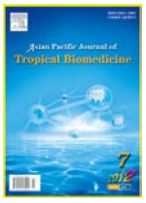
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



Document heading

Toxicological studies for some agricultural waste extracts on mosquito larvae and experimental animals

Somia El-Maghraby[1*], Galal A Nawwar[1], Reda FA Bakr[2], Nadia Helmy[2], Omnia MHM Kamel[1]

[1]*Applied organic chemistry Department, National Research Centre, Cairo, Egypt*
[2]*Department of Entomology, Faculty of Science, Ain-Shams University, Cairo, Egypt*

ARTICLE INFO

Article history:
Received 5 June 2011
Received in revised form 27 October 2011
Accepted 16 December 2011
Available online 28 July 2012

Keywords:
Agricultural wastes
Insecticides
Mosquito
Biological studies

ABSTRACT

Objective: To evaluate some agricultural waste extracts as insecticide and their effects on enzyme activities in liver and kidney of male mice. **Methods:** The insecticidal activity of five tested compounds (one crude extract and 4 waste compounds) was bioassay against the 3rd instars of the *Culex pipiens* (*Cx. pipiens*) larvae in the laboratory. The LC_{50} values of eucalyptol, apricot kernel, Rice bran, corn, black liquor and white liquor are 91.45, 1 166.1, 1 203.3, 21 449.65, 4 025.78 and 6 343.18 ppm, respectively. Selection of the compounds for the subsequent studies was not only dependent on LC_{50} values but also on the persistence of these wastes products on large scale. **Results:** White and black liquor did not produce any gross effect at 200 mg/Kg body weight. No apparent toxic symptoms were observed in tested animals during the whole period of the experiment which run out for 14 days. No statistically significance was observed in the enzyme cholinesterase activity, the activities of liver enzymes and kidney function in treated mice with black and white liquors. While, no and slight inhibition was observed after the 2 weeks of treatment period with deltamethrin and fenitrothion reached to about 24% in plasma cholinesterase enzyme activity. Significantly increase in the activities of liver enzymes and kidney function in treated mice with deltamethrin and fenitrothion. **Conclusions:** Black liquor can be used efficiently to control *Cx. pipiens* larvae under laboratory condition. Environmental problem caused by rice straw can be solved by converting the waste material to beneficial natural selective insecticide.

1. Introduction

Mosquitoes are still the world's number one vectors of human and animal diseases; and are conspicuous nuisance pests as well, even after massive efforts of eradication or control. The extensive use of chemical pesticides or insecticides resulted in inducing resistance by insect pests besides, residue contamination of human food, mammalian toxicity and environmental pollution[1]. These factors have created the need for environmental safe, degradable and target specific agents for pest control purposes. The use of deoiled rice bran and chicken feather waste, as culture medium, is highly economical, for the industrial production of the mosquito pathogenic *bacillus*[2].

Plant extracts have 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[3,4]. Several plant extracts were effective as potential acute or chronic insecticides, insect growth inhibitors or antifeedants against a variety of insect species[5,6]. Only Bakr *et al*., studied the effect of agricultural waste extracts (bran of *Oryza sativa* (*O. sativa*)) on the newly moulted 5th nymphal instars' of *Schistocerca gregaria*[5,6]. Previous studies also discussed changes in protein content and some biochemical effects of some agricultural waste extracts against *Culex pipiens* (*Cx. pipiens*) [7,8].

Several studies have shown that, pyrethroid caused alterations in biochemistry, hematology and reproduction[9]. Repeated daily oral doses of pyrethroid insecticide of α-cypermethrin at 1/10 LD_{50} altered the biochemical parameters, decreased cytochrome P450 content, antioxidant status which correlated with histopathological changes of tissues[10]. Moreover, while there is some evidence

*Corresponding author: Somia El-Maghraby, Applied Organic Chemistry Department, National Research Centre, Cairo, Egypt.
E-mail: somiaibrahem@yahoo.com

that chronic exposure to organophosphate pesticides may have adverse effects on health, there is an urgent need for high-quality observational of both occupational and environmental exposure to these compounds[11,13].

Therefore, the present study was undertaken to evaluate some agricultural waste extracts as insecticide and their effects on enzyme activities in liver and kidney of male mice for using these extracts alternate the traditional insecticides in IPM program.

2. Materials and methods

2.1. Tested compounds

2.1.1. Eucalyptol (Eucalyptus oil fraction)

Eucalyptus oil commercial grade from the local market mixed the crude oil with pet. Ether (60-80) and fractionated through silica gel column (70 mesh), the first fraction was eucalyptol.

2.1.2. Apricot kernel extract

Apricot seeds were crushed and the kernels were taken for the extraction process. One hundred grams of kernels were boiled in 1 000 mL of water for 45 min, then the water was evaporated till dryness. The resulted dry crude extracts were weighed and the overall yield from 100 g of each plant by each solvent was calculated before storage at 4 ℃ in screw capped vials, until use.

2.1.3. Rice bran extract

Rice bran was exhaustively extracted with ethanol. The extraction was accomplished by means of a Soxhlet apparatus. The solvent extract was evaporated and dried under vacuum using a rotary evaporator of water bath adjusted at 40-60 ℃. The resulted dry crude extract was weighed and the overall yield from 100 g of plant was calculated before storage at 4 ℃ in screw capped vials, until use.

2.1.4. Corn extract

Produced by patent no. (422/2008).

2.1.5. Black liquor

Black liquor produced from the paper production industry but in new way patent No. (422/2008).

2.1.6. White liquor

White liquor was effluent from bleaching paper that introduced in paper production industry[14] with modification, using sod. hyposulphite at 60 ℃ with stirring for one day.

2.2. Tested mosquitoes

2.2.1. Cx. pipiens (Culicidae: Diptera)

Larvae of *Culex pipiens* 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.

Preliminary, toxicological bioassay tests were carried out on tested compounds as a modification for the method described by Wright 1971[15]. Mortality data was analyzed by using log-probity analysis to estimate probity regression line and calculate LC_{50}, LC_{90}, slope function by applying the computer program of Unkelbach[16].

2.3. Toxicological evaluation of the selected compounds on experimental animals

2.3.1. Acute toxicity

Sixty adult Wister rats of both sexes for each compound purchased from animal house colony, in National Research Center, Giza, Egypt. The animals weighing about (200 ± 20) g were divided into six equal groups each containing 10 animals. All rats were kept under controlled conditions of temperature (22 ± 1) ℃ and humidity 60% ± 5%. They were given pellet food and drinking water ad libitum. The experimental protocol met the national guide lines on the proper care and use of animals in the laboratory research.

2.3.2. LD_{50} determination

To determine the lethal dose that kills half (50%) of the animals tested "LD_{50}" for selected compounds, black and white liquor, six doses of each compound ranged from 500 to 5 000 mg/Kg body weight were used. Each dose from both compounds was dissolved in 0.5 mL water and was administered orally to rats using stomach tube with ball tipped oral incubated needle. The LD_{50} value of the two compounds was calculated, for 24 h. mortalities following each treatment.

2.3.3. Sub-acute toxicity

Groups of Swiss albino mice of both sexes (8-10 weeks old) weighing (22 ± 1) g were housed under standard laboratory conditions and had free access to food and water.

In studies of sub-acute toxicity of white and black liquor, a group of 40 mice was used in each treatment for both compounds. In parallel, a group of 30 mice were used as control. Doses equivalent to 4 mg/mice/day of each compound were dissolved in 100 μL of water and administered orally for two weeks.

At the same time another two groups of 40 mice for each group were used to compare the toxic effect of organophosphorus and pyrethroid insecticides Fenitrothion and Deltamethrin against the two previous compounds. Mice treated daily with Fenitrothion and Deltamethrin dissolved in dimethylsulphoxide (DMSO) at dose 0.8 & 12.5 mg/mice/day, respectively corresponding to 1/20 LD_{50}. Animals were observed with regard to behavior, appetite, body weight gain

and for signs of treatment-related effects during the feeding experiments.

At the end of the experiment, 2 weeks, animals were sacrificed under ether anesthesia and blood samples were taken from the orbital plexus of treated and control mice in heparinized tubes. Blood samples were centrifuged to separate plasma for different biochemical analysis. There are at least six animals in each control and treated group.

2.3.4. Bio-chemical analysis

Acetyl cholinesterase (AChE) activity was assayed in blood samples using 0.1 M phosphate buffer. pH 7.2 and acetylthiocholine iodide as substrate, according to the method of Ellman *et al*.[17].

In dry test tubes, samples of blood were taken and centrifuged to separate serum that was used for assaying liver and kidney function enzymes. Alanine aminotransferase (ALT), aspartate aminotranferase (AST), serum alkaline phosphatase (ALP), blood urea nitrogen (BUN), and serum creatinine determination were carried out by using kits (from Biodiaganostic Company in Egypt).

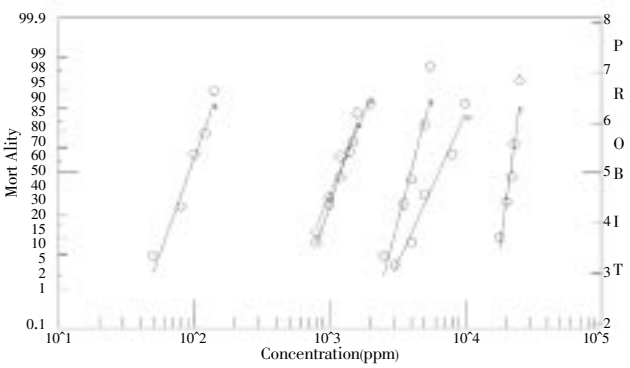


Figure 1. Susceptibility of *Cx. pipiens* larvae to some natural substances

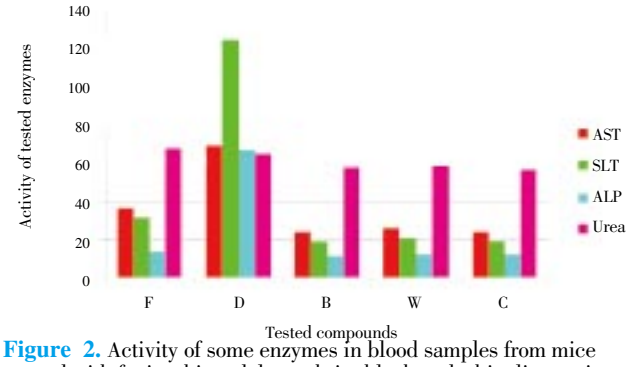


Figure 2. Activity of some enzymes in blood samples from mice treated with fenitrothion, deltamethrin, black and white liquors in comparison with non treated mice.

3. Results

Table 1.
Larval mortality and LC_{50} & LC_{95} values for the *Cx. pipiens* larvae exposed to some natural substances (west-product).

Name of sub.	Conc. (ppm)	Mortality (%)	LC_{50}	LC_{95}	Slope
Eucalyptol	50	5.0	91.45(86.54-96.63)	151.48(136.67-167.90)	1.380
	80	25.0			
	100	65.0			
	120	78.0			
	140	91.9			
Apricotkernel	800	11.7	1 166.10(1 100.29-1 236.10)	2 104.10(1 840.71-2 405.20)	1.430
	1 000	31.7			
	1 200	63.3			
	1 500	73.3			
	2 000	91.7			
Rice bran	800	8.3	1 203.30(1 145.00-1 259.20)	1 914.00(1 716.20-2 134.40)	1.324
	1 000	26.7			
	1 200	46.7			
	1 400	66.7			
	1 600	88.3			
Black liquor	2 500	5.0	4 025.78(3 872.09-4 185.57)	5 576.68(5 189.46-5 992.85)	1.250
	3 500	26.7			
	4 000	45.0			
	5 000	83.3			
	5 500	98.3			
White liquor	3 000	3.3	6 343.18(5 913.34-6 804.31)	12 052.05(10 435.15-13 920.71)	1.480
	4 000	8.3			
	5 000	33.3			
	8 000	65.0			
	10 000	91.7			
Corn	18 000	10.0	21 449.65(21 037.24-21 870.14)	26 005.44(24 942.63-27 113.57)	1.120
	20 000	28.3			
	22 000	46.7			
	23 000	71.7			
	25 000	91.7			

The insecticidal activity of five tested compounds (one crude extract and 4 waste compounds) was bioassay against the 3rd instars of the *Cx. pipiens* larvae in the laboratory. The results are presented in Table 1, and illustrated in Figure 1.

The confidential limits of each of the tested compound were statistically calculated for LC_{50} and LC_{95} at P=0.05. The tested compounds showed different toxicity. The LC_{50} values of eucalyptol, apricot kernel, rice bran, corn, black liquor and white liquor are 91.45, 1 166.1, 1 203.3, 21 449.65, 4 025.78 and 6 343.18 ppm, respectively.

Therefore the toxicity of the tested plant extracts based on LC_{50} values which could be arranged in an ascending order as follows: white liquor < black liquor < corn < rice bran < apricot kernel < eucalyptol.

3.1. The selected compounds

Selection of the compounds for the subsequent studies was not only dependent on LC_{50} (Table 1) but also on the persistence of these wastes products on large scale, lowering their coast and conversion of environment worth compounds to benefit one. Therefore black liquor and white liquor were selected for further studies.

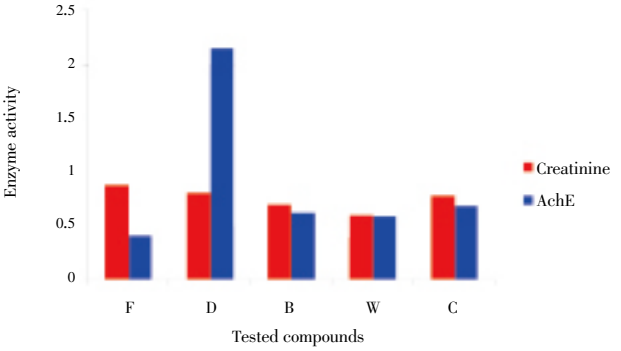


Figure 3. Activity of creatinine and AchE in blood samples from mice treated with fenitrothion, deltamethrin, black and white liquors in comparison with non treated mice.

Table 2.
Acute toxicity of black and white liquors in rats.

Black liquor		White liquor	
Dose (mg/Kg)	No. of dead rats	Dose (mg/Kg)	No. of dead rats
500	0/10	700	0/10
700	0/10	1 000	0/10
1 000	0/10	2 000	0/10
2 000	0/10	3 000	0/10
3 000	0/10	4 000	0/10
4 000	1/10	5 000	1/10

Table 3.
Effect of fenitrothion, deltamethrin, white and black liquor on certain biochemical parameters in plasma of mice after daily oral administration for 14 days (n = 40).

Enzyme activities	Control	Treated group			
		Fenitrothion(1/20 LD_{50})	Deltamethrin(1/20 LD_{50})	White liq. at 200 mg/Kg	Black liq. at 200 mg/Kg
Aspartate aminotranferase AST (U/L)	24.00 ± 1.70	36.00 ± 1.82*	124.00±4.11*	26.00 ± 2.21	24.00 ± 1.80
Alanine aminotransferase ALT (U/L)	19.00 ± 2.38	31.00 ± 0.50*	68.00±3.01*	20.00 ± 2.86	19.00 ± 1.76
Serum alkaline phosphatase ALP (U/L)	12.00 ± 0.35	13.00 ± 0.42	66.00±3.45*	12.00 ± 0.82	11.00 ± 0.66
Urea (g/dL)	56.00 ± 3.10	67.00 ± 4.54*	64.00±3.60*	58.00 ± 3.10	57.00 ± 5.64
Creatinine (g/dL)	0.78 ± 0.49	0.87 ± 0.02	0.81±0.16*	0.60 ± 0.03	0.70 ± 0.04
Acetyl cholinesteraseAchE (mmol/min/mL)	0.68 ± 0.03	0.46 ± 0.02	2.15±0.24	0.58 ± 0.03	0.62 ± 0.04

Values are mean ± S.D. *Significance at P<0.05.

3.2. Acute toxicity estimation of LD_{50}

Symptoms of acute toxicity in rat were developed within 10 min and consisted of sweating, voiding of urine and droppings and convulsions leading to death. Death occurred within 30 min and those that survived recovered within 24 h.

The mortality data during determination of LD_{50} were 1/10 for black and white liquors against the doses were 4 000, 5 000 mg/Kg body weight, respectively (Table 2).

3.3. Sub-acute toxicity

White and black liquor did not produce any gross effect at 200 mg/Kg body weight. No apparent toxic symptoms were observed in tested animals during the whole period of the experiment which run out for 14 days. Table 3 showed the effect of white and black liquor in different enzyme activities of mice treated for 14 days. No statistically significance was observed in the enzyme cholinesterase activity in treated mice as compared with control one. On the other hand, slight inhibition was observed after the 2 weeks of treatment

period with fenitrothion reached to about 24% in plasma cholinesterase enzyme activity (Table 3).

The activities of liver enzymes (AST, ALT and ALP) are shown in Table 3. During the whole experimental period, 2 weeks, no effect could be observed on the activities of these enzymes, on mice treated with white and black liquor, comparing to the control mice. Concerning the effect on kidney function, Table 3 shows the concentration of urea and creatinine.

There is no significant increase in the concentration of both above parameters was observed on the treated mice when compared with control group with the end of the treated period. On contrast, the level of alanine and aspartate aminotranferase enzymes (ALT & AST) showed a significant increase in treated mice by deltamethrin and fenitrothion to those of control group at the end of the experiment. Table 3 and Figure 2 & 3 also illustrate the toxic effect of fenitrothion and deltamethrin on kidney function of treated mice. Concerning with the concentrations of urea and creatinine, a moderate increase in urea concentration were recorded in treated animals after 14 days of experimental period comparing with the control animals.

4. Discussion

4.1. Evaluation of the larvicidal activity of some agriculture waste extracts

It was thought appreciable to evaluate some agriculture and industrial waste products from natural origin as larvaicide agents on mosquito, *Cx. pipiens*. The principal criterion in the selection of these compounds was conversion of waste materials to useful one and their productions in large scale was easy and coast less.

Chemical insecticides against mosquitoes are a major component of malaria control worldwide. Fungal entomopathogens formulated as biopesticides and applied as insecticide residual sprays could augment current control strategies and mitigate the evolution of resistance to chemical-based insecticides[18-20].

Almost most of the previous studies carried out mainly on the botanical extracts of indigenous plants of Egypt and their toxic effects on different insect species[21]. While in the present study the use of waste product to convert the useless material to benefit one.

The tested extracts revealed differences in LC_{50}, LC_{90} values and the slope functions of the regression lines. Also remarkable variations in the potency of tested extracts were observed and often attributed to the major constituents of each one.

The present data showed that despite the differences in potency of eucalyptol, apricot kernel, corn, black liquor and white liquor. They were found to possess parallel regression lines of nearly equal slope values. This may suggest that these extracts have the same mode of action against the tested insect larvae.

Similarly the black and white liquors which obtained from the same source (rice straw) and nearly equal slope values may have the same mode of action. Therefore, the difference in potency of these liquors may be referred to the quantity of the extracted materials during manufacture process rather than the quality of such materials[5].

4.2. Toxicological evaluation of the selected compounds on experimental animal

4.2.1. Acute toxicity estimation of LD_{50}

The obtained results showed no lethality till 4 000, 5 000 mg/Kg body weight for black and white liquors, respectively it means that no further testing for acute oral toxicity is needed according to EPA Health Effect Test Guidelines (1998).

4.2.2. Sub-acute toxicity

The results revealed that the absence of statistically significant differences in the biochemical parameters between control and treated groups is probably related to the low toxic effect of these compounds as they were insufficient to induce responses in these parameters. Many studies indicate that repeated exposures to relatively low level of organophosphate pesticides such as chlorpyrifos, and monocrotophos lead to protected impairments of sustained attention and an increase in impulsive behaviors in rats[22,23].

Moreover, fenitrothion and dichlorvos are a contact-acting organophosphate insecticide which inhibits acetylcholinesterase activity, thus disrupting the nervous system[24-28]. The pattern of cholinesterase inhibition was in good agreement with the probable runoff of pesticides from agricultural fields[29, 30].

Binukumar *et al*[31] studies provide an evidence of impaired mitochondrial bioenergetics which may lead to liver dysfunction after chronic exposure to organophosphate pesticide dichlorvos.

From the present study it can be concluded that, Black liquor the most potent extract from the tested waste extracts thus can be used efficiently to control *Cx. pipiens* larvae under laboratory condition. Environmental problem caused by rice straw (agricultural waste) can be solved by converting the waste material (Black liquor) to beneficial natural selective insecticide.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Domingues I, Agra AR. Monaghan K,Soares AM, Nogueira AJ. Cholinesterase and glutathione-S-transferase activities in

freshwater invertebrates as biomarkers to assess pesticide contamination. Environ Toxicol Chem 2010; **29**: 5-18.

[2] Poopath S, Abidha S. A medium for the production of biopesticides (*Bacollus Sphaericus* and *Bacillus thuringiensis* subsp.israelensis) in mosquito control. *J Econ Entomol* 2009; **102**(4): 1423-1430.

[3] Rendon-von OJ, Ortiz-Arana A, Guilhermino L, Soares AM. *In vivo* evaluation of three biomarkers in the mosquitofish (*Gambusia yucatana*) exposed to pesticides. *Chemosphere* 2005; **58**: 627-636.

[4] Mohamed MI, El-Mohamady RH, Mohamed HA. Larvicidal activity and biochemical effects of certain plant oil extracts against *Culex pipiens* larvae (Diptera: Culicidae). *J Egypt Acad Soc Environ Develop (A. Ent.)* 2003; **3**(1): 75-93.

[5] Bakr RFA, ElBermawy SM, Geneidy NAM, Emara SA, Hassan HW. Occurrence of the biological effects of some plant extracts on the cotton leaf worm *Spodoptera littoralis* (Biosd) and their physiological. *Egypt Acad Soc Environ Develop* 2006; **7**(1): 109-147.

[6] Bakr RFA, Hussein MA, Hamouda LS, Hassan, HA, Elsokary ZF. Effect of some insecticidal agents on some biological aspects and protein patterns of desert locust *Schistocerca gregaria* (Forskal). *Egypt Acad Soc Environ Develop* 2008; **9**(2): 29-42.

[7] Helmy NA, Bakr RFA, Nawwar GA, Ibrahim S El, Helmy OM. Biochemical effects of some agricultural waste extracts against *Culex pipiens* (Diptera: culicidae). *J Egypt Acad Biolog Sci* 2010; **3**(2): 75-81.

[8] Bakr RFA, Helmy NA, Nawwar GA, Ibrahim S El, Helmy OM. Changes in protein content of *Culex pipiens* mosquito treated with two agriculture waste extracts. *Biolog Sci* 2010; **3**(1): 95-103.

[9] Yousef MI, Awad TI, Mohamed EH. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by vitamin E. *Toxicology* 2006; **227**: 240-247.

[10] Manna S, Bhattacharyya D, Mandal TK. Single oral dose toxicity study of deltamethrin in rats. *Indian Vet Med J* 2004; **28**: 237-241.

[11] De-Silva HJ, Samarawickrema NA, wickremasinghe AR. Toxicity due to organophophosphorus compounds: what about chronic exposure? *Trans R Soc Trop Med Hyg* 2006; **100**: 803-806.

[12] Kaur P, Radotra B, Minz RW, Gill KD. Impaired mitochondrial energy metabolism and neuronal apoptotic cell death after chronic dichlorvos (OP) exposure in rat brain. *Neurotoxicology* 2007; **28**: 1208-1219.

[13] Binukumar BK, Gill KD. Cellular and molecular mechanisms of dichlorvos neuroyoxicity: cholinergic, noncholinergic, cell signaling, gene expression and therapeutic aspects. *Indian J Exp Biol* 2010; **48**: 697-709.

[14] Sreekrishnan MATR. Aquatic toxicity from pulp and paper mill effluentsa review. *Adv Environ Res* 2001; **5**: 175-196.

[15] Wright JW. The WHO programmer for the evaluation and testing new insecticides. *Bull Wld Hlth Org* 1971; **44**: 11-12.

[16] Unkelbach HD. *Qualitative doses/Wirkugsanalyse*. Stuttgart: Gustav Fisher Verlag; 1985.

[17] Ellman GL, Courtney KD, Andres Jr V, Featherstone RMA. New and rapid colorimetric determination of acetyl-cholinesterase activity. *Biochem Pharmacol* 1961; **7**: 88-95.

[18] George J, Blanford S, Domingue MJ, Thomas MB, Read AF, Baker TC. Reduction in host-finding behavior in fungus-infected mosquitoes is correlated with reduction in olfactory receptor neuron responsiveness. *Malar J* 2011; **10**: 219.

[19] Hancock PA. Combining fungal biopesticides and insecticide-treated bednets to enhance malaria control. *PLoS Comput Biol* 2009; **5**: e1000525.

[20] Hancock PA, Thomas MB, Godfray HC. An age-structured model to evaluate the potential of novel malaria-control interventions: a case study of fungal piopesticide spray. *Proc Biol Sci* 2009; **276**: 71-80.

[21] Kamel OM, Abd El-Baky SMM, Hassan MM, Hafez JA, Hamed MS. Synergistic and joint actions of some plant extracts on their larvicidalactivity against the mosquito *Ochlerotatus caspius* (Diptera: Culicidae). *Egypt Acad Soc Environ Develop* 2005; **6**(2): 277-289.

[22] Kashyap MP, Singh AK, Kumar V, Tripathi VK, Srivastava RK, Agrawal M, et al. Monocrotophos induced apoptosis in PC12 cells: role of xenobiotic metabolizing cytochrime P450s. *PLoS One* 2011; **6**: e17757.

[23] Middlemore-Risher ML, Buccafusco JJ, Terry AV Jr. Repeated exposures to low-level chlorpyrifos resukts in impairments in sustained attention and increased impulsivity in rats. *Neurotoxicol Teratol* 2010; **32**: 415-424.

[24] Afshar S, Farshid AA, Heidari R, Ilkhanipour M. Histopathological changes in the liver and kidney tissues of Wister albino rat exposed to fenitrothion. *Toxicol Ind Health* 2008; **24**(9): 581-586.

[25] Binukumar BK, Amanjit BAL, Ramesh kandimalla, Aditya Sunkaria Kiran Dip Gill. Mitochondrial energy metabolism impairment and liver dysfunction following chronic exposure to dichlorvos. *Toxicology* 2010; **270**: 77-84.

[26] Damasio J, Guilhermino L, Soares AMVM, Riva MC, Barata C.Biochemical mechanism of resistance in *Daphnia magna* exposed to the insecticide fenitrothion. *Chemosphere* 2007; **70**: 74-82.

[27] Farghaly M, El-Maghraby S. Investigation of chronic toxicity of 14C-fenitrothion and its degradation products on stored soybeans. *Environ Toxicol Pharmacol* 2009; **27**: 1-6.

[28] Varo I, Amat F, Navarro JC. Acute toxicity of dichlorvos to *Aphanius iberus* (Cuvier & Valenciennes, 1846) and its anti-cholinesterase effects on this species. *Aquat Toxicol* 2008; **88**: 53-61.

[29] Rendon-Von OJ, Memije MG, Ortiz A, Soares AM, Guilhermino L. An integrated approach to assess water quality and environmental contamination in the fluvial-lagoon system of the Palizada River, Mexico. *Environ Toxicol Chem* 2006; **25**: 3024-3034.

[30] Govindarajan M, Sivakumar R, Rajeswari M. Larvicidal efficacy of *Cassia fistula* Linn. leaf extract against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera:Culicidae). *Asian Pac J Trop Dis* 2011; **1**: 295-298.

[31] Binukumar BK, Bal A, Kandimalla R, Sunkaria A, Gill KD. Mitochondrial energymetabolism impairment and liver dysfunction following chronic exposure to dochlorvos. *Toxicology* 2010; **270**: 77-84.