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## RESEARCH ARTICLE

# EVALUATION OF SOME PLANT EXTRACTS IN DIFFERENT FORMULAS OF ECOFRIENDLY CHITOSAN-BASED COMPOSITES AGAINST *CULEXPIPIENS* AND *MUSCADOMESTICA* LARVAE

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**ABSTRACT**

*Achilleafragrantissima* and *Cleome droserifolia* crude extracts were blended with suitable eco-friendly polymeric materials (Chitosan, starch, glycerol and glutaraldehyde). Theseformulas have been characterized and their insecticidal activity was evaluated against *Culexpipiens* and *Muscadomestica*larvae.The series of concentrations from Chitosan and starch were mixed with glycerol and glutaralehyde for producing M1 and M2 respectively. The potency of each extract was decreased while decreasing the chitosan material. The formula which containing glutaralehyde showed more potency than the formula contained glycerol. The temporal effect of mixtures number 4 and 5 revealed that the effect of mixtures continues for more than 15 days against *Culexpipiens* while their effect is almost stopped after 6 days in case of *Muscadomestica*. Also, the formula which contained the glutaraldehyde was more persistent during application than the other formula.



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## INTRODUCTION

Carbohydrate Polymers covers the study and exploitation of the industrial applications of carbohydrate polymers in areas such as food, textiles, paper, wood, adhesives, pharmaceuticals, oil field applications and industrial chemistry. Carbohydrate polymer (Chitin, chitosan, starch, glycerol and glutaraldehyde) composited with extracted compound from nature product are biopolymers having immense structural possibilities for chemical and mechanical modifications to generate novel properties, functions and applications especially in insect control and biomedical area. Chitin and chitosan are effective materials for biomedical applications because of their biocompatibility, biodegradability and non-toxicity, apart from their antimicrobial activity and low immunogenicity, which clearly points to an immense potential for future development (Abdul Khalil, *et al.* 2012).

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These candidate biopolymers can be easily processed into gels, sponges, membranes, beads and scaffolds forms.It is already known also that the high polymers containing functional groups have attracted much attention since the beginning of the polymer chemistry on both academic and commercial levels. Also numerous natural or naturally occurring polymers such as cellulose, starch, Chitin and alginate have been chemically modified either through introduction of new functionalities or through chemical transformation of the already present functional groups. Such chemical modifications were aiming to modify their mechanical and/or physical properties of polymers to be suitable for certain applications (Long, *et al*., 2006, Abdelaal and Mohamed 2013, Abdelaal, *et al.*, 2014 and Barikani, *et al.*, 2014). Some insects (*Culexpipiens* and *Muscadomestica*) transmit serious human and animal diseases, causing millions of deaths every year Among these diseases, yellow fever, malaria, filariasis, dengue and dengue hemorrhagic fever, bacterial diseases, *Muscadomestica* salivary gland hypertrophy virus (MdSGHV) has a worldwide distribution and Rift Valley fever at endemic and epidemic

areas in many countries (WHO, 1991, Lerdthusnee, *et al.*1995, Barin, *et al.,* 2010 and Lietze, *et al.,* 2012). Many authors around the world said that plants may be alternative sources of insect control agents (Attia, 2002, Kamel, *et al*., 2005b, Pavela, 2009, El-Maghraby, *et al*., 2012 and Eldiasty, *et al*., 2014).They do many efforts to improve the potency and application of plant extracts as insecticidal agents.

## MATERIALS AND METHODS

### Tested insects

### Laboratory maintenance of the tested mosquitoes *Culexpipiens*

Mosquitoes were maintained in a walk-insectaries under controlled conditions of temperature (27 ± 2 ºC), relative humidity, R.H. (70%-80%) and light - dark period (16: 8 hrs.) under a fluorescent light. Larvae of the tested mosquito species were reared in white enamel pans (35-40 cm diameter and 10 cm depth) containing about 1.5 L of de-chlorinated tap water. Larvae were provided with tetra-amine (tropical fish food) sprinkled twice daily over the water surface of the breeding pans. The water containing larvae was gently transferred every 2 days into clean enamel pans to avoid formation of scum on the water surface or on the walls and bottoms of pans. The breeding water was gently aerated for about 5 minutes every day by means of a small air pump. Developed pupa were collected and transferred daily to plastic cups containing saline water then introduced into the breeding screened wooden cages (30x30x30 $cm^3$). Emerged adults were fed on 10% sugar solution. After three days adults were fed on blood to lay egg batches were transferred to the white enamel pans containing de-chlorinated tap water for hatching. When mosquito larvae developed to the $2^{nd}$ instars, they were poured into clean pans and observed daily. Late third larval instars were used for toxicological studies as described previously for *Culexpipiens* (Kamel, *et al.,* 2005a).

### Laboratory maintenance of the tested house flies *Muscadomestica*

Larvae of house fly can be reared in a gallon plastic container with a cloth top. The container was filled with 3-4 inches of shredded paper or wood chips (cedar, redwood, or pine were avoided as they contain insecticidal chemicals). A cup of powdered milk was mixed with 2 cups of water and poured over the wood or paper. The wood/paper should be thoroughly wet while they are about 0.5 inch above the milk level. At 25 ºC - 32 ºC the larvae are ready to pupate in about five to six days. It is best to keep the container in the dark if the larvae are to be observed, as they will crawl away into the center of the medium because of the light. The culture was checked daily and the larvae are ready to pupate when they are crawling on the sides of the container. To collect the pupae, the container of the larvae was transferred to a shallow pan. The medium containing the larvae was spread so it is within 1 inch of the top of the pan. Wetting the medium thoroughly with no water standing in the pan the larvae will be driven out of the pan. The larvae can be collected by placing the small pan containing the larvae and medium inside a larger pan with paper toweling along the bottom of the large pan. Using two paper towel or toilet paper tubes support the smaller pan above the paper toweling. The larvae will crawl out of the inner pan and pupate under the paper toweling in the dry outer pan. Collect the pupae and place them in a well-ventilated cage to await adult emergence. Larvae will eat the paper/wood/milk medium throughout their larval development. Adult flies are fed on a 1:1 mixture of granulated sugar and powdered milk. A bowl filled with wood chips and water serves as a source of water (Sawicki and Holbrook, 1961).

### Tested compounds

The tested plants were washed to remove dusts and dirt then left to dry under shade in the laboratory. Dried plant (whole plant) was cut into small pieces and ground in an electric grinder. Hundred grams of the resulting powdered materials of each plant were exhaustively extracted with absolute ethanol by means of a Soxhlet apparatus. The solvent extracts of each plant were evaporated and dried under vacuum using a rotary evaporator at 60 ºC. The dry crude extracts were stored at 4 ºC in screw capped vials until use.

### Toxicological studies

Preliminary toxicological bioassay tests were carried out to the selected plant extracts on tested insects according to a cited method after modification (Wright, 1971). Evaluation of new compounds for *Muscadomestica* was carried out according to the method reported earlier (Rabea*et al.,* 2005).

### Different formula (M1 and M2) with the tested plant extracts

The prepared solutions of different formulations were mixed with the tested plant extract (*Achilleafragrantissima* and *Cleome drosrefolia*) and then the bioassay was carried out on the tested insect.

### Temporal effect of selected formula against *Culexpipiens* and *Muscadomestica* larvae

Series of experiments were carried out to determine the stability of the larvicidal activities of the selected polymers mixtures with plant extracts at $LC_{50}$ level on temporal bases. In this experiment stock solutions and stock beast from selected materials for mosquitoes and house fly respectively according method as described (Kamel*et al.*, 2005b).

### Statistical analysis

The data were statistically analyzed by Log Propit and Excel programs.

## RESULTS AND DISCUSSION

### Larvicidal activity of plant extracts against *Culexpipiens* larvae

The insecticidal activity of two ethanolic plant extracts was bioassayed against the $3^{rd}$ instars of the *Culexpipiens* larvae in the laboratory. The results are presented in Table (1).

The confidential limits of each of the tested plant extract were statistically calculated for $LC_{50}$ and $LC_{90}$ at P= 0.05.

The $LC_{50}$ values of the ethanolic extracts *Achilleafragrantissima* and *Cleomedroserifolia* are 82.15 and 150.27 ppm, respectively.

**Larvicidal activity of plant extracts against *Muscadomestica* larvae**

The insecticidal activity of two ethanolic plant extracts was bioassayed against the 3rd instars of the *Muscadomestica* larvae in the laboratory.

**Table 1.Larvicidal activity of some plants against *Culexpipiens* larvae**

| Plant | LC 50 (Co. Limits) | LC 95 (Co. Limits) | Slope Function |
|---|---|---|---|
| *Achilleafragrantissima* | 82.15 (72.71-92.82) | 237.6 (186.6 – 302.8) | 1.905 |
| *Cleome droserifolia* | 150.27 (114.2 -197.73) | 997.2 (539.9 – 1846.9) | 3.38 |

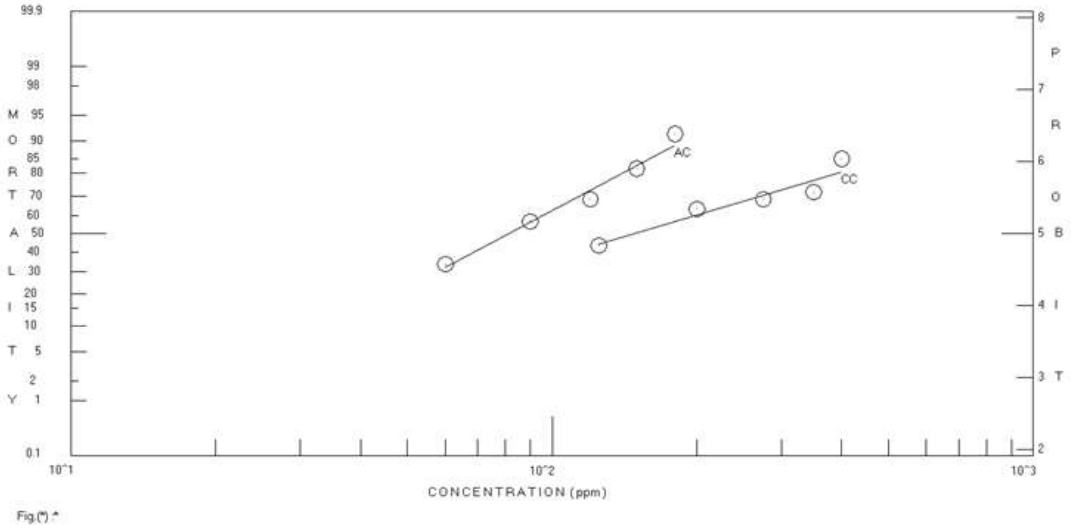


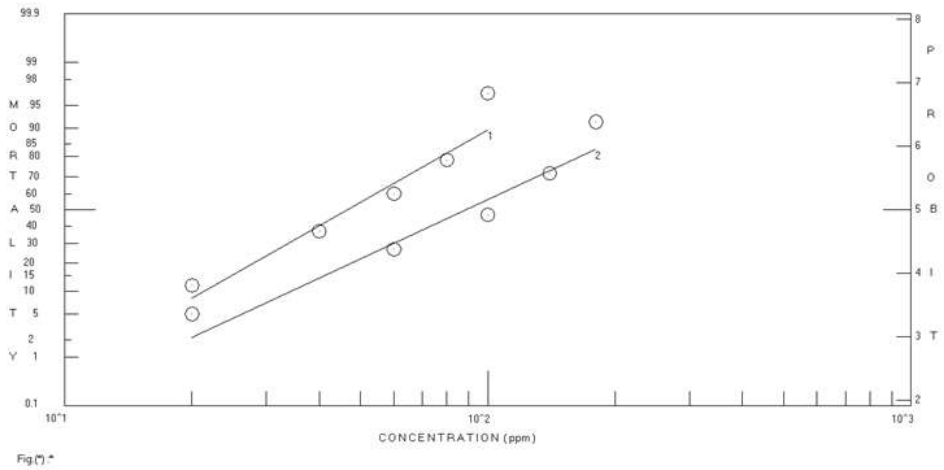

**Fig. 1. Susceptibility of *Culexpipiens*larvae to *Achilleafragrantissima* and *Cleome droserifolia* ethanolic extract**

AC = *Achilleafragrantissima* on *Culexpipiens*
CC = *Cleome droserifolia* on *Culexpipiens*

**Table 2.Larvicidal activity of some plant extracts against *Muscadomestica* larvae**

| Plant | LC 50 (Co. Limits) | LC 95 (Co. Limits) | Slope Function |
|---|---|---|---|
| *Achilleafragrantissima* | 46.61 (42 – 51.71) | 126.54 (103.43 – 155) | 3.8 |
| *Cleome droserifolia* | 89.02 (78.6 – 100.8) | 300.86 (227.69 – 398.18) | 3.1 |

The $LC_{50}$ values of the ethanolic extracts *Achilleafragrantissima* and *Cleomedroserifolia* are 46.61and 89.02ppm, respectively.




Where 1= *Achilleafragrantissima* against *Muscadomestica*
2= *Cleome droserifolia* against *Muscadomestica*

**Fig. 2. Susceptibility of *Muscadomestica*larvae to *Achilleafragrantissima*and *Cleome droserifolia* ethanolic extract**

The results are presented in Table (2) and Fig (2). The confidential limits of each of the tested plant extract were statistically calculated for $LC_{50}$ and $LC_{95}$ at P= 0.05.

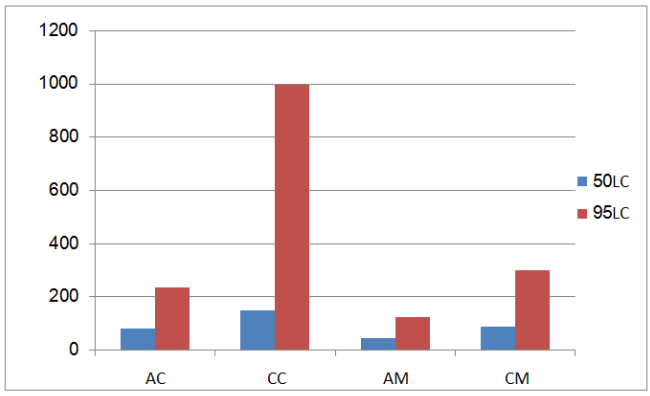



**Fig 3.Larvicidal activity of *Achilleafragrantissima* and *Cleome droserifolia* ethanolic extract against *Culexpipiens* and *Muscadomestica***

Where CM = *Cleome droserifolia* against *Muscadomestica*
AM = *Achilleafragrantissima* against *Muscadomestica*
CC = *Cleome droserifolia* against *Culexpipiens*
AC = *Achilleafragrantissima* against *Culexpipien*

**Evaluation of some plant extracts mixed with different formula of polymers against *Culexpipiens* and *Muscadomestica* larvae**

The serial of concentrations(M1 and M2) were tested against *Culexpipiens*and *Muscadomestica* larvae mixed with both extracts (*Achilleafragrantissima* and *Cleomedroserifolia*) at $LC_{50}$ level.The mixtures showed different degrees of potency represented in tables (3&4). The formula (M2) showed high potency than (M1) in different concentrations may be due to the presence of glutaraldehyde make synergism reaction with other component of mixtures (chitosan) than glycerol in (M1).

This result was agree with the studies by Paramá *et al.*, 2005 who stated that, the cross-link between chitosan & glutaraldehyde was strongly toxic to *Philasteridesdicentrarchi* is a protozoan ciliate which causes significant economic lossesin fish aquaculture. The results showed also, the decrease of potency while decreasing of chitosan concentration in all mixtures it may be attributed to the lake of chitosan material which combine with other polymer materials to promote their potency.

**Table 3. The different formulations of M1**

| Formula No | Chitosan | Strach | Glycrol | *Achillea* | *Cleome* |
|---|---|---|---|---|---|
| 1 | 50 ml | 0 | ------ | + 0.3 ml | 0.5 ml |
| 2 | 40 | 10 | 0.12 | | |
| 3 | 30 | 20 | 0.12 | | |
| 4 | 20 | 30 | 0.12 | | |
| 5 | 10 | 40 | 0.12 | | |
| 6 | 0 | 50 | 0.12 | | |

**Table 4. The different formulations of M2**

| Formula No | Chitosan | Strach | Glutaraldehyde | *Achillea* | *Cleome* |
|---|---|---|---|---|---|
| 1 | 50 ml | 0 | 0.1 | + 0.3 ml | 0.5 ml |
| 2 | 40 | 10 | 0.1 | | |
| 3 | 30 | 20 | 0.1 | | |
| 4 | 20 | 30 | 0.1 | | |
| 5 | 10 | 40 | 0.1 | | |
| 6 | 0 | 50 | ------ | | |

These results were agree with that stated by Zhang and Tan, 2003; Rabea*et al.* 2005 and Badawy and El-Aswad, 2012 which they tested chitosan against lepidopterous and homopterous insects.Tinos, *et al*., 2010 stated that, the glycerol can be used as adjuvant to pesticides to increase the potency and decrease the amount of pesticides.Results in Tables (5 - 8) showed decrease in potency with increasing concentrations of polymer material (From no. 1 to no. 6 in both extracts). Thus, the author selects no. 4&5 to test their persisting effect in field after application.

**Table 5. Larvicidal activity of *Achilleafragrantissima* at $LC_{50}$ level mixed with different concentrations of M1 polymer**

| Mix No. | % Mortality *C. pipiens* ± SE | % Mortality *M.domestica* ± SE | Mix No. | % Mortality *C. pipiens* ± SE | % Mortality *M.domestica* ± SE |
|---|---|---|---|---|---|
| 1 | 100 ± 0.0 | 100 ± 0.0 | 4 | 44.4± 0.0 | 40.33 ± 0.0 |
| 2 | 100 ± 0.0 | 98.33 ± 0.0 | 5 | 10± 0.0 | 8.7 ± 0.0 |
| 3 | 75.86± 0.0 | 70 ± 0.0 | 6 | 0 ± 0.0 | 0 ± 0.0 |

**Table 6. Larvicidal activity of *Achilleafragrantissima* at $LC_{50}$ level mixed with different concentrations of M2 polymer:**

| Mix No. | % Mortality *C. pipiens* ± SE | % Mortality *M.domestica* ± SE | Mix No. | % Mortality *C. pipiens* ± SE | % Mortality *M.domestica* ± SE |
|---|---|---|---|---|---|
| 1 | 100 ± 0.0 | 100 ± 0.0 | 4 | 70.37± 0.0 | 66.33 ± 0.0 |
| 2 | 100 ± 0.0 | 99.33 ± 0.0 | 5 | 13.33± 0.0 | 10 ± 0.0 |
| 3 | 86.67± 0.0 | 85 ± 0.0 | 6 | 6.67 ± 0.0 | 6 ± 0.0 |

**Table 7. Larvicidal activity of *Cleome droserifolia* at $LC_{50}$ level mixed with different concentrations of M1 polymer**

| Mix No. | % Mortality *C. pipiens* ± SE | % Mortality *M.domestica* ± SE | Mix No. | % Mortality *C. pipiens* ± SE | % Mortality *M.domestica* ± SE |
|---|---|---|---|---|---|
| 1 | NT | NT | 4 | 86.67± 0.0 | 84.33 ± 0.0 |
| 2 | 100 ± 0.0 | 98.67 ± 0.0 | 5 | 46.67± 0.0 | 44.7 ± 0.0 |
| 3 | 100 ± 0.0 | 97.6 ± 0.0 | 6 | NT | NT |

*NT = Not Tested

**Table 8. Larvicidal activity of *Cleome droserifolia* at $LC_{50}$ level mixed with different concentrations of M2 polymer**

| Mix No. | % Mortality *C. pipiens* ± SE | % Mortality *M.domestica* ± SE | Mix No. | % Mortality *C. pipiens* ± SE | % Mortality *M.domestica* ± SE |
|---|---|---|---|---|---|
| 1 | 100 ± 0.0 | 100 ± 0.0 | 4 | 93.67± 0.0 | 92.33 ± 0.0 |
| 2 | 100 ± 0.0 | 99.67 ± 0.0 | 5 | 63.33± 0.0 | 60 ± 0.0 |
| 3 | 96.67± 0.0 | 95.3 ± 0.0 | 6 | 23.33 ± 0.0 | 21.67 ± 0.0 |

**Table 9. Temporal effect on larvicidal activities of the selected polymer mixtures mixed with *Achilleafragrantissima* against *Culexpipiens***

| Time | Mean Percentage mortality of*Culexpipiens*larvae treated at the $LC_{50}$ level of*Achilleafragrantissima* mixed with selected polymer mixtures ± S. D. | | | |
|---|---|---|---|---|
| | *Achilleafragrantissima* 4 M1 | *Achilleafragrantissima* 4M2 | *Achilleafragrantissima* 5M1 | *Achilleafragrantissima* 5M2 |
| 48 hrs. | 50±0.0 | 50±0.0 | 50±0.0 | 50±0.0 |
| 96 hrs. | 50±0.0 | 50±0.0 | 50±0.0 | 50±0.0 |
| 144 hrs. | 50±0.0 | 50±0.0 | 50±0.0 | 50±0.0 |
| 192 hrs. | 50±0.0 | 50±0.0 | 50±0.0 | 50±0.0 |
| 240 hrs. | 50±0.0 | 50±0.0 | 50±0.0 | 50±0.0 |
| 288 hrs | 50±0.0 | 50±0.0 | 50±0.0 | 50±0.0 |
| 336hrs | 50±0.0 | 50±0.0 | 50±0.0 | 50±0.0 |
| 384hrs | 45±1.0 | 46±1.0 | 40.7±0.6 | 42.7±0.6 |

**Table 10. Temporal effect on larvicidal activities of the selectedpolymer mixtures mixed with *Achilleafragrantissima* against *Muscadomestica***

| Time | Mean Percentage mortality of *Muscadomestica* larvae treated at the $LC_{50}$ level of *Achilleafragrantissima* mixed with selected polymer mixtures ± S. D. | | | |
|---|---|---|---|---|
| | *Achilleafragrantissima* 4M1 | *Achilleafragrantissima* 4M2 | *Achilleafragrantissima* 5M1 | *Achilleafragrantissima* 5M2 |
| 48 hrs. | 50±0.0 | 50±0.0 | 50±0.0 | 50±0.0 |
| 96 hrs. | 48.9±0.4 | 49. 7±0.3 | 48.9±0.2 | 48.5±0.5 |
| 144 hrs. | 30.2±0.2 | 32.3±0.4 | 28.1±0.3 | 30.5±0.5 |
| 192 | 0.0 | 0.0 | 0.0 | 0.0 |

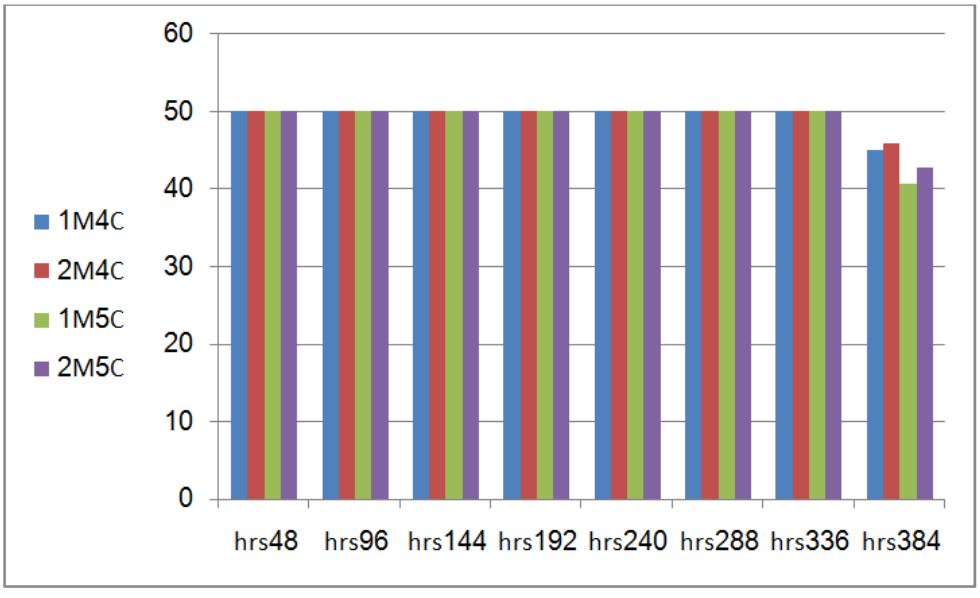



**Fig. 4. Temporal variation in percentage mortality of Culexpipiens larvae treated with selected polymers mixtures**

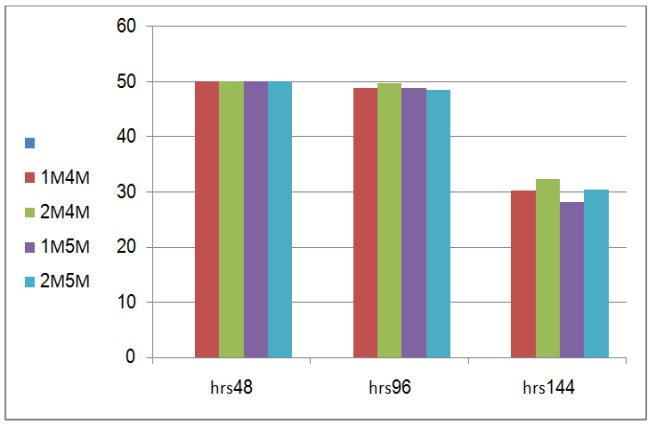



**Fig 5. Temporal variation in percentage mortality of *Muscadomestica* larvae treated with selected polymers mixtures**

**Temporal effect on larvicidal activities of the selected polymer mixtures mixed with *Achilleafragrantissima* against *Culexpipiens* and *Muscadomestica* larvae**

The purpose of this study was to determine the stability of the larvicidal activities of the selected polymer mixtures mixed with ethanolic extract of *Achilleafragrantissima* at LC50 level on temporal bases. Selection of these mixtures based on increasing potency of extract by how long it persistent in the field. The obtained results revealed differences in stability at LC50 of the selected mixtures against *Culexpipiens* & *Muscadomestica* Tables (9 - 10) & Figs. (4 - 5). The results show the effect of mixtures continues for more than 15 days against *Culexpipiens* though their effect is stopped after 6 days in case of *Muscadomestica*. Mixtures 4M2 and 5M2 are the most persistent formula in the field.

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