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## RESEARCH ARTICLE

# LARVICIDAL AND ADULTICIDAL EFFECT OF RICE STRAW EXTRACT AGAINST CULEXPIPIENS (DIPTERA: CULICIDAE)

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**ABSTRACT**

Mosquitoes constitute a major public health problem as a vector of serious human and animal diseases which causes millions of illnesses cases, and deaths. In present study the author evaluate an industrial waste product from natural origin as larvaicide and adulticide agents on mosquito, Culexpipiens. Black liquor showed potency as larvicideand its potency was increased when mixed with the emulsifier in different ratio. The LC50 and LC95 for Black liquor alone, Black liquor with emulsifier 1:1 and 1:2 were 3944.6 and5766.5, 2886.7 and 4426.6 and 2184.1 and 3985.6 respectively. Also, the waste material has adulticidal activity and its potency increased by adding the emulsifier material by ratio 1:1. Larval treatment by Black liquor and Deltamethrin showed highly significant reduction in the percentage of pupation and adultemergence. The treated larvae with Black liquor had no gonotrophic cycle because the female emerged not feeding on blood. Finally the Black liquor which release during processing the paper from Rice straw consider promising larvicidal and adulticidal agents against Culexpipiens (Diptera: Culicidae) and can be included in IVM program.



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## INTRODUCTION

Mosquitoes constitute a major public health problem as a vector of serious human diseases (Hasaballah, 2015), some of which causes millions of illnesses cases, and deaths for both humans and animals every year. Among those diseases; dengue fever, yellow fever, malaria, lymphatic filariasis, Japanese encephalitis and/or other serious diseases of humans and animals. In Egypt, genus *Culex* has a very wide distribution and is considered as the main vector of Rift valley fever, *Wuchereria bancrofti* (Darwish and Hoogstraal, 1981) and the Western Nile virus (El-Bahnasawy *et al.*, 2013). Among mosquito species, *Culexpipiens* is the most common and is widely distributed along Egypt, and has been incriminated as the main vector of bancroftian filariasis. The extensive use of chemical pesticides or insecticides resulted in inducing resistance by insect pests besides residue contamination of human food, mammalian toxicity, reducing beneficial non-target biota and environmental pollution. These factors have created the need for environmentally safe and target specific agents for pest control purposes. Since the beginning of the 20th century, plants with significant insecticidal effects have been considered a new source of pesticides (Mulla, 1997; Attiaa, 2002 and Kamel *et al*., 2005 a and b) probably with negative effects on the environment and pest resistance after applications. The use of surfactant or emulsifier materials increased the potency of insecticides and plant extracts (eg. Tween 80 and Triton 100) (Bakr, 1995; Hussein, *et al*., 2005 and Kamel, *et al*., 2005a). Someauthors using waste materials from agriculture and plastic waste (after converted to non-carcinogenic material) to decrease the cost of production of natural insecticides and convert the wastematerial to benefit one (Bakr *et al*., 2008, El-Maghraby *et al.,* 2012, Fahim, *et al.,* 2013 and Eldiasty, *et al.*, 2014). In present study the authors try to improve the potency of waste natural extract for using as larvicide or adulticide.

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## MATERIALS AND METHODS

### Tested insect

*Culexpipiens* (*Culicidae: Diptera*).

Larvae of *Culexpipiens*provided from Medical Entomology Institute and transferred to the laboratory of Entomology

Department – Faculty of Science – Ain Shams University where self-perpetuating colonies were established and maintained during the present study according to the method described by Kamel *et al.* (2005a). Late third larval instars wereused for toxicological studies.

**Tested compound**

**Black liquor**

Black liquor produced from the paper production industry but in new way patent No., (422 / 2008). Photo degradation of black liquor was carried out by using hydrogen peroxide in alkaline medium. Ethoxylated fatty acids as emulsifier prepared from chicken fat. Provided by Prof. DrGalalNawwar Green chemistry Department – NRC

**Larvicidal assay**

Efficiency of waste extract:Preliminary, toxicological bioassaytests were carried out to the selected plantextracts on tested insects as a modificationfor the method described by (Wright, 1971and Kamel *et al*., 2005b) their LC50 and LC95values were determined as well as their slopefunction, according to (Finney, 1971 and WHO, 2006).

**Adulticidalassay**

*Culexpepiens* fresh adults were exposed to filter paper treated with 0.25–2% M. azedarach leaf extract.

The paper was kept inside the beaker. Muslin cloth covering the beaker was also treated. Control insects were exposed only to methanol treated paper and muslin cloth. Mortality count was taken after 24 h (Bekele *et al.,* 2014). This method of adulticid assay was modifying by replacing the azedarach leaf extract by black liquor which introduced for present study.

**Synergistic action Ethoxylated fatty acids with Black liquor**

Black liquor was mixed with ethoxylated fatty acids (emulsifier)with the same concentration (10 %) at the ratio of 1:1 and 1:2 by volume. Evaluation the efficiency of these mixtures for larvae and adults by methods stated before. Calculate the synergistic ratio according to (Kamel *et al*., 2005a).

**Effect of sub-lethal treatment of Black liquor and Deltamethrin on some biological activities of *Culexpipiens***

The experiment was carried out according the method stated by Eldiasty *et al*., 2014.

## RESULTS

It was thought appreciable to evaluate an industrial waste product from natural origin as larvaicide and adulticide agents on mosquito, *Culexpipiens*.

**Table 1. Larval mortality and $LC_{50}$ and $LC_{95}$ values for the *Cluexpipiens* larvae exposed to Black liquor and Black liquor mixed with ethoxylated fatty acid**

| Name of sub. | Conc. ppm | Mortality % | LC50 (Co. Limits) | LC95 (Co.Limits) | Slope Function | SR* | |
|---|---|---|---|---|---|---|---|
| | | | | | | LC50 | LC95 |
| Black liquor | 2500 | 2 | 3944.6 | 5766.5 | 10.0 | | |
| | 3500 | 28.8 | (3785.2 – 4110.6) | (5332.8 – 6235.5) | | | |
| | 4000 | 51.3 | | | | | |
| | 5000 | 84.6 | | | | | |
| | 5500 | 92.5 | | | | | |
| Black and Emulsifier ratio 1:1 | 2000 | 7.5 | 2886.7 | 4426.6 | 8.9 | 1.37 | 1.30 |
| | 2500 | 28.6 | (2746.1 – 3034.6) | (4019 – 4875.6) | | | |
| | 3500 | 77.2 | | | | | |
| | 4000 | 89.8 | | | | | |
| Black and Emulsifier ratio 1:2 | 1000 | 1.5 | 2184.1 | 3985.6 | 6.3 | 1.81 | 1.45 |
| | 2000 | 40.2 | (2030 – 2350) | (3437.5 – 4621.5) | | | |
| | 2500 | 64.6 | | | | | |
| | 3500 | 90.5 | | | | | |

*Synergistic Ratio

**Table 2. Efficiency of Black liquor and Deltamethrin on some biological activities of *Cluexpipiens* larvae**

| Contribution | Control | Black | Deltamethrin |
|---|---|---|---|
| Pupation % | 98.10 (775) | 66.67 | 51.67 |
| | | 16.053* | 20.719* |
| | | $2.598 \times 10^{-58}$** | 0.000** |
| Emergence % | 99.35 | 66.25 | 79.03 |
| | | 16.598* | 12.310* |
| | | $3.438 \times 10^{-62}$ | $3.961 \times 10^{-35}$** |
| Gonotrophic cycle | 3.00 ± .000+ | | 3.47 ± .516+ |
| Fecundity | 100.80 ± 4.296+ | | 91.27 ± 1.907+ |
| Hatchability | 89.33 ± 5.354+ | | 75.47 ± 1.995+ |
| (%) | (88.62) | | (82.69) |
| | | | 4.559* |
| | | | $2.569 \times 10^{-6}$** |

+ = Mean ± S. D. * **Z** = Test of proportion. **P = Probability.

**Evaluation the waste product as larvicidal agent against *Culexpipiens* larvae**

From Table (1) the results shown potency of Black liquor as larvicide and its potency were increased when mixed with the

emulsifier in different ratio. The $LC^{50}$ and $LC^{95}$ for Black liquor alone, Black liquor with emulsifier 1:1 and 1:2 were 3944.6, 5766.5, 4426.6, 2184.1 and 3985.6 respectively and the synergistic ratio for all mixtures more than one. Regression lines represent in Fig. (1) showed parallel lines with different degree of potency.

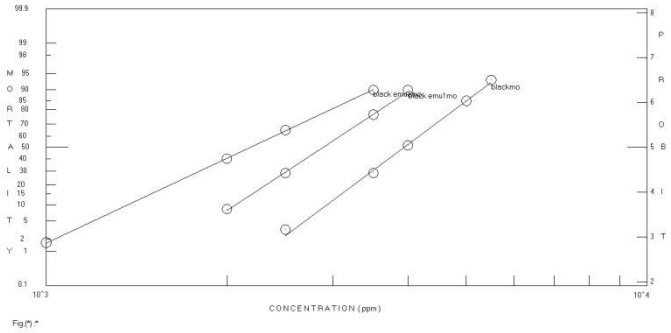


**Fig. 1. Susceptibility of *Culexpipiens* larvae toBlack liquor and Black liquor mixed with ethoxylated fatty acid**

### Effect of sub-lethal treatments of Black liquor and Deltamethrin on some biological activities of *Culexpipiens*

The results shown in Table (2) represent the pupation and adult emergence percent of Black liquor and Deltamethrin treatments. The percentage pupation from these larvae was reduced in comparison with that of the control group. Analyzing data with Z- test indicated that treatment by Black liquor and Deltamethrin showed highly significant reduction in the percentage of pupation. Estimation of adult emergence percentage is based on the ratio of the total number of emerged adults to the total number of pupae.

Statistically there are significant differences between all treatments and the control, where the percentage of adult emergence in larval treatment with Black liquor (66.25%, Z = 16.6, P = 3.44 x $10^{-62}$) and Deltamethrin (79.03%, Z = 12.31, P = 3.96 x $10^{-35}$) compared with control group (99.35 %) at P= 0.05.The length of Gonotrophic cycle of *Culexpipiens* is estimated as the number of days passed between female fed on blood after emergence and egg laying. Data presented in table (2) showed that the mean length of the gonotrophic cycle of the emerged females developing from treated larvae with Deltamethrin (3.47 days) was significantly longer than of those developed from the untreated larvae (3 days) (P = 0.05). While, the treated larvae with Black liquor had no gonotrophic cycle because the female emerged not feeding on blood.

### Evaluation the waste product as adulticidalagent against *Culexpipiens* larvae

Data from Table (3) Fig (2) clarify that the waste material has adulticidal activity and its potency increased by adding the emulsifier material by ratio 1:1.

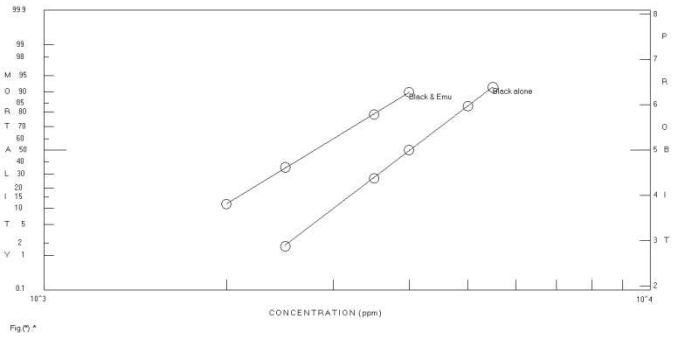


**Fig. 2. Susceptibility of *Culexpipiens*adults toBlack liquor and Black liquor mixed with Emulsifier**

## DISCUSSION

Mosquitoes are still the world wide vector of human and animal diseases, and are conspicuous nuisance pests as well, even after massive efforts of control. These factors have created the need for environmentally safe and target specific agents for pest control purposes.

Plant extracts have recently gained importance in insect control, being considered environmentally safe, less hazardous to non-target biota, simple, inexpensive and can be applied effectively by using techniques more suitable for developing countries. (Evans and Raj, 1991; Perich *et al.*, 1994; Soliman and El-Sherif, 1995; Macedo *et al.*, 1997;Messeha, 1997; Abahussain, 1999; Ansari *et al*., 2000; El-Kassas, 2001; Attiaa, 2002; Gusmäo *et al*., 2002, Mohamed *et al*., 2003, Bakr*et al*. 2006, Bakr *et al*., 2008, Habeeb *et al.* 2009,El-Maghraby *et al*. 2012 and Eldiasty *et al*. 2014). Active substances extracted from plants are used as insect repellents or synergists (Thangam and Kathiresan, 1997; El-Gougary, 1998 and Mansour *et al*., 2000), or effective as insect growth regulator and adulticide (Beehler and Mulla, 1993; Mc Carry, 1996, Kassem *et al*., 2000 and Nathan *et al.* 2006). Several investigators have reported reduction in fecundity and fertility of mosquitoes following treatment with plant extracts (Zebitz *et al*., 1987; Dhar *et al*., 1996; Reniprabha *et al*., 1999 and Mansour *et al*., 2000).

**Table 3. Adult mortality and $LC_{50}$ and $LC_{95}$ values for the *Cluexpipiens*adult exposed to Black liquor and Black liquor mixed with emulsifier 1:1**

| Name of sub. | Conc. ppm | Mortality % | LC50 (Co. Limits) | LC95 (Co.Limits) | Slope Function | SR* | |
|---|---|---|---|---|---|---|---|
| | | | | | | LC50 | LC95 |
| Black liquor | 2500 | 1.7 | 4018.2 | 5817.7 | 10.23 | | |
| | 3500 | 27.1 | (3859.8 – 4183) | (5353.8 – 6286.7) | | | |
| | 4000 | 49.7 | | | | | |
| | 5000 | 84 | | | | | |
| | 5500 | 92.3 | | | | | |
| Black and Emulsifier ratio 1:1 | 2000 | 12.1 | 2794.6 | 4446 | 8.16 | 1.41 | 1.30 |
| | 2500 | 34.8 | (2650.1 – 2947) | (4001 – 4940.8) | | | |
| | 3500 | 78.4 | | | | | |
| | 4000 | 89.4 | | | | | |

*Synergistic Ratio

In present study the use of waste products from natural origin to control mosquitoes may be decrease the pollution of environment and also decrease the coast of production for these alternative insecticides. The Black liquor material alone showed moderate larvicidal activity with slightly high slope function this result may be attributed to self-defense of each individual toward the applied material. The synergistic action resulted from mixing ethoxylated fatty acids and Black liquor with the same concentration at the ratio of 1:1 and 1:2 by volume (Black liquor: Ethoxylated fatty acids) showed considerable decrease in $LC_{50}$ values and low slope function of the tested mixtures. These results might be due to the increasing of enterance black liquor inside the insect body and decrease the natural resistant of insect with increasing the ratio of ethoxylated fatty acids. The larvicidal activity of several insecticides showed to increase greatly by adding surfactants Tween-80 or Span-80 which change the surface tension of insecticide solutions or dissolving the wax layer which covered the insects (Taylor and Schoof, 1967; Angus and Lutty, 1971; Mkhize and Gupta, 1985, Hussein, 1991, Hussein *et al*., 2005 and Kamel *et al.,* 2005).

The effect of sub-lethal concentration of Black liquor and Deltamethrin on pupation, adult emergence and reproduction potential of *Culexpipiens* were evaluated. The results obtained indicated that sub-lethal concentration of the tested toxicological agents significantly reduces the tested biological activities of *Culexpipiens*. The percentage of pupation and adult's emergence of the treated *Culexpipiens* larvae with Black liquor and Deltamethrin were significantly reduced in comparison with the control group. The significant reductions in the number of percentage of pupation, adult emergence, fecundity and fertility of the tested insect in the present study may be attributed to some physiological disorder. The reduction in some biological activities of different mosquito species treated with different plant extracts and chemical insecticides was previously recorded by several investigators (Pereira and Gurudutt, 1990; Mohsen *et al.*, 1990; El-Hag *et al.*, 1999; Attiaa, 2002, EL-Bokl, 2003, Abd El-Baky *et al*., 2005 and Eldiasty *et al.,* 2014).

The adults were emerged from larval treatment by Black liquor do not take blood meal to complete the life cycle this result may be due to paralysis or destroy the midgut as a result of using Black liquor. Meyer and Norris (1974) stated that methoxyl substitution adjacent to 4-hydroxyl group on benzaldehydes reduced feeding and replacement of a hydroxyl by a methoxyl group destroyed the feeding response.

These compounds were produced during degradation of lignin as indicated by Ksibi *et al.* (2003) which considered the main component of black liquor and minor component of white liquor. The length of the gonotrophic cycle is of crucial importance in determining the vectorial capacity of the mosquitoes in disease transmission. However, the effect of larvicidal treatment on the ability of the emerged females to acquire blood meals needed to be investigated. Marcard *et al.* (1986) had indicated that the females of *Ae. aegypti*, *Ae. togoi* and *Cx. quinquefasciatus* developed from treated larvae with methanol extracts of *Ajuga* spp. took smaller blood-meals than those developing from untreated ones.

The Black liquor material showed also adulticidal activity. The potency of Black liquor mixture with emulsifier was increased may be due to the easily penetration of material inside the insect. Hence this research is mainly focused on finding newer insecticides which will be more effective, biodegradable and also easily available at low cost. Also, Elango *et al.* 2012 evaluate the adult emergence inhibition (EI) and adulticidal activity of the leaf hexane, chloroform, ethyl acetate, acetone, and methanol extracts of *Aeglemarmelos* (Linn.) Correa ex Roxb, *Andrographis lineata* Wallich ex Nees., *Andrographis paniculata* (Burm.f.) Wall. ex Nees., *Cocculushirsutus* L. Diels, *Ecliptaprostrata* L. and *Tageteserecta* L. were tested against japanese encephalitis vector, *Culextritaeniorhynchus* Giles (Diptera: Culicidae). All plant extracts showed moderate EI and adulticidal activity effects after 24 h of exposure at 1,000 ppm.

**Conclusion**

The author concluded that, Black liquor which release during processing the paper from Rice straw consider promising larvicidal and adulticidal agents against *Culexpipiens* (Diptera: Culicidae) and can be included in IVM program.

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