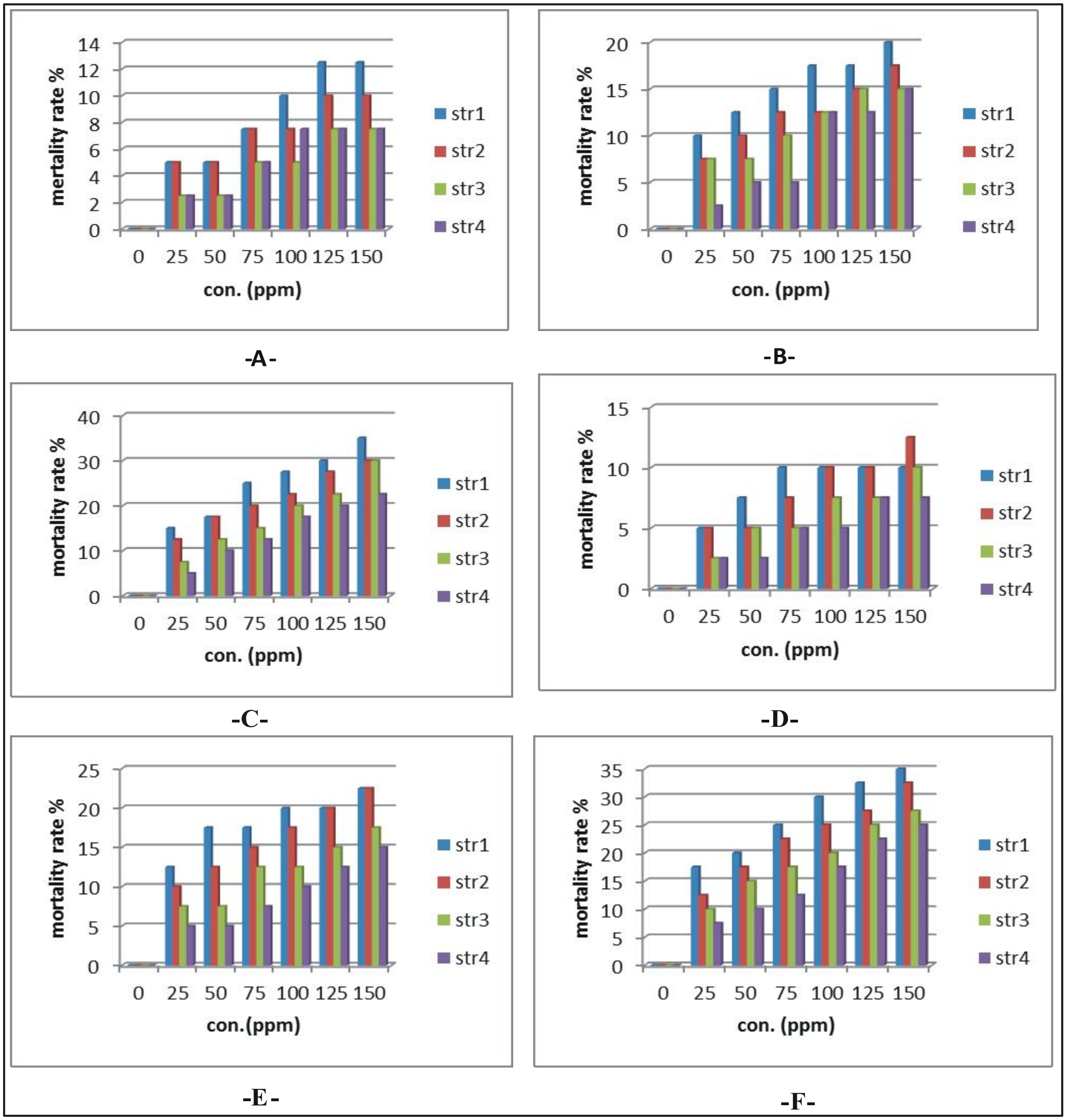

Fig. 1: Effect of secondary metabolites of the fungus with a 7- day incubation of four mosquitoes larvae *Cx. pipiens* (A, B, C) after 24, 48, 72 hours (D, E, F) of *An. Stephensi* after 24, 48, 72 hours.

In order to obtain a pure permanent culture, additional samples were taken from the different stages of mosquito larvae *Cx. pipiens* from the aforementioned collection areas, according to Mehdi and Mohsen, (1989) method; adults of *An. stephensi* were collected in the places of raising animals by the aspirator, then placed in wide-mouthed bottles covered with Altul cloth and transported to the laboratory and released in the breeding cage and their life cycle was followed until the emergence of the third generation. For preparing adequate numbers of larvae, pupae and adults, they were isolated sufficient numbers of eggs to obtain the first larval stage. The second, third and fourth in stars were prepared by isolating numbers of the larvae of the previous stage and placing them in the breeding tubes individually and monitoring them until the molting reaches the required stage for both types separately.

Preparation the crude secondary metabolites of *Cl. herbarum* with different incubation periods (7, 10, 14, 21, 28) days

Potato Dextrose Broth (PDB) was prepared and distributed in 250 ml flasks in 150 ml beaker. Inoculate

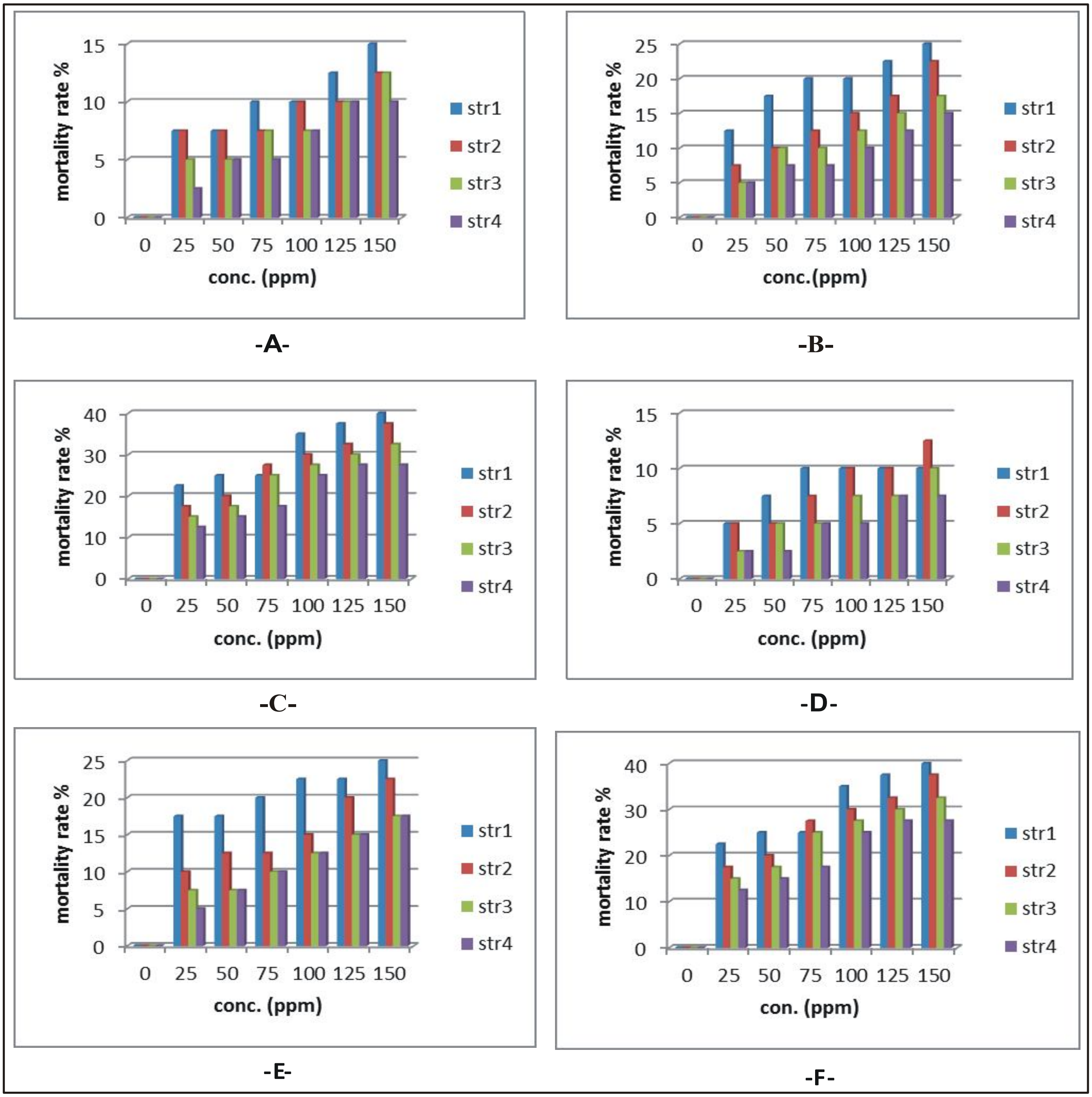

Fig. 2: Effect of secondary metabolites of the fungus with 10- day incubation of four mosquito larvae *Cx.pipiens* (A, B, C) after 24, 48, 72 hours, (D, E, F) of *An. stephensi* after 24, 48, 72 hours.

the medium with 0.5 cm diameter from the colony of fungus culture at seven days old. The flasks were incubated in a vibrating incubator 150 rpm speed at 25±2°C temperature. The incubation period was extended 7, 10, 14, 21, 28 days, after which it was filtered with Whatman No. by taking 0.25 ml of the filtrate and adding 400.75 ml of distilled water to complete it to 500 ml and from this solution the concentrations were prepared (150, 125, 100, 75, 50, 25 ppm) (Soni and Prakash, 2010).

Bioassay for the crude secondary metabolites of *Cl. herbarum* with different brood durations in the four larval stages of two type of mosquitoes *Cx. pipiens* and *An. stephensi*

Forty larvae were taken from each of the larval stages that were prepared as in paragraph (1) and for each concentration of secondary metabolites concentrations of the two types of mosquitoes (separately) and distributed into four three containers, each containing 100 ml of each concentration. The fourth it contains sterile distilled water (control treatment). Then the treated larvae were

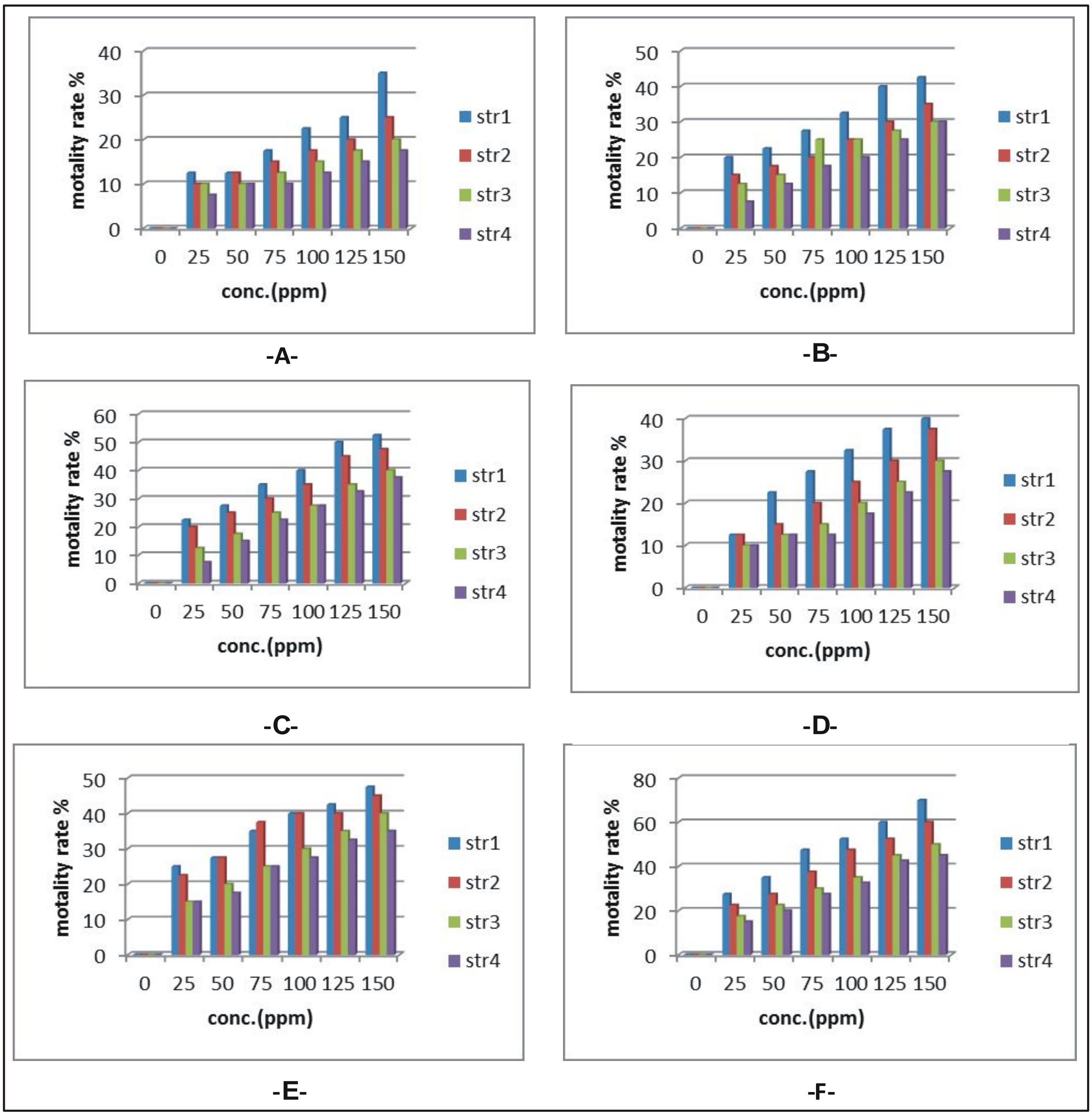

Fig. 3: Effect of secondary metabolites of the fungus with 14- day incubation of four mosquito larvae *Cx.pipiens* (A, B, C) after 24, 48, 72 hours (D, E, F) of *An. stephensi* after 24, 48, 72 hours.

transferred with a soft brush to glass containers of 250 ml containing sterile distilled water to which the larvae food was added by 10 mg. The vessels were placed in the incubator at a degree of 25 ± 2, then the percentage of mortality was calculated within 24, 48, 72 hours of treatment and percentages corrected.

Statistical analysis

The statistical program (SPSS Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) used version 23 to analyze all the results of the current study and the percentages of depreciation were calculated and corrected according to the Abbott Formula, (1925):

$$\text{\% corrected mortality} = \frac{\text{the \% of mortality in treatment - the \% of mortality in control}}{\text{100 - percentage of mortality in control}} \times 100$$

The value of LC_{50} and LC_{90} was also calculated using the Probit program according to the Finney, 1971 method. The statistical analysis also included determining the value of the Chi-square test, the P value and the regression equation.

Results and Discussion

The biological test of the crude secondary metabolites, *Cl. herbarum* in the four larval stages

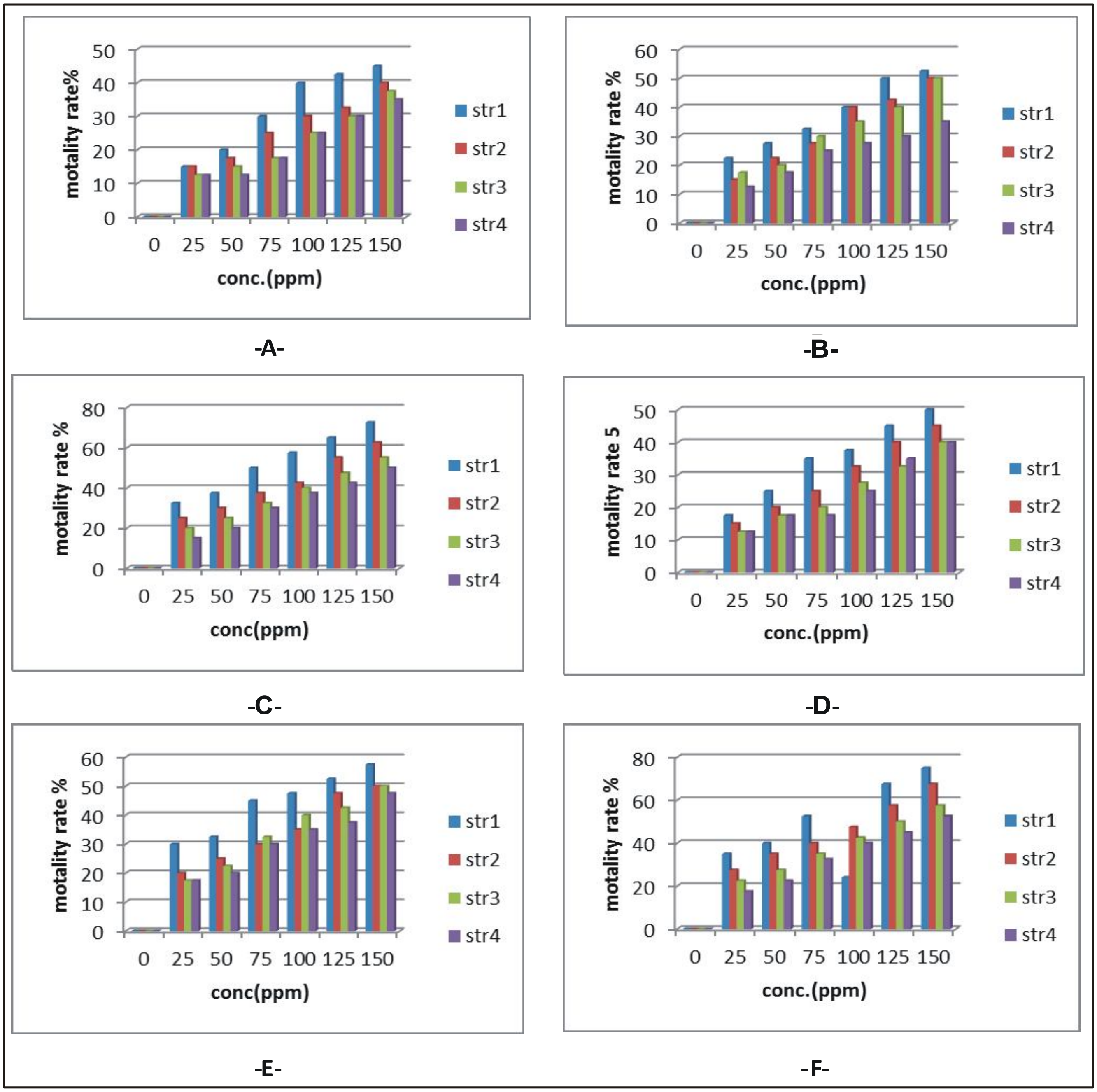

Fig. 4: Effect of secondary metabolites of the fungus with 21 day incubation of four mosquito larvae *Cx.pipiens* (A, B, C) after 24, 48, 72 hours (D, E, F) of *An. stephensi* after 24, 48, 72 hours.

of two types of mosquitoes *Cx. pipiens* and *An. stephensi* incubation in periods (7, 10, 14, 21, 28) days

Figs. 1,2,3,4 and 5, showed the effect of the secondary metabolites of *Cl. herbarum* in the four larval stages of *Cx. pipiens* and *An. Stephensi*. The mortality rates of the first larval stage of *Cx. pipiens* using the secondary metabolites were (35, 40, 67.5, 72.5, 70%) and (35, 40, 70, 75, 72.5)% of *An. stephensi* using the highest concentration of 150 ppm and with stimulation durations (7, 10, 14, 21, 28). Our results observed increase the percentage of mortality with increasing concentration, while there were no losses in the control treatment and the first phase was the most sensitive phase. Also, the present results showed significant differences between the two types of mosquitoes.; *An. stephensi* is more sensitive than *Cx. pipiens* and mortality was highest percentage during the incubation period of 21 days, which indicates that the fungus took sufficient time to produce toxic and effective substances against the insect, this evident in the values of LC_{50}, was reached to lowest value after the 21-day incubation period, which reached (112.5, 129, 148.7, 170.3) ppm of the four larval stages of *Cx. pipiens* and (103.2, 119.4, 138.8, 158.6 ppm) for

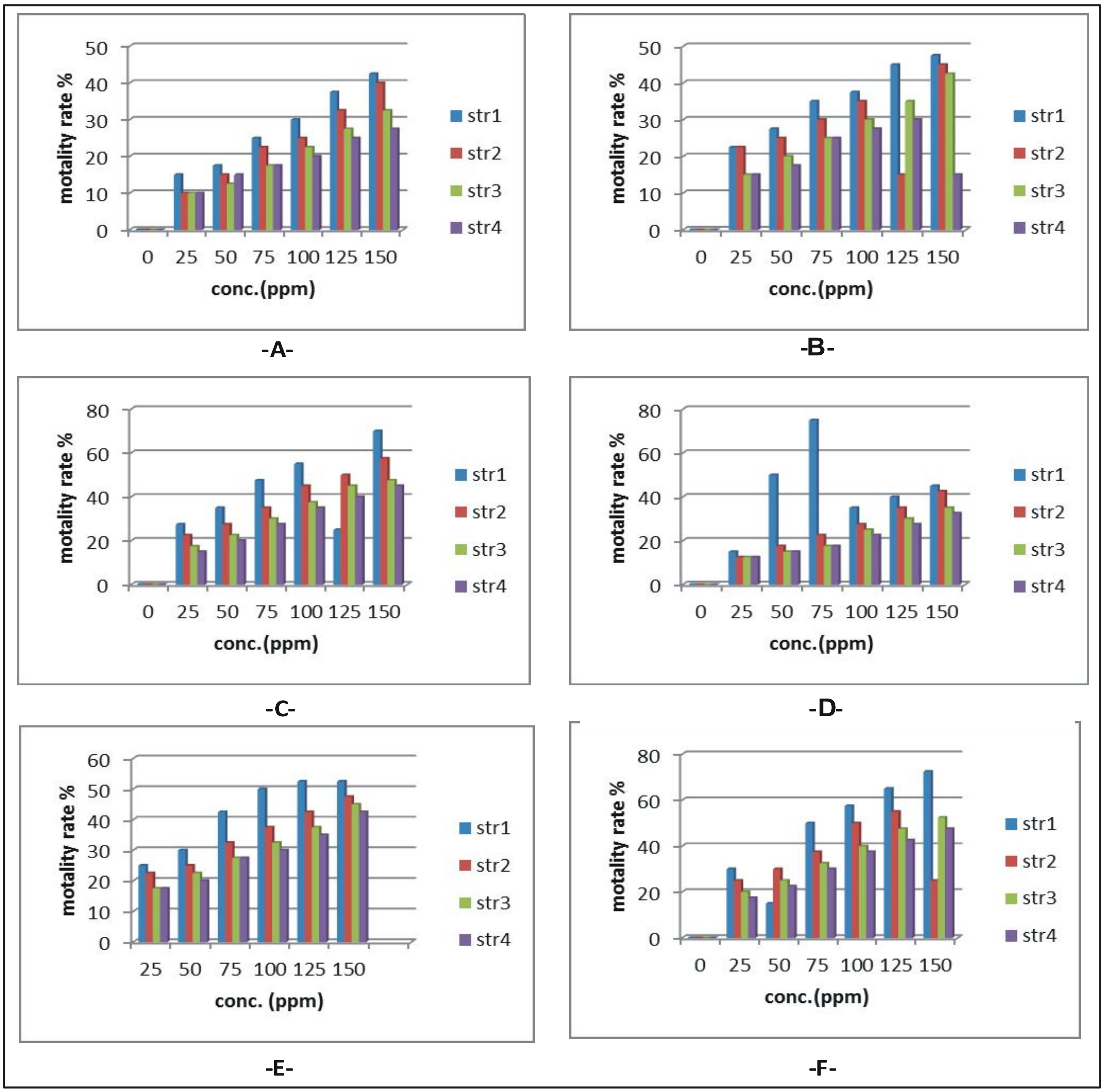

Fig. 5: Effect of secondary metabolites of the fungus with 28days incubation of four mosquito larvae *Cx.pipiens* (A, B, C) after 24, 48, 72 hours (D, E, F) of *An. stephensi* after 24, 48, 72 hours.

Table 1: Values LC_{50} and LC_{90} For the biological testing of crud metabolites of the fungus *Cl.herberum* with 7 Days incubation period for the four larval stages of the two mosquito species.

An.stephensi												*C x. p ipieus*												IC
4I			3			2I			1I			4I			3			2I			1I			
72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	
249.9	341.2	451.1	255.9	370.1	429.1	235.7	328.8	421.8	225.5	435.3	657.1	257.3	285.7	439.9	258.5	410.3	451.1	255.2	404.8	530	221.7	399.1	385.8	**IC 50 value**
181.1-674.7	259.7-667.1	426-899.2	177.3-1114.1	283.2-719.2	391.9-876.2	166.1-906.2	244.3-623.2	383.6-861.1	156.8-1237.1	403.2-896.1	504.1-1112.1	184.8-739.1	200.3-1027.5	414.2-899.2	181.8-906.1	372.2-851.3	426-899.2	173.7-1593.8	350.1-801.3	425.2=1016.2	158.6-753.8	298.1-724.6	289.1-701.3	**Limits 95%**
450.5	579.4	728.9	501.7	674.6	709.1	486.1	638.1	726.7	504.1	926.04	1192.6	456.2	464.8	715.8	482.3	746.9	728.9	523.9	754	920.1	470.6	787.4	662.4	**IC 90 value**
299.1-1422.4	457.2-3701	479.3-4425.4	313.6-2651.4	418.1-3794	477.2-4424.2	306.6-2317.5	411.2-3009.7	477.2-4424.2	308.7-360.1	626.2-5197.2	687.1-8824.3	300.7-1529.2	301.5-1922.4	461.5-4421.7	308.7-2008.9	493.2-477.2	479.3-4425.4	318.8-4064.7	498.6-4442.1	549.2-8131.1	300.2-2012.5	513.1-4125.2	394.2-4013.1	**Limits 95%**
0.093	0.090	0.225	0.222	0.236	0.225	0.214	0.019	0.155	0.134	0.236	0.430	0.442	0.823	0.488	0.300	0.154	0.225	0.113	0.103	0.135	0.240	0.124	0.205	**X2**
0.999	0.999	0.994	0.994	0.994	0.994	0.995	1	0.997	0.998	0.994	0.980	0.979	0.935	0.975	0.990	0.997	0.994	0.998	0.999	0.998	0.993	0.998	0.995	**P value**
Y=-1.06+6.42E-3*X	Y=-1.83+5.35E-3*X	Y=-2.1+4.76E-3*X	Y=-1.35+5.35E-3*X	Y=-1.57+4.27E-3*X	Y=-1.99+4.82E-3*X	Y=-1.22+5.23E-3*X	Y=-1.37+4.18E-3*X	Y=-1.78+4.24E-3*X	Y=-1.04+4.64E-3*X	Y=-1.14+2.68E-3*X	Y=-1.6+2.62E-3*X	Y=-1.7+6.84E-3*X	Y=-2.09+7.54E-3*X	Y=-2.08+4.99E-3*X	Y=-1.5+5.95E-3*X	Y=-1.57+3.84E-3*X	Y=-2.1+4.76E-3*X	Y=-1.22+4.83E-3*X	Y=-1.49+3.73E-3*X	Y=-1.75+3.32E-3*X	Y=-1.15+5.21E-3*X	Y=-1.32+3.37E-3*X	Y=-1.8+4.7E-3*X	**Regression equation**

Table 2: LC_{50} and LC_{90} values of crude metabolites for fungus *Cl. herberum* with 10 days incubation period for the four larval stages of the two mosquito species.

An.stephensi												*C x. p ipiens*												IC
4I			3			2I			1I			4I			3			2I			1I			
72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	
255.9	385.7	**439.9**	249.9	370.1	429.1	235.7	328.8	421.8	**221.7**	358.8	**399.1**	**258.5**	441.2	551.1	257.3	410.3	551.1	255.2	404.8	530	225.5	403.3	457.1	IC 50 value
177.3-1114.1	200.3-1027.5	414.2-899.2	181.1-674.7	283.2-719.2	391.9-876.2	166.1-906.2	244.3-623.2	383.6-861.1	158.6-753.8	289.1-701.3	298.1-724.6	181.8-906.1	259.7-667.1	426-899.2	184.8-739.1	372.2-851.3	426-899.2	173.7-1593.8	350.1-801.3	425.2=1016.2	156.8-1237.1	403.2-896.1	404.1-1112.1	Limits 95%
501.7	**464.8**	**715.8**	450.5	674.6	709.1	486.1	638.1	726.7	**470.6**	**662.4**	**787.4**	**482.3**	579.4	728.9	456.2	746.9	728.9	523.9	754	920.1	504.1	926.04	1192.6	IC 90 value
313.6-2651.4	301.5-1922.4	461.5-4421.7	299.1-1422.4	418.1-3794	477.2-4424.2	306.6-2317.5	411.2-3009.7	477.2-4424.2	300.2-2012.5	394.2-4013.1	513.1-4125.2	308.7-2008.9	457.2-3701	479.3-4425.4	300.7-1529.2	493.2-477.2	479.3-4425.4	318.8-4064.7	498.6-4442.1	549.2-8131.1	308.7-360.1	626.2-5197.2	687.1-8824.3	Limits 95%
0.222	**0.823**	**0.488**	0.093	0.236	0.225	0.214	0.019	0.155	**0.240**	**0.205**	**0.124**	**0.300**	0.090	0.225	0.442	0.154	0.225	0.113	0.103	0.135	0.134	0.236	0.330	X2
0.994	**0.935**	**0.975**	0.999	0.994	0.994	0.995	1	0.997	**0.993**	**0.995**	**0.998**	**0.990**	0.999	0.994	0.979	0.997	0.994	0.998	0.999	0.998	0.998	0.994	0.980	P value
Y=-1.35+5.35E-3*X	Y=-2.09+7.54E-3*X	Y=-2.08+4.99E-3*X	Y=-1.06+6.42E-3*X	Y=-1.57+4.27E-3*X	Y=-1.99+4.82E-3*X	Y=-1.22+5.23E-3*X	Y=-1.37+4.18E-3*X	Y=-1.78+4.24E-3*X	Y=-1.15+5.21E-3*X	Y=-1.8+4.7E-3*X	Y=-1.32+3.37E-3*X	Y=-1.5+5.95E-3*X	Y=-1.83+5.35E-3*X	Y=-2.1+4.76E-3*X	Y=-1.7+6.84E-3*X	Y=-1.57+3.84E-3*X	Y=-2.1+4.76E-3*X	Y=-1.22+4.83E-3*X	Y=-1.49+3.73E-3*X	Y=-1.75+3.32E-3*X	Y=-1.04+4.64E-3*X	Y=-1.14+2.68E-3*X	Y=-1.6+2.62E-3*X	AlanhdequationR

Table 3: LC_{50} and LC_{90} values of crude metabolites for fungus *Cl. herberum* with 14 days incubation period for the four larval stages of the two mosquito species.

An.stephensi												*C x. pipiens*												IC
4I			3			2I			1I			4I			r3			2I			1I			
72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	
246.9	**361.6**	**489.5**	**228.9**	**452.3**	432.5	212.5	**297.8**	451.7	**201.9**	**253.7**	**447.4**	257	374.1	543.6	235.9	360.1	533.7	221	330.9	**513.3**	202.2	330	**453.1**	IC_{50} value
175.3-812.1	206.4-3778.6	296.2-731.3	166.6-626.4	254.3-662.4	397.3-889.1	151.6-799.7	197.4-2330.1	429.2-899.7	145.6-695.7	236.4-765.2	435.1-913.2	175.6-1438	297.2-747.5	497.1-1092.3	163.3-1249.6	277.2-697.3	401-893.3	159.1-696	261.1-571.2	499.1-1098	140.9-1654.1	433.3-907.6	412.3-998.2	Limits 95%
474.8	552.3	**641.5**	**449.3**	**606.9**	760.3	474.8	**543.1**	825.6	**463.6**	748.1	**850.5**	518.7	649.9	1092.1	506.9	647.8	743.4	463.6	631.2	1165.1	505.4	1041.2	959.3	IC_{90} value
305.5-1874.6	329.8-7722.2	393.2-3914.4	295.9-1485.2	478.6-3768	476.1-4423.2	299.3-2317.6	328-5029.5	523.1-5002.7	294.4-2103.5	534.2-5122.6	598.8-5163.1	317.9-3574.5	409.1-3821	614.3-8738.2	311.4-3416.2	496.2-3921	578.3-5012.1	298.5-1821.9	387.4-4665	656.2-8791.7	303.5-5866.2	707.3-7312.5	570.2-8312.1	Limits 95%
0.232	0.074	**0.265**	**0.402**	**0.338**	0.160	0.210	**0.032**	0.114	**0.125**	**0.247**	**0.102**	0.415	0.087	0.227	0.344	0.076	0.120	0.071	0.172	0.134	0.107	0.063	0.097	X2
0.994	0.999	**0.992**	**0.982**	**0.987**	0.997	0.995	**1**	0.998	**0.998**	**0.993**	**0.999**	0.981	0.999	0.994	0.987	0.999	0.998	0.999	0.997	0.998	0.999	1	0.999	P value
Y=-**1.56**+4.**E-65**3*X	Y=-1.74+5.59E-3*X	Y=-0.99+6.02E-3*X	Y=-1.55+5.05E-3*X	Y=-1.56+3.13E-3*X	Y=-1.7+4E-3*X	Y=-1.04+4.93E-3*X	Y=-1.66+5.23E-3*X	Y=-1.55+3.44E-3*X	Y=-2.01+4.88E-3*X	Y=-1.74+3.33E-3*X	Y=-1.4+3.29E-3*X	Y=-1.27+4.96E-3*X	Y=-1.16+5.36E-3*X	Y=-1.64+2.8E-3*X	Y=-1.13+4.82E-3*X	Y=-1.6+4.42E-3*X	Y=-1.17+5.31E-3*X	Y=-2.11+5.23E-3*X	Y=-1.4+4.2E-3*X	Y=-1.35+2.37E-3*X	Y=-0.86+4.25E-3*X	Y=-1.01+2.2E-3*X	Y=-1.48+2.9E-3*X	Regression equation

Table 4: LC_{50} and LC_{90} values of crude metabolites for fungus *Cl. herberum* with 21 days incubation period for the four larval stages of the two mosquito species.

An.stephensi												*Cx . pipiens*												IC
4I			3			2I			1I			4I			3			2I			1I			
72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	
138.4	161.3	184.9	125.2	145.2	187.2	100.9	**145.1**	163.1	72	113.1	143.7	145.6	204.4	211.6	133.3	152.6	199.6	113.6	145.9	190.5	79.7	135.2	152.2	IC_{50} value
114.4-192.5	128.9-264.4	146.7-310.1	102.9-171.8	118.6-215.1	147.1-329.3	80.8-128.2	120.3-203.6	131.9-254	46.2-90.9	58.8-175.3	116.7-216.2	121.1-203.2	157.2-394.2	156.1-541.6	110.2-184.8	124.4-229.1	154.4-375.2	93.3-148.3	118.2-222.8	146-383.7	56.5-99.3	109.4-201.9	124.3-227.5	Limits 95%
298.1	347.1	357.8	292.1	317.9	368.6	257.2	**302.1**	334.9	219.2	330.2	322.1	300	391.2	436.3	296.4	323.2	385.4	272.2	326.7	395.3	227.9	319.3	321.5	IC_{90} value
227.6-503.6	251.3-702.7	260.3-708.8	221.9-502.8	236.9-581.3	264.3-773	200.7-410.4	230.7-508.7	247.5-628.5	175.2-329.7	233.2-771.1	238.1-607.1	230.1-499.5	275-892.9	289.3-1377.3	225.7-505.4	240.7-590.5	272.3-859.2	210.6-443.5	240.4-624.1	273.4-977.2	181.4-345.4	235-612.2	239.9-584.4	Limits 95%
0.233	0.377	0.486	0.014	0.430	0.084	0.221	**0.465**	0.057	0.147	0.434	0.463	0.285	0.320	0.440	0.013	0.252	0.168	0.242	0.310	0.193	0.152	0.203	1.080	X2
0.994	0.984	0.975	1	0.980	0.999	0.994	**0.997**	1	0.997	0.980	0.977	0.991	0.989	0.979	1	0.993	0.997	0.993	0.989	0.996	0.997	0.995	0.897	P value
Y=-1.12+8.09E-3*X	Y=-1.12+6.93E-3*X	Y=-1.36+7.31E-3*X	Y=-0.96+7.67E-3*X	Y=-1.09+7.15E-3*X	Y=-1.32+7.05E-3*X	Y=-0.83+8.2E-3*X	Y=-1.19+8.26E-3*X	Y=-1.22+7.47E-3*X	Y=-0.63+8.7E-3*X	Y=-0.67+5.92E-3*X	Y=-1.05+7.3E-3*X	Y=-1.22+8.39E-3*X	Y=-1.4+6.81E-3*X	Y=-1.22+5.87E-3*X	Y=-1.05+7.85E-3*X	Y=-1.15+7.52E-3*X	Y=-1.37+6.81E-3*X	Y=-0.91+8.05E-3*X	Y=-1.03+7.07E-3*X	Y=-1.2+6.29E-3*X	Y=-0.69+8.64E-3*X	Y=-0.94+6.95E-3*X	Y=-1.17+7.75E-3*X	Regression equation

Table 5: LC_{50} and LC_{90} values of crude metabolites for fungus *Cl. herberum* with 28 days incubation period for the four larval stages of the two mosquito species.

An.stephensi												*C x. pipiens*												IC
4I			**3**			**2I**			**1I**			**4I**			**13**			**2I**			**1I**			
72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	72	48	24	
152.7	185.3	230.6	136.9	172.1	208.6	109.1	158.3	176	81.3	120.1	159.9	160.4	209.7	260.6	149.2	180.2	214.1	123.5	180.4	181.6	88.7	153.9	173.5	IC_{50} value
123.2-238.1	141.1-392.4	167.3-642.5	112.2-196.8	134-320.3	158.2-435.9	89.3-139.8	122.5-308.8	141.2-283	59.8-100.2	94.5-180.8	129-251.7	129.7-249.8	154.3-552.6	179.3-1226.1	121.3-225.5	140.6-330.6	162.8-436.5	101.5-168	133.2-517.1	145.9-290.4	69-109	118.7-305.8	138.1-289.1	Limits 95%
333.2	402.1	453.9	308.4	376.2	406.7	263.8	380.5	345.5	222.9	321.4	336.7	334.2	440.1	509.1	324.4	375.5	402.6	289.1	437.2	344.6	230.5	382	353.6	IC_{90} value
244.3-644.4	274.3-1071.1	297.9-1527.2	231.3-552.1	263.4-879.9	280.6-1015.4	205.8-420.3	261.3-990.5	254.1-658.9	179.3-328.4	231.9-675.7	247.3-647.1	246.5-631.3	290.1-1442.6	316.1-2902.9	240.3-604.5	265.1-844.4	280.1-967.3	220.2-493.2	282.7-1707.5	254.7-645.2	184.9-341.2	260.6-1038.1	256.4-710.6	Limits 95%
0.171	0.143	0.049	0.077	0.025	0.138	0.226	0.087	0.022	0.144	1.346	0.422	0.212	0.236	0.145	0.267	0.029	0.039	0.112	0.087	0.204	0.173	0.189	0.108	X2
0.997	0.998	1	0.999	1	0.998	0.994	0.999	1	0.998	0.854	0.981	0.995	0.994	0.997	0.992	1	1	0.998	0.999	0.995	0.996	0.996	0.999	P value
Y=-1.09+7.61E-3*X	Y=-1.1+5.9E-3*X	Y=-1.32+5.69E-3*X	Y=-1.03+7.4E-3*X	Y=-1.08+6.2E-3*X	Y=-1.35+6.42E-3*X	Y=-0.9+8.27E-3*X	Y=-0.91+5.78E-3*X	Y=-1.33+7.561E-3*X	Y=-0.74+9.04E-3*X	Y=-0.77+6.44E-3*X	Y=-1.17+7.38E-3*X	Y=-1.19+7.46E-3*X	Y=-1.17+5.6E-3*X	Y=-1.35+5.2E-3*X	Y=-1.1+7.3E-3*X	Y=-1.18+6.58E-3*X	Y=-1.46+6.83E-3*X	Y=-0.96+7.78E-3*X	Y=-0.9+4.986E-3*X	Y=-1.44+7.97E-3*X	Y=-0.8+9.04E-3*X	Y=-0.87+5.65E-3*X	Y=-1.24+7.13E-3*X	Regression equation

An.stephensi table 1, 2, 3, 4, 5. The present results agreed with the findings of Grove and Pople, (1980) who used 20 g of the crude extract of Beauvercin toxin produced by *B. bassiana* against the larvae of *Ae. aegypti*, resulting in 86% mortality. Weiser and Matha, (1988) achieved a 100% mortality rate using a crude extract of Tolypin produced from *Tolypocladiumniveum* at a concentration of 100 mg / ml against the *Cx. pipiens*. Vivekanandhan *et al*., (2018) mentioned fungus leaching of *Fusarium oxysporium* affected the larvae of my *An. stephensi* and *Cx. quinquefasciatus*, the value of LC_{50} (109.24, 320.30) mg/ml for the two mosquitoes, respectively, after 24 hours of treatment.

Conclusion

The secondary metabolites of fungus *Cl. Herbarum* was a high effect on different larval stage mosquitoes *Cx. pipiens* and *An stephensi*, this metabolites can use as insecticide against other types of insects .

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