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Mortality Rate of Houseflies Exposed to some Selected Insecticides

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Abstract

Houseflies are pest in homes, dairies, barns, poultry houses, recreation areas and food processing sites. The study is to evaluate the susceptibility of Musca domestica to deltamethrin, cypermethrin and DDVP insecticides. Topical bioassay was used to perform adulticidal test, while dipping method was used for larva and pupa, different concentrations were used which ranges from 0.5ml/L-10.0 ml/L for larvae and pupae while 5.0 µL/fly-50.0 µL/fly was used for adult Musca domestica. The mortality of larvae on application of deltamethrin ranges between 23-53% (LC_{50} 8.125 ml/L) cypermethrin ranges between 29-76% (3.401 ml/L). DDVP ranges between 25-91% (LC_{50} 2.36 ml/L). DDVP was more effective against larvae. The mortality of pupae on application of Deltamethrin ranges between 70-97% (LC_{50} 0.087 ml/L), cypermethrin ranges between 40-77% (LC_{50} 1.186 ml/L) while DDVP ranges between 43-83% (LC_{50} 0.777 ml/L. Deltamethrin had better pupacidal activity compared to DDVP and cypermethrin. The mortality for deltamethrin against adulticidal study ranges between 27-73% (LC_{50} 14.626 µL/Fly) while cypermethrin had mortality of 23-77% (LC_{50} 18.272 µL/Fly) and DDVP ranges between 20-73% (LC_{50} 19.182 µL/Fly), deltamethrin was more effective. Notable level of susceptibility of DDVP, cypermethrin and deltamethrin was found in the present study, as deltamethrin, cypermethrin and DDVP were toxic to Musca domestica in a dose dependent manner.

Keywords: Cypermethrin, Deltamethrin, Dichlorvos, Insecticides, Mortality, *Musca domestica*, Toxicity

INTRODUCTION

The common housefly, *Musca domestica* Linnaeus (Diptera: Muscidae) is found all over the world but most adaptable in warm areas (Bala *et al*., 2014). Houseflies are pest in homes and other places like dairies, barns, poultry houses, recreation areas and food processing sites (Sanchez and Capinera, 2008). Houseflies undergo complete metamorphosis, which starts from egg, larva, pupa and adult stage. Housefly can reproduce; develop a generation within two weeks in summer, as they have great breeding potentials during this period (Sawar, 2020). Houseflies are a threat to human and animal health as they can transmit more than 100 diseases including protozoans, helminthes, bacterial and viral infections (Ragaa, 2019).

Houseflies can spread a deadly strain of bacteria like *Escherichia coli* and transmit life threatening antibiotic-resistant bacteria, which are an ever-increasing threat in healthcare facilities (Macovei and Zurek, 2006). Houseflies can also transmit pathogens responsible for eye diseases such as trachoma and epidemic conjunctivitis and infect wounds or skin with diseases such as cutaneous diphtheria, mycoses, yaws and leprosy (Motazedian *et al.* 2014).

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The mobility of houseflies, towards regular excreta, carcasses and garbage and other septic matter, and intimate association with animal pathogens and humans because of their strong sense for odor all contribute to their roles in transmission of these diseases of various forms (Onyenwe *et al*., 2016).

Environmental sanitation is the most important aspect in controlling houseflies, though the use of insecticides is still considered the mainstay of agricultural pest control. Unfortunately, the intensive and injudicious use of insecticides has resulted to the development of resistance to all four major classes of insecticides like organochlorine, organophosphate, carbamates and pyrethroids (Memmi, 2010)

The principle of bioassay studies to evaluate the toxicity of insecticides with diverse mode of action to the same species under the same test conditions is very important. There are several reports on the development of resistance to conventional insecticides and insect growth regulators in houseflies from different parts of the world like the USA (Kaufman *et al*., 2010), the UK (Bell *et al*., 2010), Central Europe (Londer *et al*., 1996), Argentina (Acevedo *et al*., 2009), Brazil (Pinto and do Prado, 2001), Japan (Lee *et al*., 2006), Denmark (Kristensen *et al*., 2001), China (Zhang *et al*., 2000), Turkey (Akiner, 2006) and Pakistan (Shah *et al*., 2014).

In China, housefly resistance to insecticides has been demonstrated in the field by Huang *et al.* (2015) and is monitored annually by the Department for Disease Control and Prevention Georghiou *et al*. (1963) reported dieldrin resistance in houseflies from Kano Nigeria; however, the current susceptibility status of *Musca domestica* to insecticides in Nigeria is largely unknown or underreported.

The present study sought to determine the toxicity of cypermethrin, deltamethrin and DDVP against *Musca domestica*, adult, larva, and pupa reared in the laboratory in the department of Zoology, Ahmadu Bello University, Zaria.

MATERIALS AND METHOD

Study Area

The study was conducted in the Parasitology and Entomology Laboratory, Department of Zoology, Ahmadu Bello University Samaru Zaria which is located in Sabon Gari Local Government Area of Kaduna State. It is located between latitude 11° 15′N to 11°3′N and longitude 7° 30′E to 7°45′E

Collection of samples

Chicken droppings (dry form) from the poultry house of Anderson poultry house Samaru Zaria, Nigeria, was used as the medium for rearing the flies.

The commercial-grade formulated insecticides used for bioassays, were: synthetic pyrethroid (Cypermethrin 10EC and Deltamethrin 10EC) and DDVP 10EC. Distilled water was used as control.

Dichlorvos was manufactured by Hubei Sanonda Co. ltd. No.93 Beijing Road Jingzhou Hubei China, manufactured for WACOT Ltd. 14, Chivita Avenue, Ajao Estate, Apakun, Oshodi, Lagos Nigeria. Toxicity class WHO (AI).
Distilled water was purchased from the Department of Botany Ahmadu Bello University Zaria.

Cypermethrin with registration number CIR-21,410/95/Cypermethrin (EC)-1113 was purchased from Lionseal Industries Ltd Km 8, Tokorawa Industries Area, Hadejia Road Kano Nigeria along with deltamethrin.
All the insecticides were certified by Chemist at Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine Ahmadu Bello University, Zaria

Procedures
Adult flies of *Musca domestica* were collected from the garbage site in Samaru using a sweep net**.** Fifty (25 males and 25 females) adult were identified using the method of Soonwera and Sinthusir (2014), selected, and transported in a small cage to the laboratory. Flies were maintained in cages (30 × 30 × 30 cm) and provided with granulated sugar. Petri dishes containing cotton pads soaked in milk powder dissolved in water (10 % w/v) and jars (500 mL)

Larval media consist of yeast, dry milk powder, wheat bran and water according to the method described by Pavela (2006). The jars were removed from cages after 2-3 days when eggs were visible, after which the larva was provided with wood dust for pupation and kept in separate cages for fly emergence.

Adult bioassays
The bioassay was performed using the topical application method of WHO (1980), four days old adults were individually transferred from the rearing cage (16 × 16 × 16 cm) using a transparent test tube. The cage was covered with a transparent plastic bag in order to anesthetize the flies by fumigation with CO_2 for three minutes. The flies were allowed to recover before being treated with the insecticides used. The flies were tested across five concentrations; 5 µl/fly, 10µl/fly, 20 µl/fly, 40 µl/fly and 50 µl/fly for each insecticide and control. Flies were gently placed onto a plastic ice cube, after which each insecticide concentration was topically applied at the dorsal thorax (mesonotum) of each fly using a microapplicator. (Microlux, USA) Control flies were treated with distilled water. After being tested, the flies in all groups were transferred into each rearing cage and provided with adult food such as sugar, milk, chicken dungs. Mortality of flies was scored after post exposure. All treated flies were kept at standard room laboratory conditions (25±1 °C, 57–75 % RH, and a photoperiod of 12:12 (L/D). Flies that showed no response after soft pricking were considered dead. The treatment was carried out in three replications and three concentrations. All treated flies were kept at standard room laboratory conditions (25±1 °C, 57–75 % RH, and a photoperiod of 12:12 (L/D)

Pupicidal bioassay
The **p**upicidal bioassay was evaluated by using the dipping method of Sinthusiri and Soonwera (2013). Ten pupae were dipped into 10 mL of cypermethrin, deltamethrin and DDVP for 20 seconds after which they were transferred into a filter paper in plastic box, 7.5 × 10 × 7.5 cm. Five concentrations 0.5 ml/L, 1.25 ml/L, 2.5 ml/L, 5.0 ml/L and 10.0 ml/L and three replicates were used, the concentration were chosen after preliminary studies. Ten pupae were dipped into distilled water in three replicates for twenty seconds, as control. Occurrence of mortality was recorded seven days after treatment

Larvicidal bioassay
The larval bioassay was evaluated using a dipping method according to Sinthusiri and Soonwera (2010). Fifteen of the third instar larva were dipped into 10 mL of each tested concentrations for 30 seconds and then transferred to a filter paper in a plastic cup. Five concentrations 0.50 ml/L, 1.25 ml/L, 2.50 ml/L, 5.0 ml/L and 10.00 ml/L with five replicates

were prepared. The concentrations were chosen after preliminary studies, control larvae were dipped in distilled water for 30 seconds. Larval mortality was recorded by larval wasting and immobility after 24 hours of the treatment. The lethal concentration (LC_{50}) was calculated using probit analysis by Finney (1971). Sterilized distilled water was used as control

Data analyses

Mortality was expressed in percentages. The concentration response data was analyzed by probit analysis (Finney, 1971) with POLO software (LeOra Software 2005) to determine the LC_{50} values and standard errors. One- way ANOVA was used to determine the significant mean difference of *Musca domestica* to various concentrations. Duncan Multiple Range Test (DMRT) was used to separate the mean of different insecticides

RESULTS

Larvicidal assay

The larvicidal assay revealed higher mean mortality for deltamethrin at a concentration of 5 and 10 ml/L (7.20 ± 0.37 and 8.00±0.78 respectively) and significantly different ($p<0.05$) from that observed at a concentration of 0.5, 1.25 and 2.50 ml/L (3.40 ± 0.25, 4.40 ± 0.40 and 4.60 ± 0.51 respectively) (Table1). The highest mean mortality for cypermethrin was observed at a concentration of 10 ml/L (11.40 ± 0.60) and significantly different ($p<0.05$) from that observed at a concentration of 0.5, 1.25, 2.50 and 5 ml/L (4.40 ± 0.51 4.6 ± 0.25, 5.60 ± 0.51 and 7.40 ± 0.93 respectively). The mean mortality for cypermethrin at a concentration of 5 ml/L was significantly different ($p<0.05$) from that observed at a concentration of 0.5, 1.25 and 2.50 ml/L. The highest mean mortality was observed for DDVP at a concentration of 10ml/L (13.60 ± 0.40) and significantly different from that observed at a concentration of 0.5, 1.25, 2.50 and 5 ml/L (3.80 ± 0.80, 4.80 ± 0.49, 6.60 ± 0.75 and 7.60±0.51 respectively), however that observed at a concentration of 2.50 and 5 ml/L were significantly different ($P<0.05$) from that observed at a concentration of 0.5 and 1.25 ml/L. In general, increase in mean mortality was observed as concentration of deltamethrin, cypermethrin and DDVP. The LC_{50} for deltamethrin, cypermethrin and DDVP were 8.04, 3.40, 2.42 ml/L respectively

Pupicidal assay

The pupicidal assay revealed higher mean mortality for deltamethrin at a concentration of 1.25, 2.50, 5.00 and 10 ml/L (8.67 ± 0.88, 9.00 ± 0.58, 9.33 ± 0.67 and 9.67 ± 0.33 respectively) and significantly different ($p<0.05$) from that observed at a dosage of 0.5 ml/L (7.00 ± 0.58) (Table 2). The highest mean mortality for cypermethrin was observed at a concentration of 2.50, 5.0 and 10 ml/L (6.33 ± 0.88, 6.67 ± 0.88 and 7.67±0.88 respectively), although mean mortality for cypermethrin at a concentration of 10 ml/L was significantly different ($p<0.05$) from that observed at a concentration of 0.5 and 1.25 ml/L (4.00 ± 0.58 and 4.67 ± 1.20 respectively). The highest mean mortality was observed for DDVP at a concentration of 2.50, 5.0 and 10 ml/L (7.33 ± 0.33, 8.00 ± 0.58 and 8.33 ± 1.67 respectively), and significantly different from that observed at a concentration of 0.5 and 1.25 ml/L (4.33 ± 0.33 and 5.3 3± 0.33 respectively). In general, the result of the pupicidal assay revealed increase in percentage mortality as concentration of deltamethrin, cypermethrin and DDVP increases. The computed LC_{50} for deltamethrin, cypermethrin, DDVP were 0.087, 1.186 and 0.777 ml/L respectively, therefore a deltamethrin had more efficient pupicidal effect.

Adulticidal assay

The adulticidal assay revealed higher mean mortality for deltamethrin at a dosage of 2.50, 5.0 and 10 ml/L (5.67 ± 0.33, 6.67 ± 0.88 and 7.33 ± 0.88 respectively) and significantly different ($p<0.05$) from that observed at a dosage of 0.5 and 1.25 ml/L (2.67 ± 0.67 and 4.67 ± 0.33

respectively). The higher mean mortality for cypermethrin was observed at a concentration of 5.0 and 10ml/L (6.33 ± 0.88 and 7.33 ± 0.88 respectively) and significantly different ($p<0.05$) from that observed at a concentration of 0.5 and 1.25 ml/L (2.00 ± 0.58 and 4.00 ± 0.58 respectively). On the other hand, mean mortality for cypermethrin at a concentration of 10 ml/L was significantly different ($p<0.05$) from that observed at a concentration of 2.50 ml/L (5.00 ± 0.58). The highest mean mortality was observed for DDVP at a concentration of 10 ml/L (7.67 ± 0.00) and significantly different ($p<0.05$) from that observed at a concentration of 0.5, 1.25 and 2.50 ml/L (2.33 ± 0.88, 4.33 ± 0.67 and 4.67 ± 0.67 respectively), however that observed at a concentration 5 ml/L (6.00 ± 0.58) was significantly different ($p<0.05$) from that observed at a concentration of 0.5 ml/L only. In general, increase in percentage mortality was observed as concentration of deltamethrin, cypermethrin and DDVP. The reported LC_{50} for deltamethrin, cypermethrin and DDVP were 14.626, 19.182 and 18.272 µL respectively, therefore deltamethrin had more efficient larvicidal effect, while deltamethrin was least efficient

Table 1: Mortality of *Musca domestica*'s Larva after exposure to deltamethrin, cypermethrin and Dichlorvos for 24 hours

Variable		Insecticides		
	Conc. (ml/L)	Deltamethrin	Cypermethrin	DDVP
Mean mortality	Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
	0.50	3.40 ± 0.25^{b}	4.40 ± 0.51^{c}	3.80 ± 0.80^{c}
	1.25	4.40 ± 0.40^{b}	4.60 ± 0.25^{c}	4.80 ± 0.49^{c}
	2.50	4.60 ± 0.51^{b}	5.60 ± 0.51^{c}	6.60 ± 0.75^{b}
	5.00	7.20 ± 0.37^{a}	7.40 ± 0.93^{b}	7.60 ± 0.51^{b}
	10.00	8.00 ± 0.78^{a}	11.40 ± 0.60^{a}	13.60 ± 0.40^{a}
	p-value	0.000	0.000	0.000
% Mortality	Control	0	0	0
	0.50	23	29	25
	1.25	29	31	32
	2.50	31	37	44
	5.00	48	49	51
	10.00	53	76	91
	Equation	$y = 0.662x + 4.3977$	$y = 0.9128x + 4.5148$	$y = 1.3861x + 4.4918$
	LC_{50}	8.125 ml/L	3.401 ml/L	2.326 ml/L

DDVP: 2, 2-dichlorovinyl dimethyl phosphate; n: 75; Conc: Concentration
Superscripts with different letters a, b, c, d are significantly different at $p<0.05$.

Table 2: Mortality of Pupa of *Musca domestica*'s Pupa after exposure to deltamethrin, cypermethrin and Dichlorvos for seven days

Variable	Conc. (ml/L)	No flies	Insecticides Delta	Cyper	DDVP
Mean mortality	Control	30	0.33±0.33^{c}	0.33±0.33^{c}	0.33±0.33^{d}
	0.50	30	7.00±0.58^{b}	4.00±0.58^{b}	4.33±0.33^{c}
	1.25	30	8.67±0.88^{ab}	4.67±1.20^{b}	5.33±0.33^{bc}
	2.50	30	9.00±0.58^{a}	6.33±0.88^{ab}	7.33±0.33^{ab}
	5.00	30	9.33±0.67^{a}	6.67±0.88^{ab}	8.00±0.58^{a}
	10.00	30	9.67±0.33^{a}	7.67±0.88^{a}	8.33±1.67^{a}
	P-value			0.001	0.000
% Mortality	Control		3	3	3
	0.50		70	40	43
	1.25		87	47	53
	2.50		90	63	73
	5.00		93	67	80
	10.00		97	77	83
			y = 0.8015x + 5.8486	y = 0.7761x + 4.9422	y = 0.9425x + 5.1032
	LC_{50}		0.087ml/L	1.186 ml/L	0.777 ml/L

DDVP: 2, 2-dichlorovinyl dimethyl phosphate; n: 30; Conc: Concentration
Superscripts with different letters a, b, c, d are significantly different at p<0.05.

Table 3: Mortality of *Musca domestica* after exposure to deltamethrin, cypermethrin and Dichlorvos for 24 hours

Variable	Conc. µL/Fly	Insecticides Deltamethrin	DDVP	Cypermethrin
Mean mortality	Control	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{d}
	5.0	2.67±0.67^{c}	2.00±0.58^{de}	2.33±0.88^{c}
	10.0	4.67±0.33^{b}	4.00±0.58^{cd}	4.33±0.67^{b}
	20.0	5.67±0.33^{ab}	5.00±0.58^{bc}	4.67±0.67^{b}
	40.0	6.67±0.88^{a}	6.33±0.88^{ab}	6.00±0.58^{ab}
	50.00	7.33±0.88^{a}	7.33±0.88^{a}	7.67±0.33^{a}
	P-value	0.000	0.000	0.000
% Mortality	Control	0	0	0
	5.0	27	20	23
	10.0	47	40	43
	20.0	57	50	47
	40.0	67	63	60
	50.00	73	73	77
	Equation	y = 1.1367x + 3.6756	y = 1.3217x + 3.3044	y = 1.2526x + 3.4195
	LC_{50}	14.626 µL/fly	19.182 µL/fly	18.272 µL/fly

DDVP: 2, 2-dichlorovinyl dimethyl phosphate; n: 30; Conc: Concentration
Superscripts with different letters a, b, c, d are significantly different at p<0.05.

DISCUSSION

All the insecticides used had varying degree of mortality on the larva, pupa and adult. Deltamethrin, cypermethrin and DDVP on housefly larvae showed an increase in percentage mortality with increase in concentration of the insecticides, which shows that the higher the concentration the more active or toxic the insecticide becomes which can lead to a higher mortality in the housefly, this result agrees with the findings of Muhammed and Syed (2000) who worked on toxicity of crude neem extract as against DDVP and found out that increase in concentration leads to increase in percentage mortality.

Considering the LC_{5O} of deltamethrin, cypermethrin and DDVP, DDVP had low LC_{50} which shows that DDVP was more effective in controlling the *Musca domestica* larva as against others. This may be due to the mode of action of DDVP, an organophosphate insecticides that affect acetylcholinesterase, a cholinergic enzyme primarily found at postsynaptic neuromuscular junctions, especially in muscles and nerves against pyrethroids which are known to alter the normal function of insect nerves by modifying the kinetics of voltage-sensitive sodium channels, which mediate the transient increase in the sodium permeability of the nerve membrane that underlies the nerve action potential (Kristopher *et al*., 2014). The work do not agree with the work of Sukontason *et al.* (2004) who worked on toxicity of Eucalptol on housefly and blowfly where all the larvae dipped into the chemical died.

Considering the activities of cypermethrin, deltamethrin and DDVP, they act either by absorption to the cuticle via the respiratory tract, or either through the process of ingestion into the gastrointestinal tract. Once inside the body of the larvae the substances can reach the site of action or cause systemic effects by diffusion into different tissues. Further effect of higher concentration was also distinguished due to tanning of body colour from whitish to brownish black during the treatment. However, at low concentration, larvae showed mortality but without any change in the body color, also morphological abnormalities like swelling of body pigmentation weakens cuticle and irregular body shape was reported. The LC_{50} value observed showed that there is a high activity of cypermethrin, deltamethrin and DDVP on the larvae which is an indication of the sensitivity of the larvae to the insecticides used which require little concentration to cause mortality.

Mortality of pupae was evaluated using deltamethrin, cypermethrin and DDVP insecticides. The results came out in a concentration dependent manner, the higher the concentration the high mortality rate. Deltamethrin shows high pupacidal activity compared to cypermethrin even though they have a similar mode of action as compared to DDVP, with a different mode of action. Although the pupa stage is the non-feeding in the lifecycle of housefly this chemical were still effective against the pupae this could be attributed to the method used in carrying out the test (dipping bioassay) which allow the insecticide to stay on the pupa for sometimes thereby hindering the emergence of it to adult . Epstein and Hollingsworth (2013) made an observation in his research work while working with insecticides against the pupa stage of housefly that they all died after emergence from the pupa stage due to the effect of the chemical. The increase in toxicity among the pupa also could be attributed to temperature; temperature is regarded as one of the most important abiotic environmental factors affecting biological process and physiological function. However, the relationship between temperature and insecticides toxicity may vary among insecticides classes because temperature may increase the rate of metabolism thereby increasing detoxification and excretion of some insecticides as the case for pyrethroid, as in the case of this work deltamethrin which had the lowest LC_{50} may be attributed to temperature because insecticides with negative temperature coefficient tend to perform well when the temperature is low while insecticides with positive temperature coefficient perform well when the temperature is high, the reason being that sodium influx increases due to the stability of open sodium channels at low temperature, being axonics poison deltamethrin control the movement of sodium ion during movement of nerve impulse, the sensitivity of neuron increases between 15-30˚C which result in repetitive nerve firing but the reverse has been observe in higher temperature (Scott, 1995).

The results gotten from this studies are in accordance with already reported results on the impact of temperature on pyrethroid toxicity with different insect species and also with Khan and Akram (2014) who worked on the effect of temperature on the toxicity of insecticides against *Musca domestica* L.: Implication for the effective management of diarrhea who attributed the increase in toxicity of the insecticides to temperature. The mortality rate of houseflies as a result of exposure to deltamethrin, cypermethrin, and DDVP on adult houseflies shows a concentration-dependent relationship, higher concentration gives a high mortality rate in all the insecticides used, this may be due to the high concentration of the active ingredient, while lower concentration gives low mortality rate, this result is in agreement with the work of Levchenko *et al*. (2017) who worked on the susceptibility of houseflies from a livestock farm in Tyumen region, Russia. It also agrees with Zahidul and Khalequzzaman (2002) who tested DDVP, cypermethrin, diazinon, lambda-cyhalothrin and propoxur after 24hours by topical bioassay application method and find out that all insecticides increase in toxicity, they attributed it to increase in cuticular penetration and the sensitivity of housefly to insecticides used, some houseflies in lower concentration that were still alive showed lack of movement coordination, lethargy and inability to fly

CONCLUSION

Dichlorvos is toxic to larva, deltamethrin is more effective against pupa and adult housefly, it can also be summarized that all the insecticides can be used in controlling houseflies as they all causes death of housefly, thereby reducing the risk of contracting houseflies related diseases Monitoring of the susceptibility of *M domestica* population to the conventional insecticides and new insecticides for effective houseflies control should be encouraged.

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