Species composition, blood meal sources and insecticide susceptibility status of *Culex* mosquitoes from Jimma area, Ethiopia

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Abstract

Different species of *Culex* mosquitoes are present in high numbers in many other countries in addition to sub-Saharan Africa and Asian countries. *Culex* mosquitoes are serious nuisance which also transmit a range of pathogens including several viruses such as West Nile virus, Saint Louis encephalitis, and Rift Valley fever, as well as parasites such as avian malaria, and filarial worms. In Ethiopia, unlike *Anopheles* mosquitoes, little effort was given to study habitat, species composition, blood meal sources and insecticide susceptibility status of *Culex* mosquitoes. Therefore, this study aimed to assess the species composition, their blood meal source and insecticide susceptibility status of *Culex* mosquitoes to some of selected insecticides in Jimma town southwest Ethiopia. Culicine mosquito larvae were collected using a standard dipper (by dipping) from a range of breeding sites and reared to adults. Species identification was carried out using standard keys. Bioassay tests were performed on adults to assess the susceptibility of *Culex* mosquitoes to insecticide-impregnated papers with Dichlorodiphenyltrichloroethane (DDT (4%), malathion (5%), bendiocarb (0.1%), propoxur (0.1%), deltamethrin (0.05%) and pirimiphos-methyl (0.25) following World Health Organization Pesticide Evaluation Scheme (WHOPES) guideline. Moreover, 184 blood fed (BF) *Culex* mosquitoes were collected using aspirator from indoor and outdoor resting and assayed to assess blood meal sources using Enzyme-Linked Immunosorbent Assay (ELISA). The result of the study showed that among the collected *Culex* mosquitoes, two species were identified as *Culex quinquefasciatus* and *Cx. antennatus* whereas the remaining one could not be identified to species level. *Culex* mosquitoes were found to be resistant to DDT, malathion, bendiocarb, propoxur, and deltamethrin whereas susceptible to pirimiphos-methyl. The blood meal source analysis using Enzyme-Linked Immunosorbent Assay (ELISA) showed higher blood source of human (33.2%) than bovine (15.2%). Thus, the observed resistance to the most of the insecticides coupled with higher human blood meal source calls further studies to be carried out in *Culex* mosquito populations of Ethiopia.

Keywords *Culex* mosquito · Species composition · Insecticide resistance · Blood meal · Ethiopia

Introduction

Culex mosquitoes are distributed more or less worldwide except the extreme northern parts of the temperate zone (Service, 2012). Various species of *Culex* mosquitoes are vectors of different disease. Twenty five sub genera and 769 species in the world fauna belong to the genus *Culex* L. (Harbach 2016). Some of the species of the genus *Culex* are known for their biting nuisance and certain species involved in the transmission of different arboviral and filarial diseases to humans and domestic animals (Tsai and Mitchell 1989; Goddard et al. 2002; Weissenböck et al. 2010). *Culex tritaeniorhynchus* and *Cx. vishnui* transmit Japanese encephalitis (JE) (Hammon et al. 1949; Seo et al. 2013; Su et al. 2014; Sahu et al. 2018). *Culex vishnui* transmit West Nile virus (WNV) in some Asian countries (Mishra and Mourya 2001; Pani, 2013).

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Fig. 1 Map showing study area

Culex quinquefasciatus is a main vector of *bancroftian filariasis* (Jayasekera et al. 1991; Mwakitalu et al. 2013; Khan et al. 2015; Derua et al. 2017) and a recognized vector of Rift Valley fever virus (RVFV) (Fall et al. 2016;). *Culex pipiens* is known for the transmission of RVFV (Moutailler et al. 2008; Brustolin et al. 2017) and WNV (Kwan et al. 2010).

Temperate and tropical areas constantly faced with the threat of out breaks of diseases transmitted by different species of mosquitoes (Clements 1992). This has led to widespread usage of insecticides in order to help control over mosquito populations. Insecticide treated nets (ITNs) and indoor residual sprayings (IRS), are progressively used in Africa for malaria control and have benefited in protecting people from filarial and arboviral diseases (Manga 2002). However, subsequent application of insecticides has led mosquitoes to develop resistance against many types of insecticides (Hemingway et al. 1990; Liu et al. 2019; Lopes, et al. 2019; Rai et al. 2019). Metabolic and target site resistance mechanisms are the most common and widely distributed types of mechanisms in *Culex* mosquitoes (Hemingway and Karunaratne 1998; Che-Mendoza et al. 2009; Scott et al. 2015; Lopes et al. 2019).

Culex mosquitoes are known to feed on several species of birds, mammals and rarely reptiles and amphibians (Mackay et al. 2010). *Culex restuans* and *Cx. pipiens* acquired blood mostly from birds where as *Cx. salinarius* fed commonly from mammals and birds (Molaei et al. 2006). In Ethiopia relatively there is ample information on the habitat characterization, feeding behavior and resistance status of malaria vector (Yewhalaw et al. 2010; Mereta et al. 2013; Alemayehu et al. 2017; Messenger et al., 2017; Simma et al. 2019) than culicine mosquitoes (Birhanu et al., 2019). In Ethiopia *Anopheles* mosquito species are believed to be the main vectors of lymphatic filariasis (Bockarie et al. 2009). However detail studies need to be carried out. Little effort was given to study habitat characterization, feeding behavior and susceptibility status of *Culex* mosquitoes to various public health important insecticides in the country. Moreover, species composition of *Culex* mosquitoes and blood meal sources not yet studied. Therefore, this study aimed to assess species composition of *Culex* mosquitoes, their blood meal source and insecticide susceptibility status to various public health important insecticides in Jimma town, Southwest Ethiopia.

Table 1 *Culex* mosquito species and abundance in Jimma town (April to August, 2018)

Site	Species	Abundance	
		Number	(%)
Jimma	<i>Cx. antennatus</i>	511	50.5%
	<i>Cx. quinquefasciatus</i>	439	43.2%
	Other <i>Cx.</i> spp.	61	6.0%

Materials and methods

Study site, monthly mosquito sampling and identification

The study was conducted in Jimma town southwestern Ethiopia, located 352 km south-west of Addis Ababa (Fig. 1). Culicine mosquitoes larvae were collected from a range of breeding sites rich with organic debris (rotting vegetation, household refuse and excreta), road puddles, surface water harvest and ditches using a standard dipper (by dipping) April to August 2018. Larvae were collected once per week for five months. The collected larvae were reared to adults at Becho bore site (Jimma town) and fed with brewery yeast. Adults were provided a 10% sucrose solution soaked into cotton pads (Gerberg et al. 1994). Adult *Culex* mosquitoes were identified under dissecting and compound microscope using African standard key and pictorial keys (Reuben et al. 1994; Kent 2006). Identified *Culex* mosquito species were counted and individually preserved in Eppendorf tubes over silica-gel.

Insecticide bioassays

Insecticide susceptibility bioassays were performed as per WHO standard guideline (WHO 2016). Three to five days old, non-blood-fed female *Culex* mosquitoes were exposed to insecticide impregnated papers with discriminating concentrations of DDT (4%), deltamethrin (0.05%), bendiocarb (0.1%), pirimiphos-methyl (0.25), malathion (5%) and propoxur (0.1%) using WHO standard assays (WHO 2016). The insecticide impregnated and control papers were obtained

from Sekoru Tropical and infectious disease research center Jimma University. A total of 900 mosquitoes in 6 replicates were used for the insecticide bioassays. Batches of 25 mosquitoes in 4 replicates were exposed in test kit tubes for all bioassays against 6 insecticides for 1 h and knockdown was recorded at 10, 15, 20, 30, 40, 50, and 60 min. Equal numbers of mosquitoes in 2 replicates were exposed to the parallel control papers impregnated with olive oil (Organophosphate/Carbamate control). After one hour, mosquitoes were transferred into holding tubes and provided 10% sucrose solution with cotton pads. Mortality was recorded after 24 h of exposure.

Culex mosquitoes blood meal analysis

Direct ELISAs were developed for identification of human and bovine blood meals. One hundred eighty-four FF *Culex* mosquitoes were collected using aspirator from indoor and outdoor resting and assayed for human and bovine blood antigens by ELISA (Beier and Koros 1988). Abdomen of each FF mosquito was ground in 50 µl phosphate-buffered saline (PBS) and final volume brought to 200 µl with PBS buffer. 50 µl of the triturate was coated in duplicate wells on two separate U-bottomed 96-well micro titer plate simultaneously; plates were allowed for 2 h incubation at room temperature and washed twice with PBS-Tween20. 50 µl peroxidase-conjugated anti-human IgG was added and incubated for one hour at room temperature and washed thrice with PBS-Tween 20. Finally, 100 µl ABTS peroxidase substrate was added, incubated at room temperature for 30 min and observed for green color reaction visually and absorbance read at 405 nm by ELISA reader. A two-step procedure was developed for determining a second host (bovine) source in the same micro titer plate well, where mosquitoes were screened for human blood. A second conjugate phosphatase labeled anti bovine IgG (1:250 dilution of 0.5 ml stock solution) was added to the peroxidase labeled anti-human IgG solution. Blood meals were screened first for human IgG by the addition of peroxidase substrate. After reading absorbance at: 30 min the wells were washed three times with PBS-Tween 20 and 100 µl phosphatase substrate was added to each well. Plates were read after 1 h to determine positive bovine reactions.

Data analysis

Mean mortality of replicate with 95% CI was determined for each insecticide using SPSS version 23. The WHO criterion for evaluating resistance or susceptibility was used (WHO 2016); mortality of less than 80% indicate resistance, while those greater than 98% indicate susceptibility. Mortality between 80%–98% suggests the possibility of resistance that needs to be verified. The blood

Table 2 Temporal variation of *Culex* mosquito species in Jimma town (April to August, 2018)

<i>Culex</i> mosquito species	April	May	June	July	August	Total
<i>Cx. antennatus</i>	90	38	27	256	100	511
<i>Cx. quinquefasciatus</i>	93	60	42	244	-	439
Other <i>Cx.</i> spp.	35	15	11	-	-	61
Total	218	113	80	500	100	1011

Table 3 Insecticide susceptibility status of *Culex* mosquitoes in Jimma town (April to August, 2018)

Insecticide	Mortality (%)	mean mortality	95%CI	Resistance phenotype
DDT (4%)	17%(100)	4.25	1.24 7.26	Resistant(R)
Malathion (5%)	77%(100)	20.25	17.86 22.64	Resistant(R)
Bendiocarb (0.1%)	79%(100)	6.75	3.22 10.28	Resistant(R)
Propoxur (0.1%)	27%(100)	19.75	15.18 24.32	Resistant(R)
Deltamethrin (0.05%)	81%(100)	19.25	14.87 23.33	Resistant(R)
Pirimiphos-methyl (0.25%)	98%(100)	24.50	23.58 25	Susceptible(S)

meal source of *Culex* mosquitoes during ELISA test, was determined using the cut off value (average of negative control times two), in which the blood meal sample was considered as positive if the absorbance value equivalent and exceeded the cut off value.

Results

Species composition and abundance of *Culex* mosquitoes

Three *Culex* mosquito species were recognized in the study area. Two of them identified as *Culex quinquefasciatus* and *Cx. antennatus* and the remaining one species could not be identified to the species level. Among 1011 female adult *Culex* mosquitoes 439(43.2%) were identified as *Cx. quinquefasciatus*, 511(50.5%) were *Cx. antennatus* and the remaining 61(6.0%) could not be identified to species level (Table 1).

Monthly mosquito dynamics and abundance

Culex mosquito larvae collected between April and August 2018 showed temporal variation in species composition (Table 2). *Culex. quinquefasciatus* was the predominant species from April to June 2018 whereas *Cx. antennatus* replaced *Cx. quinquefasciatus* and found the most abundant in July and August 2018.

Table 4 Blood meal origin of *Culex* mosquito in Jimma town (April to August, 2018)

Study Site	Place of Collection	Number Tested	Positive for human n (%)	Positive for bovine n (%)	Other blood source n (%)
Jimma	Outdoor	44	8 (18.2%)	5 (11.4%)	31 (70.4%)
	Indoor	140	53 (37.9%)	23 (16.4%)	64 (45.7%)
	Total	184	61 (33.2%)	28 (15.2%)	95 (51.6%)

Insecticide bioassay test

Among *Culex* mosquitoes exposed to DDT, malathion, bendiocarb and propoxur over 64% them were identified as *Cx. quinquefasciatus* while mosquitoes exposed to pirimiphos-methyl and deltamethrin were fully *Cx. antennatus*. *Culex quinquefasciatus* and *Cx. antennatus* developed resistance against organochlorine (DDT), pyrethroid (deltamethrin) and carbamate (bendiocarb and propoxur). Mortality rates for DDT, deltamethrin, bendiocarb, propoxur and malathion was 17%, 81%, 79%, 27% and 77% respectively. *Culex antennatus* populations were found to be susceptible to organophosphate (pirimiphos-methyl) with mortality rate of 98% (Table 3).

Blood meal analysis

One hundred eighty-four FF *Culex* mosquitoes (44 collected from outdoor and the remaining 144 collected from indoor) were assayed for human and bovine blood antigens by direct Enzyme-Linked Immunosorbent Assay (ELISA). The outdoor as well as indoor collected *Culex* mosquitoes showed higher blood source of human (Table 4). Human blood was the most common source of vertebrate blood for *Culex* mosquitoes in the study area.

Discussion

The occurrence and distribution of *Culex* mosquitoes vary from place to place depending on ecological, environmental

and human factors. This study documented *Cx. quinquefasciatus* and *Cx. antennatus* in the study area. *Culex quinquefasciatus*, an arboviral and filarial vector, is widely spread across sub-Saharan Africa and Asian countries (Jayasekera et al. 1991; Mwakitulu et al. 2013; Khan et al. 2015). *Culex quinquefasciatus* is the most common species in East, West and central African countries like Kenya (Muturi et al. 2008) Tanzania (Jones et al. 2012; Derua et al. 2017;) Senegal (Fall et al. 2014, 2016) Benin (Yadouléton et al. 2015) and Zambia (Kent 2006). *Culex antennatus* is dominant in Egypt (Hanafi et al. 2011; Emtithal and Thanaa 2012) Zambia (Kent 2006); Madagascar (Tantely et al. 2015) and some other places in Africa.

Understanding the extent of temporal variability and consistency is an important factor in vector competence. *Culex quinquefasciatus* was dominant in the study area from April to June 2018 but later replaced by *Cx. antennatus* in July and August 2018. Environmental factors such as rainfall, temperature and biotic factor like vegetation were found to influence *Cx. quinquefasciatus* population at different time (Grech et al. 2013). Environmental and population genetics might influence vector competence (Kilpatrick et al. 2010). Vector competence of *Cx. pipiens* and *Cx. restuans* for WNV showed variation in transmitting a virus at different periods (Kilpatrick et al. 2010) highlights the effect of environmental factors for the vectorial competence of *Culex* mosquitoes.

Culex quinquefasciatus and *Cx. antennatus* in the study area have developed resistance to organochlorine (DDT), pyrethroid (deltamethrin) and carbamate (bendiocarb and propoxur). The resistance is very strong with DDT and propoxur with mortality rates less than 30%, but moderate with deltamethrin, bendiocarb and malathion and susceptible to organophosphate (pirimiphos-methyl). Similarly, previous study conducted in two districts (Omo-Nada and Tiro-Afeta) in Jimma zone revealed pyrethroid resistance in populations of *Cx. quinquefasciatus* (Birhanu et al. 2019). Various studies from Africa documented resistance in *Culex* mosquitoes. *Culex* mosquitoes have developed resistance to permethrin, deltamethrin, DDT and bendiocarb in Benin (Yadouléton et al. 2015). Similar findings from Zambia revealed *Cx. quinquefasciatus* showed resistance to all insecticides (Laura and Douglas 2011). In Ghana *Culex* mosquitoes displayed large variation in resistance to organophosphates (malathion, fenitrothion) carbamates (propoxur, bendiocarb) and organophosphates (Andreas 2015). Similar findings were also reported from India where *Cx. quinquefasciatus* were resistant to multiple insecticides (deltamethrin, cyfluthrin, permethrin, lambda-cyhalothrin, malathion and DDT) (Kumar et al. 2011; Rai et al. 2019).

Human activities can change environment (Wang and Lui 2013) which contributes to an increase in the density of

mosquito vectors. The anthropogenic activities could also facilitate larval and adult vector abundance and human-mosquito interactions (Vanwambeke et al. 2007). Mosquito's blood meal analysis is an important assay for the identification of possible and preferred hosts. Feeding preference is one of the selection criteria for host selection that influence the probability of a host being exposed to a mosquito. In our finding of the known blood sources, human fed mosquitoes were doubled than bovine fed mosquitoes. Similarly, *Cx. quinquefasciatus* from Kenya was the only species with higher frequency of human blood from indoor collected populations compared with outdoor-collected populations (Muturi et al. 2008). *Culex pipiens* and *Cx. antennatus* fed exclusively on mammals while few *Cx. pipiens* fed on birds (Zimmerman et al. 1985). *Culex antennatus* and *Cx. tritaeniorhynchus* took much higher blood from human although there are some variations in some areas in Senegal (Gordon et al. 1991). In Ethiopia, even though *Anopheles* mosquito species is the main vector of lymphatic filariasis (Bockarie et al. 2009), detail advanced investigation is needed to understand the role of *Culex* mosquitoes.

Conclusions

The findings of this study revealed that *Cx. quinquefasciatus* and *Cx. antennatus* are the most abundant species with some temporal variation. *Culex* mosquitoes have developed resistance to DDT, malathion, bendiocarb, propoxur and deltamethrin but susceptible to pirimiphos-methyl. Higher human blood source than bovine was identified. Thus, identifying potential blood meal source is important to further determine feeding preference and vectorial capacity of *Culex* mosquitoes in the study area. The application of molecular techniques will have paramount importance for proper identification of species, investigation of blood meals and characterization of resistance mechanisms in *Culex* mosquitoes. The observed resistance to the most of the insecticides coupled with higher human blood meal source calls further study to be carried out in *Culex* mosquito populations of Ethiopia.

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Authors' contributions WG, EA and DY conceived and designed the study. WG performed the field and laboratory experiments and drafted the manuscript. EA supervised the field activities and the bioassays. EA and DY critically reviewed the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets supporting the conclusions of this article are included within the article.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no competing interests.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Code availability Not applicable.

Abbreviations

ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; DDT, Dichlorodiphenyltrichloroethane; ELISA, Enzyme-Linked Immunosorbent Assay; FF, Female fed; IRS, Indoor residual spraying; ITNs, Insecticide treated bed nets; PBS, Phosphate-Buffered Saline; WHO, World health organization; WHOPES, World health organization Pesticide Evaluation Scheme

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