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Factors Associated with Pesticide Resistance in Culex Mosquitoes

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2019

Abstract

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by

Jude Akpan

BS, Walden University, 2013

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

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Abstract

In recent years mosquito-borne diseases have reemerged, largely because of pesticide resistance. The mosquitoes develop resistance to pesticides because of broad and repeated uses of pesticides. Preventing the development of pesticide resistance requires proper understanding of the environmental factors potentially associated with the development of the resistance. The purpose of this study was to investigate the environmental factors associated with the resistance to pesticides by the Culex mosquitoes. This correlational study included the analysis of archived data samples ($N = 29{,}794$) from the Field Cage Tests results conducted between 2013 and 2017 by a large county public health department. The compartmental model was used to help understand and interpret study findings. To examine the associations between the independent variables (i.e., wind speed, temperature, humidity, time, month, and weather conditions) and the dependent variable (i.e., pesticide resistance), chi-square and multiple logistic regression analyses were performed. The results showed that the odds of mosquitoes developing resistance were 2.1 times higher during high temperatures than at low temperatures [$x^2(1) = 346.5$, $p = .000$]; the resistance was 1.5 times higher during high humidity than in low humidity [$x^2(1) = 7.23$, $p = .007$]; and the odds of mosquitoes developing resistance to pesticides in August were 3 times higher than when sprayed in June or July [$x^2\ (2) = 702.606$, $p = .000$]. Study findings may be used to help with the development of more effective methods for vector control thereby reducing the numbers of nuisance and disease-carrying mosquitoes along with a possible reduction in the incidence of mosquito-borne diseases and related human morbidity and mortality.

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Dedication

To the Almighty God is the Glory. I dedicate this dissertation to my parents: Jude Akpan Sr. (late father) and Augusta Jude Akpan (mother). Also, to my beloved children: Cardinal Jude Akpan, Alex Jude Akpan, and Gloria Jude Akpan.

Acknowledgments

I want to thank several persons who have made it possible for me to finish this stage of my academic and professional life: my dissertation committee chair, Dr. Ernest Ekong; my committee member, Dr. Richard Jimenez; and Dr. James Rohrer, my URR.

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Chapter 1: Introduction to the Study

Introduction

The focus of this study was to investigate the effects that environmental factors (i.e., temperature, wind, humidity, time, months, and weather) have on the development of pesticide resistance by the Culex mosquito populations. Controlling mosquito populations can result in fewer cases of disease-carrying mosquitoes. Mosquito-borne diseases, such as malaria, dengue, chikungunya, West Nile virus (WNV), St. Louis encephalitis, yellow fever, and the Zika virus, are global public health problems because about half the world's population, living in over 100 countries, remains at a high risk of contracting these diseases (Acevedo, Prosper, Lopiano, Ruktanonchai, Caughlin, Martcheva and Smith, 2015). In addition, mosquito-borne diseases carry a high financial burden, estimated to be at least $15 billion per year, mostly in the poorer countries of the world (Ranson and Lissenden, 2016). Besides, the socioeconomic burden associated with it, mosquito-borne diseases present a significant impediment to development in many African countries. For instance, diseases, such as malaria, remain the major cause of poverty in Africa and other developing countries, and it is estimated that malaria alone has reduced the gross national product of the African continent by more than 30% (World Health Organization [WHO], 2016).

The Culex mosquitoes and other species are the vector of many destructive mosquito-borne diseases such as dengue, malaria, and yellow fever (Platt, Kwiatkowska, Irving, Diabaté, Dabire and Wondji, 2015). In the continental United States, Culex mosquitoes are the leading vector of West Nile virus, encephalitis, and filariasis (CDC, 2016). However, pesticide resistance affects all classes of pesticides, all species of mosquitoes, and all mosquito-borne diseases (Barbosa and Hastings, 2012). In 2017, there were approximately 3,000 cases of West Nile virus reported in the United States (CDC, 2018). Globally, the populations at the greatest risk of vector-borne diseases are those that reside in the continents of Africa, Asia and America, with African populations accounting for the largest burden of the disease (Koella, Lynch, Thomas and Read, 2012). Soko, Chimbari and Mukaratirwa (2015), noted that in 2013, close to 230 million malaria cases were reported, leading to 600,000 deaths globally. About 90% of the mortality recorded from the WHO database was from the African region, and 80% of these occurred in children under the age of five (Samy, Elaagip, Kenawy, Ayres, Peterson and Soliman, 2016). The application of pesticide has been documented as the most effective method of controlling mosquitoes and has been adopted globally by the WHO (Cisse, Keita, Dicko, Dengela, Coleman, Lucas, Beach, 2015). The strategy is yielding substantial progress; however, the development of resistance by the mosquitoes is threatening to reverse the progress made by the use of pesticide (Corbel, Achee, Chandre, Coulibaly, Dusfour, Fonseca and David, 2016). Additionally, according to Terrell (2016), the Zika virus has been linked to microcephaly and Guillain-Barre syndrome. The Zika virus is also known to be transmitted by sexual relationships (Basundra, Hiremath, Khajuria and Ghodke, 2016). Additionally, the mosquitoes hinder many of our outdoor activities especially in the evenings and in the areas with warm temperatures (Ali, Gugliemini, Harber, Harrison, Houle, Ivory, Mordecai, 2017)

The important social change that will come as a result of this study is the reduction in the numbers of nuisance and disease-carrying mosquitoes, thereby increasing people's outdoor activities and possibly minimizing incidence of mosquito-borne diseases. Additionally, I intend to submit the findings of this study for publication in some peer-reviewed scientific and professional association journals, including the *Journal of American Entomological Association*, the *Journal of American Mosquito Control Association*, and the *Vector Ecology*, among others. The findings of the study will also be presented in many public health seminars and continued education forums. Such presentations and publications have the potential of adding to the existing knowledge about pesticide resistance in the mosquito populations (see Ali et al., 2017). Previous studies have focused only on the mechanisms of pesticide resistance and the treatment of mosquito-borne diseases but not the environmental factors associated the development of pesticide resistance in Culex mosquito populations. The mathematical model was the framework employed in this study.

Background of the Study

Mosquitoes are some of the most efficient vectors of many diseases in the animal kingdom and are responsible for more deaths than any other insect or animal (Saddler and Koella, 2015). Despite decades of control efforts, vector-borne diseases are still a major public health problem, especially in the tropical and subtropical regions of the world (Corbel et al., 2016). They are more common in the developing- and resource limited countries and inflict enormous burden in terms of morbidity and mortality (Kilpatrick and Randolph, 2012). The majority of their victims are from the less privilege members of our society (Koffi, Ahoua Alou, Adja, Chandre and Pennetier, 2013). Besides the health impact, mosquito-borne diseases also contribute to extensive poverty, underdevelopment, and school absenteeism and dropout in the developing countries (Oriento, Koske and Mutiso, 2016). These diseases are responsible for massive economic losses both in terms of health care costs and loss of productivity; again, mostly in countries that can least afford those (Wilson and Nguyen 2015).

When mosquitos touch the exposed skin and unleash the needlelike proboscis to pierce a blood vessel, they are not just sucking up a blood meal; they are also leaving something behind with their saliva: the mosquito-borne diseases (Acevedo et al., 2015). In many cases, the bites could present harmless anticoagulant that keeps the blood flowing and causes nothing but itchy skin (Corbel et al., 2016). In other cases, mosquitos carry serious and sometimes deadly diseases. Beyond the malaria carried by Anopheles mosquitoes, there is also the Zika virus carried by the Aedes aegypti and St. Louis encephalitis causes by Culex mosquitoes (Terrell, 2017). The three viruses combined with other mosquito-borne diseases are creating nightmare worldwide. Beside the disease potentials, mosquitoes are creating nuisances through their bites. In many parts of the world, including the Texas Gulf Coast, mosquito bites are interrupting outdoor activities (Murray, Ruktanonchai, Hesalroad, Fonken and Nolan, 2013).

To date, the most effective method of controlling mosquitoes has been through the use of pesticides. However, after years of progress, the application of pesticide to control the mosquito populations is becoming a strategy that scientists say is beginning to be less effective (Edi, Koudou, Jones, Weetman and Ranson, 2012). The effectiveness of pesticides that once helped in controlling the mosquitoes is diminishing. Public health professionals are estimating that the pesticide-treated nets, the WHO gold standard for stopping mosquito-borne diseases could lose much of its effectiveness before the year 2020 (Agossa, Gnanguenon, Anagonou, Azondekon, Aïzoun, Sovi and Akogbéto, 2015; Russell, Beebe, Bugoro, Apairamo, Chow, Cooper, Burkot, 2016). Urbanization and other man-made changes are making it easy for the mosquitoes to expand their range, even in developed countries like the United States (Chabi, Baidoo, Datsomor, Okyere, Ablorde, Iddrisu and Diclaro, 2016).

Mosquito-borne diseases are now reemerging partly because of the pesticide resistance that has developed in mosquito vectors and the drug resistance of pathogens (Barbosa and Hastings, 2012). Hence, addressing the problem of pesticide resistance developed by the Culex mosquito is a sound public health policy. Previous studies have focused extensively on controlling the nuisance mosquito populations and mosquito-borne diseases as they emerged. Since pesticide is the primary method of mosquito control, Corbel et al. (2016) stated that future studies should investigate the effects of environmental conditions (i.e., wind, temperature, and humidity) on the development of pesticide resistance by the mosquitoes. This is the gap that I investigated in this study.

Problem Statement

Mosquitoes and the diseases they carry have exerted enormous burdens, especially on poor, developing countries (Deming, Manrique-Saide, Barreiro, Koyoc-Cardeña, Che-Mendoza, Jones and Lenhart, 2016). For instance, West Nile virus infections have caused serious fatal neurological diseases in both humans and animals

(Mann, McMullen, Swetnam, Salvato, Reyna, Guzman, Barrett, 2013). About 80% of those infected with WNV will not present any symptoms (Acevedo et al., 2015). Like many other mosquito-borne diseases, there are no vaccinations available for WNV in humans (CDC, 2016). However, vaccines are available for horses (Chene, Houard, Nielsen, Hund, D'Alessio, Sirima and Viebig, 2016). In the United States in 2012, there was an unusual outbreak of the West Nile virus which resulted in about 6,100 human cases of infections, 500 deaths, and millions of dollars in economic loss (Murray et al., 2015). According to the latest estimates from the WHO (2016), in 2015 for another vector-borne disease, there were about 310 million cases of malaria worldwide and 640,000 subsequent deaths with 90% occurring in Africa. Additionally, the outbreak of the Zika virus in the Americas was declared a public health emergency by the WHO in 2015 (Plourde and Bloch, 2016). Available evidence has linked the virus to Guillain-Barre syndrome and microcephaly (Paploski, Prates, Cardoso, Kikuti, Silva, Waller and Ribeiro, 2016).

The impact of these diseases as well as other mosquito-borne diseases calls for the need for public health communities to do what they can to prevent future outbreaks of mosquito-borne diseases (Basundra et al., 2016). While it may not be possible to prevent all the disease outbreaks, at least the intensity of epidemics can be minimized. Delbue, Ferrante, Mariotto, Zanusso, Pavone, Chinaglia and Ferrari (2014) pointed out that it was difficult to treat mosquito-borne diseases because they could develop resistance to drugs. The authors concluded that the best method to control the mosquito-borne diseases is to control the mosquito vectors themselves. The most effective method available to control the mosquitoes is through the application of pesticides (Caputo, Ienco, Manica, Petrarca, Rosà and Torre, 2015). Unfortunately, the mosquitoes also develop resistance to the pesticides, thereby rendering them ineffective (Wanjala, Mbugi, Ototo, Gesuge, Afrane, Atieli and Yan, 2015). Therefore, there is the need for future studies to investigate the effects of pesticide resistance on the mosquito vectors.

Purpose of the Study

The purpose of this study was to investigate the association between the environmental factors (i.e., wind, temperature, humidity, time, months, and weather) and pesticide resistance in Culex mosquito populations. This was a quantitative, retrospective, cross-sectional, correlational study. The study populations were both the susceptible and feral Culex mosquitoes. The feral mosquitoes were those that were exposed to pesticides and collected from different geographical areas of a large county in the United States. The susceptible Culex mosquito populations were the Culex mosquitoes maintained in public health laboratories and insectaries. The primary independent variables were wind direction, outside temperature, humidity, months of pesticide application, time of day, and weather conditions, while the primary dependent variable was pesticide resistance, which was measured in mosquito mortality.

The modification of our natural environment creates the situation in which mosquitoes, the vector of many of the diseases that affect both human and animals, thrive if unabated with the potential to affect public health negatively. An example of the modification includes hurricanes, such as Harvey, with the accompanied flooding throughout the Gulf Coast area. Other cases of environmental modification include dams, irrigation systems, and impoundments, create fertile grounds for the mosquitoes that transmit diseases such as malaria, West Nile virus, dengue, and Zika virus. The control of these diseases requires the use of pesticides; however, the use of pesticides to control these diseases is a triple-edged sword. In one hand, the use of pesticide can help control the mosquito populations, while on the other hand, the mosquito can develop resistance that can render the pesticide ineffective, and pesticides can equally damage our environment (Barbosa and Hastings, 2015).

I performed descriptive and inferential analyses on the data set using SPSS Version 21. The descriptive statistical analyses included the measures of central tendency (i.e., mean, medium, and mode) to describe the study populations. Percentages were calculated and the numerical results summarized with tables and graphs including my interpretation. The descriptive statistics also included computing frequency statistics, summarizing a group of scores with a single number, and the range that helped in determining the spread of scores within the group. According to Brix, Bruland, Sarfraz, Ernsting, Neuhaus, Storck, and Dugas (2018), descriptive statistics are important because they present the data in meaningful ways easy for the audience to visualize.

The inferential statistics used for this study included both bivariate statistical analyses using chi-squared and multivariate statistical analysis using logistic regression. Bivariate statistical analysis involved cross tabs and chi square. Cross tabulation is a frequency statistic that displays the relationship between two variables in a single table (Adjakossa, Sadissou, Hounkonnou and Nuel, 2016). I computed measures of association to calculate the strength between one nominal variable with another nominal variable.

The Pearson chi-squared test was calculated as a correlation test for categorical variables to tell if they were statistically significant. The multivariate logistic regression model was the statistical technique used for modeling and analyzing the effect of multiple independent variables, that is the predicting criterion on a dependent or outcome variable.

Research Questions and Hypotheses

My overarching goal with this study was to investigate the association between environmental factors (i.e., wind, temperature, humidity, time, months, and weather) and the development of pesticide resistance by the Culex mosquitoes. The six research questions and corresponding hypotheses I developed to guide this study was as follows:

Research Question 1: What is the association between wind direction and pesticide resistance in Culex mosquitoes?

$H_0$1: There is no association between wind direction and pesticide resistance in Culex mosquitoes.

H_a1: There is an association between wind direction and pesticide resistance in Culex mosquitoes.

Research Question 2: What is the association between temperature and pesticide resistance in Culex mosquitoes?

$H_0$2: There is no association between temperature and pesticide resistance in Culex mosquitoes.

H_a2: There is an association between temperature and pesticide resistance in Culex mosquitoes.

Research Question 3: What is the association between humidity and pesticide resistance in Culex mosquitoes?

$H_0$3: There is no association between humidity and pesticide resistance in Culex mosquitoes.

H_a3: There is an association between humidity and pesticide resistance in Culex mosquitoes.

Research Question 4: What is the association between month of pesticide application and pesticide resistance in Culex mosquitoes?

$H_0$4: There is no association between month of pesticide application and pesticide resistance in Culex mosquitoes.

H_a4: There is an association between month of pesticide application and pesticide resistance in Culex mosquitoes.

Research Question 5: What is the association between time of the day that pesticide is applied and the development of resistance by the Culex mosquitoes?

$H_0$5: There is no association between time of the day that pesticide is applied and the development of resistance by the Culex mosquitoes.

H_a5: There is an association between time of the day that pesticide is applied and the development of resistance by the Culex mosquitoes.

Research Question 6: What is the association between weather (i.e., rain, snow, and ice) at the time of pesticide application and pesticide resistance in Culex mosquitoes?

$H_0$6: There is no association between weather (i.e., rain, snow, and ice) at the time of pesticide application and pesticide resistance in Culex mosquitoes.

H_a6: There is an association between weather (i.e., rain, snow, and ice) at the time of pesticide application and pesticide resistance in Culex mosquitoes.

To answer the six research questions, I obtained the data set from a large county's Public Health Department. The data came from the yearly Field Cage Test results conducted by the Public Health Department to examine the efficacy of the pesticides they applied to control mosquito populations between the years of 2013 to 2017.

With the first set of research questions, I examined the effects of environmental factors (i.e., wind, temperature, humidity, time, months, and weather) in resistance development by the Culex mosquitoes. To address these Research Questions, percentages were calculated, and the numerical results were summarized with tables and graphs including my interpretations. The descriptive statistics used included computing frequency statistics, summarizing a group of scores with a single number, and the range that helped in determining the spread of scores within the group. In addition, I calculated frequency distribution tables representing the effectiveness of the two classes of pesticides on mosquito mortality in each of the five years under investigation. The department applies two pesticides (i.e., Fyfanon and Permenon) on weekly rotations. I determined whether the potential predictor variable (i.e., pesticide) had a significant correlation with the criterion variable (i.e., the mosquito mortality). With the second set of research questions, I examined the effects of timing on pesticide efficacy, and this involved the use of inferential statistics: chi square and logistic regression.

Conceptual Framework

One of the negative outcomes of mosquito bites is the transmission of diseases, which can affect mosquitoes, birds, and humans. In this study, I used the mathematical model (also called the compartmental model) to explain the dynamics of mosquito control and disease transmission. The mathematical model of mosquito control divides the affected human populations into groups called compartments (Barbosa & Hasting, 2012). According to Mandal, Sarkar and Sinha (2011), the mathematical model has been used to provide the framework for understanding the mosquito control and transmission dynamics of mosquito-borne diseases in humans for more than a century. In this model, the transmission of the infectious agents to the susceptible hosts is the central element (Gourley, Liu, and Wu, 2012). When the mosquito-borne pathogens appear, the pathogens partition the populations into compartments depending on the density of the parasites and the type of infections (Cummins, Cortez, Foppa, Walbeck and Hyman, 2012). The compartments are represented by the notations susceptible, exposed, infectious, and recovery SEIR (Voirin, Barrat, Cattuto, Colizza, Isella, Régis, Pinton, Khanate, Broeck and Vanhems, 2011). The first compartment represents the populations that are susceptible, while the next compartment is the exposed class, those people infected by the pathogens but are unable to transfer the infection to others during the latent period (Aldila, Nuraini, Soewono and Supriatna, 2014). The third compartment is the infectious group who are capable of transferring the infections to others through the interactions with the susceptible group (Perkins, Scott, Le Menach and Smith, 2013). Finally, those that can recover from the disease are the last compartment in the notation (Green, 2014). In this study, I used the mathematical model to explore the role of SEIR in the transmission dynamic of the mosquito vectors on pesticide resistance and the human hosts.

The use of compartments or the mathematical model separates different stages of mosquito-borne infection, the parasite in the population density, and susceptibility to pesticide (Mandal et al., 2011). The model uses two features in the prediction of the disease's progression in the host and vector populations (Aldila et al., 2014). The mathematical model represents the dynamics of pesticide concentration as the chemical properties change over time (Haario and Nannyonga, 2014). My purpose of using the compartmental model was two-fold: (a) the first was the genetics, which is the process that the pesticides go through at the physiological level and (b) the second was the intensive application of the pesticides, the mechanism of resistance to pesticides related to the changes in pharmacology.

The advantage of using mathematical model in this study was that the mathematical representation of biological processes allowed for accuracy regarding the epidemiological assumptions of the effects of pesticides. That afforded me a greater understanding of the mosquito-borne disease epidemiology by comparing SEIR and the mosquitoes that survive pesticide application during the test. Evaluation of the mortality rate of the mosquitoes under investigation was then made possible through the use of this model.

Nature of the Study

This was a quantitative, cross-sectional study. The quantitative approach allowed for the use of numerical data to investigate the factors associated with the development of pesticide resistance by mosquitoes. The primary independent variables were wind direction, outside temperature, humidity, months of pesticide application, time of day, and weather conditions, while the primary dependent variable was pesticide resistance, which was measured in mosquito mortality.

The Southern Region of USA represent an excellent model to study mosquitoes and the diseases they vector. Several factors contribute to this. During the 2012 West Nile virus outbreak in the United States, the area accounted for over 35% of both the numbers of cases and fatalities (Mann et al., 2013).The southern area of the United States also serves as a temporary roosting site on a major flyway for migratory birds in transit between the temperate and tropical regions of the world (Murray et al., 2013). There was also constant surveillance of the St. Louis encephalitis virus in local mosquito populations by the county Mosquito and Vector Control Division since the mid-1960s, which provided an excellent infrastructure for the detection of other mosquito-borne disease activity in the mosquito vector and the wild bird reservoir on this major bird migratory pathway (Delbue et al., 2014).).

The transmission of mosquito-borne disease pathogens is maintained in an enzootic cycle between the mosquitoes, birds, and equine (Murray et al., 2013). The routine collections of West Nile virus-positive birds and mosquito pools for decades have provided an outstanding opportunity to investigate other viruses. Moreover, the testing county represents an interesting ecosystem, namely a warm year-round climate with unique resident mosquito and avian species capable of transmitting any mosquito-borne disease comparable to those available in the mosquito endemic countries. Additionally, this region hosts more avian species (and possibly more mosquito-borne disease reservoir hosts) than anywhere else in the United States (Mann et al., 2013).

Finally, pesticides and mosquito resistance to them are universal (Edi et al., 2012). The same pesticide used in controlling Anopheles mosquitoes that transmit malaria in Africa is the same pesticide used in controlling Culex mosquitoes that vectors West Nile virus in the southeastern part of US as well as for controlling Aedes mosquitoes that transmit the Zika virus in Brazil. Therefore, the results of this study of pesticide resistance can be of benefit to other areas and regions in the world. Hence, the data from resource-rich county Public Health Department could be of great benefit to the resource-limited, third world countries in their efforts to control malaria.

In this study, I used a quantitative, retrospective, cross-sectional, correlational design to describe the effects of pesticide resistance on the mosquito populations. The data set came from the results of yearly Field Cage Tests conducted by the large county's Public Health Department, Mosquito and Vector Control Division between 2013 and 2017 to assess the effectiveness of pesticide the department applies to control mosquitoes in the county. The test had been performed by the county's Public Health Department since mid-1960s, and the results are available to the public. In the Field Cage Tests, the feral Culex mosquito populations were assessed to determine whether they were exposed to the pesticides and whether they had the outcome of interest: resistance.

Definitions

Pesticide resistance: Agossa et al., (2015) defined pesticide resistance as the acquisition of heritable characteristics in mosquitoes that results in the repeated failure of pesticide to provide the intended level of control when used according to recommendations. Also, it is the decrease in susceptibility of the mosquito populations to the pesticide that was previously effective at controlling the pest (Yadav, Rabha, Dhiman and Veer, 2015).

Wind: Albeny-Simoes et al., (2014) defined wind as the quantity of air that is set in motion naturally and that is blowing with any degree of violence.

Temperature: According to Polson et al., (2012) temperature is the measurement of hotness or coldness, which is expressed in terms of any of the arbitrary scales and indicates the direction with which the heat energy will spontaneously flow.

Humidity: Humidity is the quantity of water vapor present in the atmospheres. The level of humidity in the atmospheres indicates the possibility of precipitation, fog, or dew (Caputo et al., 2015).

Climate: Samy et al., (2016) defined climate as the long-term averages and variations in weather measured over a period of several decades.

Weather: It is short-term condition of the atmosphere in the past, present, or future. It is described in terms of humidity, wind, temperature, precipitation, cloudiness, as well as other variables (Brown et al., 2015).

Timing of pesticide application: The time of the day or month of the year that pesticides are applied. For instance, applying pesticides at the time of the day in which the temperature falls between 60 and 85 degrees is preferable. On the other hand, applying pesticides at the month of the year with mosquito postinfestation is ideal (Naranjo, Qualls, Jurado, Perez, Xue, Gomez and Beier, 2014).

Mosquito-borne diseases: The diseases transmitted by mosquitoes that include among others West Nile virus, malaria, chikungunya, and dengue (Kasai et al., 2014).

*Vecto*r: An organism that is capable of transmitting a disease (Regis et al., 2013).

Mosquito mortality: The state and conditions of being subject to death through the use of pesticides (Godfray and Coulson, 2013).

Metabolic resistance: Menze et al., (2016) defined metabolic resistance as the ability of a resistant mosquito to detoxify or destroy pesticide faster than the susceptible mosquitoes or get rid from their bodies of the toxic effect of pesticide molecules.

Target-site resistance: According to Platt et al., (2015), target site resistance is the ability of mosquitoes to alter the target-site where pesticides act in the mosquitoes that would genetically modify the pesticide and prevent it from interacting and binding at the site of action, thereby reducing or eliminating the toxic effects of the pesticide.

Mathematical model: Voirin et al., (2012) noted that the mathematical model is also known as compartmental model because it divides the affected populations into compartments in an event of a disease outbreak. According to the authors, the model enables scientists to calculate from the available facts about the state and possibly the development of an outbreak.

Assumptions

In the analysis of the factors associated with the development of pesticide resistance by Culex mosquitoes, I relied on archived data. The following assumptions characterized the study:

- By using the mathematical model, I assumed that the total affected population was constant.
- There was the possibility of missing data, a situation that is well-known when using secondary data.
- I also assumed that the information in the database contained valid and reliable data constructed from the results collected through a rigorous and valid process. During the Field Cage Tests, several agencies participate.

Scope and Delimitations

The scope of this study was limited to the use of archived secondary data to investigate the possible effects that pesticide resistance had on the control of mosquito populations. The data came from a large county's Mosquito and Vector Control Operations obtained during their Field Cage Testing. The data were limited to those collected between 2013 and 2017. I conducted this study by examining the mortality rates in the wild mosquitoes collected from nine of the 268 geographical areas of the county.

Limitations

The focus of this study and the data used were specific to the Culex mosquito populations, the Southern Area of US, and the two classes of pesticides: Malathion and Pyrethrin. The findings of this study cannot be generalized to any mosquito species other than the Culex mosquitoes. Even within the Culex mosquito populations, the findings of this study cannot be generalized beyond the testing areas and the four summer months (i.e., June, July, August, and September). Though most countries in sub-Saharan Africa and most other developing countries face similar and even worse mosquito problems, the results cannot be generalized to other mosquito endemic regions. The response of the Culex mosquitoes to pesticides were not tested during the winter months. This is the period when the entomologists believe that susceptibility is bred back into the mosquito populations (Smart et al., 2016). The results cannot be generalized to other mosquito species and other classes of pesticides than those used in this study and analyses.

In this study, I used secondary data from a large county's Public Health Department, Mosquito and Vector Control Division to investigate the association between the dependent and independent variables. The limitations inherent in the use of secondary data applied. I identified the following limitations in this study:

- Data collected for other purposes were used.
- The original data collection was not influenced by the current research topic; hence, some data might be missing or incomplete.
- Additional data manipulations were done to account for the missing and incomplete data.
- A cause and effect outcome was not possible; rather the associations between the independent and dependent variables were explored. Since the primary dependence variable for this study was pesticide resistance, which was measured by mosquito mortality that minimized the chances of other confounders. The result had potentially higher internal validity.

Significance of the Study

The results of this study have the potential to advance the knowledge about mosquito control in the field of medical entomology and promote sound public health policies in controlling both the nuisance and disease-carrying mosquitoes. The findings can also be used to reduce the incidence of mosquito-borne diseases and help in increasing the numbers of outdoor activities in the evening world-wide. Mosquitos and the diseases they transmit are difficult to control because of the adaptable nature of the parasites, hosts, and vectors (Velazquez-Castro, Anzo-Hernández, Bonilla-Capilla, Soto-Bajo and Fraguela-Collar, 2018). While some effective methods have been developed to combat the vectors, the mosquitoes and parasites equally continue to elude those methods if used ineffectively (Millius, 2016). According to Reid and McKenzie (2016), malaria is among the most destructive infections in human history and resulted in about 640,000 deaths globally in 2015. Malaria has significant effects on the health of infants, young children, and pregnant women and contributes to malnutrition, indirectly causing the deaths of over half of all the children under the age of 5 in third world countries (WHO, 2016). In Africa, one fifth of all cases of severe maternal anemia and low birth weights are linked to malaria (Koella et al., 2012). This disease has spread because mosquitoes have developed resistance to pesticides, thereby making it difficult to effectively control the diseases they vector (Agossa et al., 2015).

In 2017, there were about 3,000 cases of WNV in the United States (Mann et al., 2013). Out of that 3,000, there were approximately 1,500 neuro-invasive and 800 non-neuro-invasive cases of WNV, resulting in 150 deaths (CDC, 2018). Also in 2015, there were about 310 million cases of malaria with 640,000 deaths globally with about 90% of the deaths occurring in African countries (WHO, 2016). Given these statistics, it becomes necessary that solutions other than those of a temporary nature be implemented. In this study, I investigated the factors associated with the development of pesticide resistance by Culex mosquito populations.

The results of this study could be used to reduce the numbers of nuisance and disease-carrying mosquitoes, thereby creating healthy environments and possibly reducing the incidence of mosquito-borne diseases. Social change can also come from drawing the attention of public health practitioners and pest control professionals to the use of other means of controlling the mosquito populations such as the Integrated Mosquito Control System (IMCS). The IMCS emphasizes the use of preventive and alternative means to control the mosquito such as wolbachia (i.e., mosquito-eating parasites) and other mosquito predators (Hancock, Sinkins and Godfray, 2011). Additionally, I intend to present the results of this study for publication in several peer-reviewed, scientific and professional association journals. The findings of the study will also be presented in public health seminars and continuing education forums. Such presentations and publications have the potential of adding to the existing knowledge about pesticide resistance in the mosquito populations.

Significance to Social Change

The findings of this study could result in potential positive social change through a reduction in the numbers of nuisance and disease-carrying mosquitoes, thereby creating healthier environments and a possible reduction in the incidence of mosquito-borne diseases. Over time, with the repeated and broad application of pesticides in an area, resistance can develop in the target mosquito populations; hence, if the development of pesticide resistance by the mosquito populations can be minimized, that would represent a positive social change. Furthermore, understanding the environmental factors associated with the development of resistance by mosquitoes could increase public health knowledge about mosquito vector control strategies, which could promote the wise use of pesticide (see Ali et al., 2017). For instance, if high wind speed, high humidity, and high temperature can adversely affect pesticide application, then applying pesticide at times and conditions more conducive to minimizing the adverse effects of resistance development would be a wise use of pesticide and an important positive social change (.see Nkya, Akhouayri, Kisinza and David, 2015).

Summary and Transition

Since the mid-1940s, pesticides have been used to control mosquitoes and are still an important component of mosquito control operations by public health professionals. The uses of pesticides have resulted in the significant reduction in the numbers of nuisance mosquitoes as well as those of the disease-carrying mosquito populations (Naranjo et al., 2014). However, the more pesticides are applied, the more the mosquitoes develop resistance to them. As such, pesticide resistance can profoundly affect public health if the applied pesticides are ineffective in controlling the mosquitoes. An increase in mosquitoes will increase mosquito bites, which can transmit mosquito-borne diseases and can prevent individuals from engaging in many outdoors activities such as sporting events, camping, as well as many others. Studies have shown that mosquito parasite virulence (i.e., the harm mosquito parasites cause in the human hosts) is positively and directly correlated with the numbers of biting mosquitoes (Barnard, Knue, Dickerson, Bernier and Kline, 2011). . Therefore, I investigated the association between environmental factors and the development of pesticide resistance by Culex mosquito populations. In Chapter 2, I will provide a review of literature in relation to the topic under study and the theoretical framework.

Chapter 2: Literature Review

Introduction

Mosquito bites can cause mosquito-borne diseases and prevent individuals from engaging in many outdoors activities, such as sporting events or camping. Studies have shown that mosquito parasite virulence (i.e., the harm mosquito parasite cause in the human hosts) is positively and directly correlated with the numbers of biting mosquitoes (Albeny-Sinoes et al., 2014. Mosquito-borne diseases and viruses cause some of the most destructive infectious diseases in the world and disproportionately affect economically disadvantaged countries (Hancock et al., 2011). In the interconnected global community, vectors and pathogens can quickly spread around the world. Mosquito-borne diseases, such as malaria, West Nile virus, and Zika virus are experiencing a reemergence (Menzel et al., 2016). The global increases in trade, climate changes, and land use have made many parts of the world more vulnerable to the mosquitoes and mosquito-borne diseases (Deming et al., 2015). According to Yadav et al., 2015), the World Health Organization estimated that there are over 100 million cases of dengue fever reported annually and about 75% of the cases arise from Asia. Strode, Donegan, Garner, Enayati and Hemingway (2014) also mentioned that in 2010, there were over 310 million clinical cases of malaria globally, resulting in 640,000 deaths, with 90% of the cases and fatalities on the continent of Africa. Pesticide resistance takes multiple forms in the mosquito populations, including metabolic resistance, cuticular resistance, and target-site resistance. In this Chapter, I will review the literature related to pesticide resistance and mosquito control.

Literature Search Strategy

I found the extant literature and articles used for this study in many electronic databases accessible through the Walden University Library and other credible sites. The databases included PubMed, Medline, and EBSCO, and journals focusing on malaria and emerging infectious diseases. I also reviewed information from credible organizations, such as the CDC; WHO; the state's Department of Health Services; and the county's Public Health Department, Mosquito and Vector Control Division. The professional journals that published articles included in this study were *Malaria Journal, the American Journal of Tropical Medicine and Hygiene, the East African Journal of Public Health, the International Journal of Epidemiology, and the American Journal of Tropical Health and Hygiene*.

The key search criteria was articles and materials published between 2011 and 2019. The search terms used were *mosquitoes, mosquito-borne diseases, vector-borne diseases, malaria, dengue, chikungunya, West Nile virus, Zika virus*, and *pesticide resistance*. The literature search and review produced more than 70 scholarly articles that helped in the investigation of the association between pesticide resistance and the control of mosquitoes.

Theoretical Foundation

The history of mankind is shaped by various outbreaks of infectious diseases. Many nations and civilizations have been destroyed through the ages because of infectious diseases. The list of pandemics is too long to mention. It started from the biblical Pharaonic Plagues of Egypt in 1715 BC (Lotfy, 2015). The Cocolizth epidemic of Athens in 430 BC resulted in the death of 13 million people, while the Black Death bubonic plague of Europe resulted in the death of over 25 million people in five years: 1346–1351 (Shipman, 2014). The pandemic influenza of 1919 that swept through Africa, Asia, Europe, and the Americas killed over 40 million people (Shanks and Waller, 2019). In the last several decades, the emergence and reemergence of infectious diseases, such as HIV, measles, malaria, and tuberculosis, have equally caused deaths to millions of people annually (Wanjala et al., 2015). The WHO (2016) estimated that as of 2010, about 640,000 people died from malaria and 34 million died from the global HIV epidemic that included 3.5 million children. The rapid theoretical advancement has enhanced public health capabilities in the fight against these pandemics. At the center of the fight is the mathematical model that plays critical roles in shedding lights on the problems of infectious diseases while helping policy makers in the decision-making process.

Mathematical Model in Epidemiology

The first publication that addressed the mathematical model in epidemiology dated back to 1766. In that year, Daniel Bernoulli used the mathematical model to help in analyzing the mortality due to smallpox in England (Aldila et al., 2014). At the time, Bernoulli used the mathematical model to show that vaccination against the smallpox virus would increase the life expectancy by up to three years (Barbosa and Hastings 2012). That was followed by Ross, Kermack, and McKenderick in their publication of a seminal paper that created the mathematical model, sometimes called the compartmental model of epidemiology (Acevedo et al., 2015), which was used as the conceptual framework of this study.

According to Mandal et al. (2011), the compartmental model has been used to provide the framework for understanding the transmission dynamics of mosquito population and mosquito-borne diseases in human for more than 100 years. In the compartmental model, the transmission of the infectious agents to the susceptible hosts is the central element (Gourley et al., 2011). When the mosquito-borne pathogens appear, they partition the populations into categories depending on the density of the parasites and the type of infections (Colvin, Peterson, Kent and Schreck, 2015). The categories called compartments are represented by the notation: SEIR (Voirin et al., 2011).

Literature Review

Metabolic resistance is the most important resistance in mosquitoes concerning the use of pesticides; this resistance is a result of the activities of the genetic makeup of the mosquito families (Menze et al., 2016). Those genes can metabolize the pesticides, thereby preventing them from reaching the target sites in the mosquitoes (Strode et al., 2014). Cytochromes P450s are responsible for most of the metabolic resistance in mosquitoes (Platt et al., 2015). Metabolic resistance is also a result of the nonsilent point mutation in the sodium channel genes (Corbel et al., 2016). The resistance blocks any successful binding of pesticide molecules to the sodium channels (Deming et al., 2016).

The Mosquito Feeding Cycle

A mosquito searches for hosts until they are successful or die in the process (Kasai et al., 2014). At every attempt, the mosquito search for the human victim with the probability of Q, and the animals with the probability of 1-Q. If the mosquitoes are searching for humans, they encounter a pesticide treated host with the probability of I, the mosquito is either repelled and repeats the host searching attempt with the probability of R, or they are repelled and feed on the susceptible host successfully at the probability of (1-r)s. If they are not repelled, they are killed by the pesticide with the probability of (1-r)(1-s). With such assumption, the probability of the mosquito achieving a successful bite in a single attempt is as follows:

$$1-Q\,[1-(1-R)\,s]$$

(Ross, 1915)

Mathematical Model and Pesticide Resistance

Pesticide resistance in the mosquito is caused by three primary mechanisms: (a) the reduced sensitivity of the target site, (b) reduced penetration of the pesticides due to altered cuticles in the mosquitoes, and (c) the increased level of detoxification enzymes (Kassai et al., 2014). Mosquitoes develop the resistance by acquiring one or more of those mechanisms. Metabolic-mediated resistance is the key resistance mechanism in mosquitoes (Li, Guo, Zhang, Dong, Xing, Yan and Zhao, 2016). There are three classes of metabolic enzymes involved in the metabolism of the pesticides: esterases, glutathione transferees and P450s (Riveron et al., 2014).

In 1915, Ross published the compartmental model for mosquito-borne in the malaria infection. In his model, there are two infected classes- the infected human and the infected mosquitoes. The infected human population (Ih) increases when the non-infected humans (1- Ih) come into contact with infected mosquitoes (Im)and are bitten at a rate α.

λ is the proportion of those bites that result in human infection, and ω is the ratio of female mosquitoes to that of humans. Humans then recover at an average rate γ. The equation is as follows:

$$\frac{dI_h}{dt} = \alpha\lambda\omega I_m (1 - I_h) - \gamma I_h$$

$$\frac{dI_m}{dt} = \alpha v I_h (1 - I_m) - m I_m$$

Ross (1915)

The mosquitoes are infected when they bite the infected human at the rate of αν, the product of the biting rate and the proportion of bites by which mosquitoes becomes infected. Mosquitoes die at a rate m (Mandral et al., 2011). Otieno, Koske and Mutiso (2016) used the mathematical model to explain how pesticide resistance influences the transmission of mosquito-borne diseases.

Literature Review Related to Mosquito Biology

Mosquitoes are two-winged insects belonging to the family Culicidae and the order Diptera. The family of Culicidae is further divided into three other sub-families: Toxorhynchitinae, Anophalinae, and Culicinae. Globally, there are about 3,500 species and 37 genera of mosquitoes (Sinden, 2015). Mosquitoes like humid, warm, and moist conditions for survival; they can survive everywhere except the areas that are permanently frozen. There are two major characteristics of mosquitoes- the Floodwater mosquitoes and the Permanent water mosquitoes. The floodwater mosquitoes lay their eggs on the moist substrate, and not on the standing water. The biological requirement is that the eggs must first dry out completely for a period before they can become viable eggs. Once the drying period has been achieved, the eggs can hatch if the area is flooded with water (Cardo, Vezzani and Carbajo, 2012). The resurgence of mosquitoes from nowhere once there is any rainfall is a result of flood water mosquitoes. On the other hand, the permanent water mosquitoes lay their eggs on the surface of water either in a raft that can contain as many as 300 mosquitoes or singly (Godfray and Coulson, 2013). Generally, the eggs of the permanent water mosquitoes may hatch within 24 hours (Cardo et al., 2012).

The Life Cycle of Mosquitoes

Mosquitoes have four distinctive stages in their life cycle: eggs, larvae, pupae, and adults (Godfray and Coulson, 2013). Depending on the species of mosquito involved, mosquitoes can lay their eggs either on the surface of water or the moist soils and other objects capable of flooding (Cardo et al., 2012). The larva and pupa are the aquatic stages of the lifecycle because they can only survive in water (McBride, Vosshall, Baier, Spitzer, Omondi, Ignell and Sang, 2014).The adults are the active flying insects (Sinka M. E, Bangs M. J, Manguin S, et al., 2012). Some mosquito species may go through the entire life cycle in as little as five days to as long as one month (Godfray and Coulson, 2013).

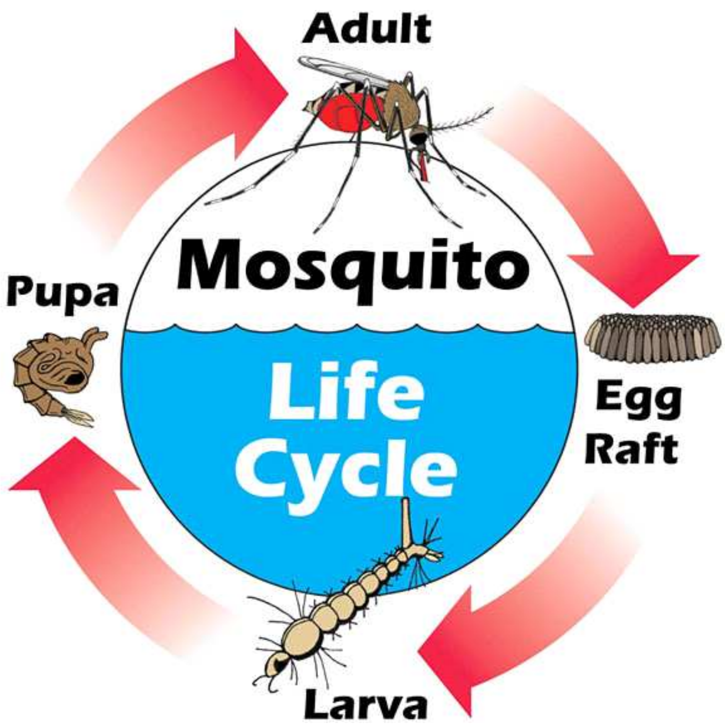

Figure 1. Life cycle of mosquitoes; retrieved from http://www.portsmouthva.gov/456/Mosquito-Life-Cycle.

The Adult Mosquito

Female adult mosquitoes require a blood meal for their egg production; therefore, they bite birds and animals, both warm and cold blooded (Cardo et al., 2012). There are some factors that influence the biting habit of mosquitoes, including the availability of carbon dioxide, smell, temperature, color, and moisture (Agosa et al., 2015). Since male mosquitos do not require blood for egg production, they do not bite (Sinden, 2015). Both male and female mosquitoes feed on the nectars of flowers and other suitable sugar sources (Kasai et al., 2015). The females of the mosquito species known as Toxorhynchitis do not bite or obtain the blood meal for egg production (Godfray and Coulson, 2015). The female mosquitoes capable of feeding prefer to obtain their blood meals from birds, horses, and small mammals (Yadav et al., 2015). Human blood meals are their second choice (Sinden, 2015). Aedes and Ochlerotatus are the species that bite persistently and painfully (Wang et al., 2015). The preferred time for their blood feed is the early morning, at dust and the early evenings (Albeny-Simoes et al., 2015). Culex mosquitoes may bite also; they prefer to bite at dust and dark (Agosa et al., 2015). Their preferred feeding animals are birds, cow, and horses (Acevedo et al., 2015).

Figure 2. Adult female mosquito. Copyright c 2016. Retrieved from http://www.portsmouthva.gov/456/Mosquito-Life-Cycle.

Blood-Feeding Behavior of Mosquitoes and Disease Transmission

Spillings, Coetzee, Koekemoer and Brook (2008) observed that mosquitoes may be generalists and or opportunistic in feeding habits. On the one hand, they might be the specialist in their preference for some hosts species. For instance, the primary vector of West Nile virus- the *Culex* mosquitoes prefer feeding on some avian and mammalian hosts. Therefore, determining the feeding preferences of the mosquito species is important in the prediction of their disease transmission. The effective bridge vectors of many mosquito-borne diseases feed on the host preferably the avian hosts during their first blood meals to become infected with the parasites. However, the same mosquito species will obtain the second blood meal from the human to transmit the infected virus. On the whole, the *Culex tarsales* are more generalized feeders than the rest of the *Culex* complexes. As a result, the temporal alternation in their feeding behavior leads to the spillover of the diseases from the enzootic cycle in the early parts of mosquito season to human in the later part of the season (Ciota and Kramer, 2013).

Moreover, the genetic characteristics of the *Culex* mosquitoes are an important factor affecting the host feeding preferences. The European populations of the *Culex* species are pure while the Culex populations in the United States contain various Culex molecules characteristics. It is the properties that correlate to the geographical variations and the ability to feed on mammals and the possibility of spillover to humans (Doucoure and Drame, 2015). The mosquito vectors that prefer avian blood meals will be affected by a number of factors including the avian nesting places, the time of the day the avian are active, as well as where the forage. The availability of avian population and distribution is a critical factor in the prediction of mosquito-borne diseases. When there is a drought in an area, the result is the aggregation of mosquito species and wild birds, thereby resulting in a high level of mosquito-borne disease transmission such as the case of the 2012 West Nile virus situation in the Texas Gulf Coast (Kilpatrick & Randolph, 2012).

Insect Growth Regulators

The (IGR) are the pesticide that mimics the hormones of the young mosquitoes. The regulators disrupt the growth and reproduction cycle of mosquitoes. They rarely produce fatalities in mosquitoes; their effects are seen on preventing the eggs production and hatching of the eggs. Beyond that, the IGR prevents the molting of the mosquitoes from the larval stage to pupal stage. These are the class of pesticides that disrupt the critical physiological functions associated with the normal growth, development, and reproduction of mosquitoes. The adult mosquitoes exposed to IGR do not suffer adverse effects of the pesticides (Caputo et al., 2015).

The Genesis of Pesticide Resistance

The individual genes of an organism determine the physical and behavioral traits of the organism. In the case of mosquitoes, when they reproduce, they pass on their unique combination of the genes to their offspring. Certain environmental conditions favor some physical and behavioral traits in mosquito populations. The mosquitoes with genes that can improve their survival will probably pass on those genes to their offspring (Silva et al., 2014). Those traits include the resistance and can develop when a portion of mosquito population can survive the destructive effects of the pesticide. These mosquitoes will reproduce and pass on their pesticide resistance to their offspring. If the same pesticide with the same mode of action is repeatedly used against the resistant group of mosquitoes, a greater proportion of the mosquito will likely survive. Wang et al. (2015) noted that the result is that once effective pesticides can no longer control the mosquito populations.

The repeated use of the same class of the pesticide to control mosquitoes can cause changes to the mosquito genes pools and lead to resistance. When the pesticide is first applied to mosquitoes, at least, a small proportion of the mosquito population may survive the exposures because of their genetic makeup. Those mosquitoes will pass on the genes for resistance to the next generations. The subsequent application of pesticides will increase the proportion of the less susceptible mosquitoes. Through a process such as that, the mosquitoes gradually develop resistance to the pesticides (Wang et al., 2015).

The resistance to pesticides used in controlling the mosquito populations is now a regular occurrence. But, how does mosquito develop the resistance to pesticides? Scientists believe that it is due to the mutations that provide the mosquitoes that probability of surviving the applied pesticides at the toxic doses that would be normal under usual conditions (Kasai et al., 2014). Entomologists have studied the case of *Culex pipens* mosquitoes that become resistant to some classes of pesticides through overproduction of the secretion of the two main enzymes- esterase A and esterase B. The two enzymes trap the pesticides before they can enter the nervous systems of the mosquitoes (Silva et al., 2014). The overproduction of the two enzymes is due to two factors. The first factor may be attributable to a simultaneous increase in the numbers of the duplicate of two or one of the corresponding genes. Or it may be due to the phenomenon that the expression of esterase A gene is controlled. Understanding the dynamic, origin, and mechanisms of mosquito resistance to pesticides can have significant effects on the strategies of combatting the mosquitoes and the diseases they vector (Li et al., 2016)

Summary and Conclusions

Mosquitoes transmit some of the most dangerous diseases to both the humans and animals. After many decades of repeated use of pesticides to control mosquitoes, some species have shown the ability to evolve the resistance to pesticides. Whenever mosquitoes develop resistance to pesticides, it undermines the control of diseases they transmit because in many cases, it increases the numbers of mosquitoes that survive the treatment. There are two scenarios here- the potential for disease control failure or vector control failure. Even when there is a potential for mosquito-borne disease failure, we must work hard to succeed in the control of mosquito populations.

Pesticide resistance has impacts on the quality of mosquito vectors and on the primary determinants of parasite transmission: the vector competency, longevity, and behavior. The chances are that pesticide resistances will likely result in either decrease mosquito longevity, a decrease in the infectious capacity or the changes in behavior. All those will certainly reduce the vectorial capability of mosquitoes. If such effects are large enough, then the impact of pesticide resistance may not be as grave. On the other hand, if pesticide resistance has the opposite effects- that are increasing the vectorial capacity of mosquitoes, the result may be a dramatic increase in the mosquito-borne diseases. In many cases, the prevalence may be higher than in the absence of pesticides. In either way, there is no simple solution. The consequences of the evolution of pesticide resistance for mosquito-borne diseases deserves this much attention because the lives and welfare of millions of people especially in the Continent of Africa deserve attention. The next chapter will examine the methodology which will include the study population, sampling procedures, and the data collection instrument.

Chapter 3: Research Method

Introduction

The purpose of this quantitative, cross-sectional, correlational study was to investigate the environmental factors that may be associated with Culex mosquitoes' resistance to pesticides. In this quantitative study, I explored the association between the independent variables (i.e., wind direction, outside temperature, humidity, time of the day, month of the year, and weather condition at the time of pesticide application) and the dependent variable or outcome of interest (i.e., pesticide resistance among Culex mosquito populations). The study populations were both the susceptible and feral Culex mosquitoes. The feral mosquitoes were those that were exposed to pesticides and collected from different geographical areas of the large county under study. The susceptible Culex mosquito populations were the Culex mosquitoes maintained in public health laboratories and insectaries. The management of pesticide resistance in the mosquito vector population is essential for the public health efforts to contain the emergence and reemergence of mosquito-borne diseases such as malaria, St. Louis encephalitis, West Nile virus, and the Zika virus, among others.

This Chapter will focus on the quantitative methodology and cross-sectional retrospective correlational design employed in the study. The design and approach, setting and sample, the inclusion and exclusion criteria, reliability and validity, variables, data collection, and data analysis approach will be discussed. I will also describe the research design and its appropriateness to the problem statement. The chapter will also include a description of the data analysis approach, ethical issues associated with non-human subjects, and conclude with a summary.

Research Design and Rationale

In this retrospective, cross-sectional, correlational study, I investigated the feral Culex mosquito populations collected from nine geographical areas for the presence of pesticide resistance. The independent variables for this study included wind speed, outside temperature, humidity, time of the day, month of the year, and weather condition at the time of pesticide application. The dependent variable or outcome of interest was pesticide resistance among Culex mosquitos. The data for this study was drawn from the yearly Field Cage Test results for pesticide efficacy conducted by a large, urban county's Public Health Department, Mosquito and Vector Control Division to determine the effectiveness of the pesticides used by the department to control the mosquito populations. I used the retrospective design because the data were entered into database files at the county's Public Health Department, Mosquito and Vector Control Division. A cross-sectional approach was appropriate because with it, different population groups could be compared at a single point in time. Additionally, the major characteristics of a cross-sectional study design are that it allows researchers to compare many different variables at the same time (Djalalinia, Modirian, Sheidaei, Yoosefı, Zokaiee, Damirchilu and Abachizadeh, 2017). I used the archived, retrospective secondary data approach because it saved time and financial resources.

The database from which the sample case for this study was drawn is maintained by a large, urban county's Public Health Department, Mosquito and Vector Control

Division. The data are available free to the public on request. I analyzed the data using both descriptive and inferential statistical analyses, which I will describe in detail in the following sections.

Methodology

Population

The target population for this study was the Culex quinquefasciatus mosquito. This mosquito species is also known as the southern house mosquito. It is brown in color and medium size mosquito found throughout the Southern and the Gulf Coast areas of the United States (Forrester and Gardner, 2013). This mosquito is active at night and early evening hours and is the primary vector of St. Louis encephalitis virus and West Nile virus (Ciota and Kramer, 2013). The Culex mosquito species is important because it is known to carry many pathogens and capable of transmitting many diseases.

Data collection

Sampling and Sampling Procedures

The study populations were the Culex mosquito populations and the sample populations were those mosquitoes that had shown resistance to pesticides. Therefore, the primary question to address was what factors were associated with the resistance to pesticides in the Culex mosquito populations. The study populations included the wild Culex mosquitoes collected from various locations and reared in the county's Public Health Department, specifically for the efficacy testing purposes. These were the mosquito populations with the potential exposures to both the diseases and pesticides.

A correlational, cross-sectional study takes a picture of the population of interest; it is one of the most common and well-known study designs (Creswell, 2009). In this case, out of the Culex populations of interest, those mosquitoes that had shown resistance to pesticides were analyzed for the factors associated with the resistance.

The use of a cross-sectional quantitative design in this study offered two advantages: It was less expensive and less time consuming because the secondary data were already available. This approach allowed for the exploration of the associations between the variables of interest and the possibility to identify predictors of the outcome of interest: pesticide resistance

Sampling Strategy

The sampling strategy I adopted ensured representation of the key characteristics of the target Culex mosquito populations and helped to minimize both bias and errors. The samples were drawn from three geographical areas in the large, urban county under study. To test for pesticide resistance, approximately 25 Culex mosquitoes from three selected geographical areas were pipetted into cages, each representing an area. The mosquitoes were then placed at the distance of 100 feet, 200 feet, and 300 feet, totaling 48 cages containing at least 5,000 mosquitoes. The test was conducted in an open field measuring at least 500 feet x 500 feet. At the appropriate time, pesticides were applied through truck-mounted equipment. After about 10 minutes post-pesticide application, the cages containing mosquitoes were retrieved and counted to see how many of the mosquitoes died as a result of pesticides applied. The dead mosquitoes were counted, and surviving mosquitoes were those resistant to pesticides.

Permission to Use Data

The data for this study was based on the yearly Field Cage Test results, carried out to evaluate the efficacy of the pesticides used in controlling mosquitoes in the years 2013 through 2017 by the county Public Health Department. The data are publicly available and can be obtained upon request to the public health department. For this study, I submitted the Walden University Data Use Agreement form to the director of Mosquito and Vector Control Division with a letter that included the title of my study, a brief description, and purpose of the study in relation to the data requested. The director of the Public Health Department, Mosquito and Vector Control Division signed the Data Use Agreement. The data use agreement is available in Appendix. Permission to use the Field Cage Test data was also granted.

Inclusion and Exclusion Criteria

The primary function of male mosquitoes is to mate with the females, and they can die anytime afterwards (Acevedo et al., 2015). Therefore, the male mosquitoes were excluded from the study. The inclusion criteria were those mosquitoes (primarily females) that were exposed to pesticides at 7 to 10 days of age. Additionally, the data set was those mosquitoes collected between 2013 and 2017.

Post Hoc Power Analysis

I performed post hoc power analysis after the completion of the final analyses of the study to determine the true power of the study. Chi-square and Pearson's correlation helped in testing the association between two variables and also to determine whether there was a significant relationship and correlation between the variables as well as the extent of their association. Equally, logistic regression multivariate analyses were applied to predict the relationship in the group or categories and to test for predictors in the study population.

The Research Questions and Hypotheses

I developed six research questions and corresponding hypotheses to guide my investigation in this study:

Research Question 1: What is the association between wind direction and pesticide resistance in Culex mosquitoes?

$H_0$1: There is no association between wind direction and pesticide resistance in Culex mosquitoes.

H_a1: There is an association between wind direction and pesticide resistance in Culex mosquitoes.

Research Question 2: What is the association between temperature and pesticide resistance in Culex mosquitoes?

$H_0$2: There is no association between temperature and pesticide resistance in Culex mosquitoes.

H_a2: There is an association between temperature and pesticide resistance in Culex mosquitoes.

Research Question 3: What is the association between humidity and pesticide resistance in Culex mosquitoes?

H_03: There is no association between humidity and pesticide resistance in Culex mosquitoes.

H_a3: There is an association between humidity and pesticide resistance in Culex mosquitoes.

Research Question 4: What is the association between month of pesticide application and pesticide resistance in Culex mosquitoes?

H_04: There is no association between month of pesticide application and pesticide resistance in Culex mosquitoes.

H_a4: There is an association between month of pesticide application and pesticide resistance in Culex mosquitoes.

Research Question 5: What is the association between time of the day that pesticide is applied and the development of resistance by the Culex mosquitoes?

H_05: There is no association between time of the day that pesticide is applied and the development of resistance by the Culex mosquitoes.

H_a5: There is an association between time of the day that pesticide is applied and the development of resistance by the Culex mosquitoes.

Research Question 6: What is the association between weather (i.e., rain, snow, and ice) at the time of pesticide application and pesticide resistance in Culex mosquitoes?

$H_0$6: There is no association between weather (i.e., rain, snow, and ice) at the time of pesticide application and pesticide resistance in Culex mosquitoes.

H_a6: There is an association between weather (i.e., rain, snow, and ice) at the time of pesticide application and pesticide resistance in Culex mosquitoes.

I used various statistical analyses to answer these research questions. SPSS Version 21 was used to perform descriptive and inferential statistical analyses, including descriptions of the sample as well as inferential tests aligned with the research questions. Bivariate analyses were conducted using chi square or binary logistic regression to determine the association between the dependent and independent variables. A multivariate logistic regression analysis was used to determine associations among variables in a predictor model.

The six research questions required two main analyses. In the first set of analysis, I investigated the effects of environmental factors (i.e., wind, temperature, and humidity, time, months, and weather) in pesticide resistance development by Culex mosquitoes. To address the research questions, I examined the frequency distribution tables representing the effectiveness of the two classes of pesticides on mosquito mortality in each of the five years under investigation. The department applies two pesticides (i.e., Fyfanon and

Permenon) on a weekly rotation. The association was analyzed to determine whether the potential predictor variable (i.e., pesticide) had a significant correlation with the criterion variables (i.e., independent variables), which was shown in the mosquito mortality. With the second set of analysis, I investigated the effects of instrumentation and timing on pesticide efficacy.

Data Analyses Plan

To answer the six research questions, the dataset from a large County Public Health was obtained. The data came from the yearly Field Cage Test Results conducted by the Public Health Department to examine the efficacy of the pesticides they apply to control mosquito populations between the years 2013 to 2017. Descriptive and inferential analyses were performed on the dataset using SPSS Version 21. The data had a total study population of 29,794.

Data Cleaning and Preparation

The first data management performed was to screen the selected data sample to identify potential patterns of any missing data and outliers. Normally, if less than 5% of data missing for each variable, then it would be assumed that the data were missing by chance rather than that of systemic errors and would substitute a mean value for that variable missing data. Otherwise, the missing data accounted for by the use of the SPSS.

Another issue of concern was outliers, and outliers are case scores that are extreme. Outliers have the potential of having high impact on the outcome of the study statistical analysis if found in the data set. Therefore, to avoid biased results, the data set would be screened for both univariate and multivariate outliers. Outliers could be screened by following SPSS method-analyze then click on descriptive statistics, then explore, and finally click on outliers.

On the other hand, the outliers in the data set identified as a result of an error or a false measurement could have been handles by simply removing them. The information for this study was from the County Public Health Department Excel spreadsheets. Since the SPSS is much better at handling numeric variables than string variables the Excel spreadsheets data was decoded. Before the analysis, many of the categories and sub-groups of the independent variables were coded. First, the wind speed. In the dataset, the wind speed varied between 3 to 10 miles per hour (mph). In the analysis, the wind speed was coded low wind speed (0-5 mph) and high wind speed (5-10 mph). In the dataset, the temperature varied between 65^{0f} and 95^{0f}. In the SPSS analysis temperature was coded low temperature (65^{0f}-80^{0f}) and high (80^{0f} to 95^{0f}). Also, in the dataset, humidity varied between 50% and 70% and was coded low (50%-60%) and high (60%-70%). The time of pesticide application were 7pm to 10pm and was coded (1) for 7pm to 8pm and (2) for 8pm to 10pm. The months for pesticide application were June, July, and August.

Descriptive Analysis

The descriptive statistical analysis was performed to describe the population being investigated (See Tables 1-5 below.). The descriptive analysis used SPSS by computing a frequency statistics to measure frequency for measures of central tendency which include mean, mode, and median. Frequency distributions were used to analyze the data and they include histogram, bars, tables, and graphs. The descriptive statistics for each of the independent variables under investigation were generated. Equally important was the fact that the frequency distribution histogram assisted in visual presentation of the means, median, mode, as well as the percentages of each of the six independent variables. Refer to the Data Analyses Matrix in Table 1 and Tables 2 through 5 for mock descriptive statistical analyses.

Table 1

Analysis Matrix for the Pesticide Resistance Study

RQs and Objectives	**Concept**	**Data Source**	**Level of Measurement**	**Analysis Procedures**
RQ4	Distribution of Months and Resistance	County Health Department	Nominal	%
RQ2	Distribution of Temps and Resistance		Nominal	Frequency
RQ6	Distribution between Weather and Resistance		Ordinal	Mean
OBJ 1	Characteristics and frequencies of Resistance by Pesticides		Ordinal	%
OBJ 2	Summary of Resistance by year (2013-2017)		Ordinal	Frequency
OBJ 3	Differences in Resistance by Locations		Nominal	Mean

Table 2

Study Population by Pesticides

Pesticides	Yes (Number)%	No (Number)%	Total (Number)%
A	%	%	%
B	%	%	%
Totals	%	%	100%

Table 3

Study Population by Test Months

Months	Resistance	No Resistance
April	Yes	
May	Yes	
June		Yes
July	Yes	
August		Yes
September	Yes	

Table 4

Study Population by Temperature

Pesticides	30- 50^0	51- 70^0	71- 100^0	Total

A	%	%	%	%
B	%	%	%	%
Totals	%	%	%	%

Table 5

Study Populations by Areas

Areas	**March- April**	**May- August**	**Sept.- Dec.**	**Total**
Area 51	%	%	%	%
Area 93	%	%	%	%
Area 225	%	%	%	%
Totals	%	%	%	%

Inferential Analysis

Inferential analyses were used in making inferences about the population and analyses of the sample (See Tables 6-10). The inferential statistics used for the study include both bivariate statistical analysis and multivariate logistic regression statistical analysis. The Bivariate Analysis was used in testing the association between each of the six independent variables and the dependent variable using chi square statistic. The bivariate statistical analysis used cross tabs and chi square. Cross tabulation is a frequency statistics that displays the relationship between two variables in a single table as in Tables 6, 7, 8, 9, and 10. Following the computation of the bivariate analysis, the multivariate logistic regression analysis was computed in SPSS to explore correlation by predicting the value of a variable based on the value of another variable. Multivariate statistical analysis was used to test for a predictive model for pesticide resistance using multiple logistic regressions.

A bivariate statistical analysis for each independent variable was applied to see if there was an association between the independent variable and the dependent variable. The multivariate analysis method was performed to identify the predictor values of any combination of two independent variables. A logistic regression analysis performed and is appropriate when the criteria variable is dichotomous with two possible outcomes. The data was presented in narrative and table format that described the characteristics of the culex mosquito populations. Refer to Tables 6, 7, 8, 9, and 10 for inferential statistical analyses.

Table 6

Chi square analysis by numbers and pesticides

Pesticides	**Yes (Number) %**	**No (Number) %**	**Chi Square**	**P Value**
A	%	%		
B	%	%		
C	%	%		

Table 7

Chi square analysis by areas

Areas	**Yes (Number)**	**No (Number)**	**Chi Square**	**P Value**

	%	%
55		
109		
225		

Table 8

Chi square analysis for pesticide resistance by years (2013- 2017)

Pesticides	**2013**	**2014**	**2015**	**2016**	**2017**
Pesticide A	%	%	%	%	%
Pesticide B	%	%	%	%	%

Table 9

Predictor model for all IVs

IVs	**Resistance**	**No Resistance**
Wind	Yes	
Temperature		No
Humidity	Yes	
Time of day		No
Months of Year	Yes	
Weather (Rain, Snow)		No

Table 10

Regression analysis for variables predicting pesticide resistance

IVs	*B*	*SE B*	*Beta*
Wind			
Temperature			
Humidity			
Time of the Day			
Month of the Year			
Weather (Rain, Snow)			

Treat to Validity

According to Garcia-Perez (2012), the use of quantitative retrospective design archive data can lead to threats to external validity. Historical accuracy of the data collection can decrease the threat to external and internal validity and increase the reliability of the data. Statistical regression remains a possible threat to interval validity with nonrandom samples, such as in the case of archived secondary data analysis and two measurements with no association. According to Mel, Lim, Soekojo, Thow, Lang, Lee and Tan (2017), in this situation, the statistical regression occurs causing a threat to the internal validity and reliability of the study. The accuracy of the data may also be skewed by providing inaccurate data due to reasons such as distractions

The archived secondary data from the results of the Field Cage Test was used for this study. The reliability of the statistics of the data may have changed over time and the system of collecting the data may equally have changed. Also, the original researcher collected the data for purposes other than that of this research. These may have effects on the internal validity of this study. Since the study involves mosquitoes, the small insect can die before or during the test with no one noticing. This threat was extraneous effects and maturation. It is extraneous because the participants were exposed to events other than pesticide with the potential to affect the independent variables. It would be maturation because the mosquitoes participants changed with passing of time in a way unrelated to independent variables. To correct the experimental mortality effects, the duration of the experimental test was greatly reduced to limit the mosquitos' exposures to factors other than the independent variables under investigation.

Ethical Consideration

The primary populations for this study were the mosquitoes populations both feral and laboratory maintained. Therefore, many of the ethical concerns associated with the research involving human subjects were not applicable here in the research. While there were no ethical considerations, as a researcher, I conduct research study in transparent and rigorous manner and in accordance with the department standard operation procedures: the Code Book. Also, the Institutional Review Board (IRB) approval number for this study is 10-25-18-0354560

Summary

This chapter included a description of the methodology used to evaluate the hypotheses and answer the research questions. This was a retrospective correlational cross-sectional study which the primary focus was to determine if there were significant associations between the dependent variable (pesticide resistance) and the independent variables (wind direction, outside temperature, humidity, month of the year, time of the month, and weather condition). To that end, the descriptive and inferential statistics was used in the analyses. The descriptive statistical analysis was conducted using SPSS version 21. Measure of central tendency was performed to include means, mode, median, and standard deviation. Frequency distribution (histogram, bars, tables, and graphs) was conducted.

A bivariate statistical analysis was conducted on each independent variables and dependent variable. The bivariate analyses used include chi square tests that determine the significant relationships and correlations between the independent variables and dependent variable. Also discussed was the source of the data, research questions, hypotheses, data collection, validity and reliability, and data analysis information pertinent to this study. Multivariate statistical analysis was performed which included regression techniques to determine the predictor model for the outcome of interest, pesticide resistance. In Chapter Four the results of the analyses and interpretation will be presented.

Chapter 4: Results

Introduction

The purpose of this quantitative, cross-sectional, correlational study was to investigate the environmental factors associated with the pesticide resistance of Culex mosquito populations. I explored the association between the independent variables (i.e., wind speed, outside temperature, humidity, time of the day, month of the year, and weather condition at the time of pesticide application) and the dependent variable or outcome of interest (i.e., pesticide resistance in Culex mosquito populations). The study populations were both the susceptible and feral Culex mosquitoes. The feral mosquitoes were those that were exposed to pesticides and collected from different geographical areas of a large county. The susceptible Culex mosquito populations were those maintained in the public health laboratories and insectaries.

In recent years, mosquito-borne diseases have been resurgent, largely because of the pesticide resistance developed by the mosquito vectors due to broad and repeated applications of pesticides (Agossa et al., 2015). Improving pesticide resistance management involves better understanding of the environmental factors potentially associated with the development of the resistance (Nkya et al., 2015). I developed the following six research questions and corresponding hypotheses to investigate in this study:

Research Question 1: What is the association between wind speed and pesticide resistance in culex mosquitoes?

$H_0$1: There is no association between wind speed and pesticide resistance in Culex mosquitoes.

H_a1: There is an association between wind speed and pesticide resistance in Culex mosquitoes.

Research Question 2: What is the association between temperature and pesticide resistance in Culex mosquitoes?

$H_0$2: There is no association between temperature and pesticide resistance in culex mosquitoes

H_a2: There is an association between temperature and pesticide resistance in Culex mosquitoes

Research Question 3: What is the association between humidity and pesticide resistance in Culex mosquitoes?

$H_0$3: There is no association between humidity and pesticide resistance in Culex mosquitoes

H_a3: There an association between humidity and pesticide resistance in Culex mosquitoes

Research Question 4: What is the association between month of pesticide application and pesticide resistance in Culex mosquitoes?

$H_0$4: There no association between month of pesticide application and pesticide resistance in culex mosquitoes

H_a4: There is an association between month of pesticide application and pesticide resistance in Culex mosquitoes.

Research Question 5: What is the association between time of the day that pesticide is applied and the development of resistance by the Culex mosquitoes?

$H_0$5: There is no association between time of the day that pesticide is applied and the development of resistance by the Culex mosquitoes.

H_a5: There is an association between time of the day that pesticide is applied and the development of resistance by the Culex mosquitoes.

Research Question 6: What is the association between weather (rain, snow, and ice) at the time of pesticide application and pesticide resistance in Culex mosquitoes?

$H_0$6: There is no association between weather (rain, snow, and ice) at the time of pesticide application and pesticide resistance in culex mosquitoes.

H_a6: There is an association between weather (rain, snow, and ice) at the time of pesticide application and pesticide resistance in culex mosquitoes.

The data gathered for the study enabled my analyses of the association between the independent variables and the dependent variable. I performed analyses of the archived secondary data from the results of Field Cage Tests conducted between 2013 through 2017. The original study was conducted by the county Public Health Department, Mosquito and Vector Control Division. The data set contained a sample of 29,794. The pesticide resistance management in the mosquito vectors is essential for the public health efforts to contain the emergence and reemergence of mosquito-borne diseases such as malaria, St. Louis encephalitis, West Nile virus, and the Zika virus, among others. I used

SPSS Version 21 software to address the research questions and hypotheses and run chi-square and logistic regression analyses.

This chapter is divided into three sections and the findings of the study will be presented in the same order: descriptive statistical analysis, inferential bivariate statistical analysis, and inferential multivariate statistical analysis. The first section will include the descriptive data analysis of the data set for the years under investigation (i.e., 2013–2017). It will also include the frequency distributions that illustrate the means and percentages of the mosquito mortalities in the geographical areas and years. That will be followed by the presentation of total numbers of Culex mosquitoes used in the studies as well as the numbers assigned to each of the classes of pesticides used in the study. Then, the efficacy of the pesticides will be shown for each of the years: 2013–2017. The chapter will also include the inferential bivariate statistical analyses used to establish the association between two variables. According to Adjakossa et al. (2016), bivariate statistical analysis is used to establish an association between two variables, while multivariate statistical analysis is used in the establishment of the association of more than two variables. The bivariate association of the two variables will be illustrated using the chi-square analysis to address each of the six research questions and corresponding pairs of hypotheses. Finally, I will provide summary analyses of all the independent variables as related to the dependent variable with the help of logistic regression.

Data Collection

The data for this study was based on the yearly Field Cage Test results carried out to evaluate the efficacy of the pesticides used in controlling mosquitoes in the years 2013 through 2017 by the county Public Health Department. The data are publicly available and can be obtained upon request to the public health department. For this study, I submitted the Walden University Data Use Agreement form to the director of Mosquito and Vector Control Division with a letter that included the title of my study, brief description, and purpose of the study in relation to the data requested. The director of the Public Health Department, Mosquito and Vector Control Division signed the Data Use Agreement. The data use agreement and the permission to use the results of Field Cage Test Data was granted.

The original data samples were collected using the established protocol by the public health department in evaluating the effectiveness of the pesticides applied to control mosquitoes. The tests were conducted at least three times a year with at least three tests per day. In the Field Cage Test, at least 25 Culex mosquitoes from the laboratory were maintained and three selected geographical areas in the county were pipetted into cages each representing an area. The study populations were put in 48 cages and placed at the distance of 100 feet, 200 feet, and 300 feet. The 48 cages contained at least 5,000 mosquitoes. The test was conducted in an open field measuring at approximately 500 feet x 500 feet. At the appropriate time, pesticides were applied through truck-mounted equipment. After about 10 minutes post-pesticide application, the cages containing Culex mosquitoes were retrieved and counted (i.e., 12 to 24 hours afterwards) to see how many of the mosquitoes died as a result of pesticides application. The dead mosquitoes were counted, and surviving mosquitoes were those resistant to pesticides. The sampling strategy adopted ensured the representation of the key characteristics of the target Culex mosquito populations with the advantage of minimizing both errors and bias in the study. The samples were drawn from nine geographical areas in the large county under study.

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Data Analyses

Descriptive Analysis

I performed descriptive and inferential analyses on the data set using SPSS Version 21. The descriptive statistical analyses included the measures of central tendency (i.e., mean, medium, and mode) to describe the study populations. Percentages were calculated, and the numerical results were summarized with tables and graphs including my interpretations. The descriptive statistics also included computing frequency statistics, summarizing a group of scores with a single number, and the range that helped in determining the spread of scores within the group. According to Brix et al. (2018), descriptive statistics are important because they present the data in meaningful ways easy for the audience to visualize.

Inferential Analysis

The inferential statistics used for the study included both bivariate statistical analyses using chi-square and multivariate statistical analysis using logistic regression. Bivariate statistical analysis involved cross tabs and chi square. Cross tabulation is a frequency statistic that displays the relationship between two variables in a single table (Bradley and Brand, 2016). I computed measures of association to calculate the strength between one nominal variable with another nominal variable. The Pearson chi-squared test was calculated as a correlation test for categorical variables to tell if they are statistically significant. The multivariate logistic regression model was the statistical technique used for modeling and analyzing the effect of multiple independent variables (i.e., the predicting criterion) on a dependent or outcome variable.

Confounders: Restriction

The confounders for the study were controlled by the study design. In this study, the potential confounding factors were sex and age of the Culex mosquito populations. This was addressed in the study design through restrictions. The Culex mosquitoes used in this study were all females and all about 7–10 days of age. No male mosquitoes were admitted, and all the mosquitoes were reared in a laboratory to ensure proper age distribution. These restrictions ensured that the sex and age distributions were similar so that the confounding effects would be eliminated or minimized.

Post-Hoc Analysis

I used a power analysis to calculate the minimum sample size required to detect the possible effect of the population size. The statistical power is the probability of the researcher to reject the null hypothesis [i.e., accepting the alternative hypothesis when the alternative hypothesis is true (Sharp, 2013)]. Low statistical power can reduce the probability of rejecting the null hypothesis and ultimately increase the probability of committing a Type II error (Onwuegbuzie and Leech, 2004). Sharp (2013), in accordance with Onwuegbuzie and Leech (2004), recommended a probability of .80 or greater for correctly rejecting the null hypothesis. A statistical power can either be retrospective (post hoc) or prospective (a priori) and is denoted by 1-ß, where ß (beta) is the Type II error (Onwuegbuzie and Leech, 2004). The Type II error is the probability of failing to reject the null hypothesis when it is false (Johnsen, 2015). A study with power greater than ß, where ß is less than 0.2 or .8 is considered statistically powerful (Johnsen, 2007). A priori power analysis is conducted before the research study, while the post-hoc power analysis is performed after the study (Onwuegbuzie and Leech, 2004). The alpha size for this study was .05 and power was .80. The estimated population size was 29,794, with a confidence level of 95% and 5% margin of error, based on the power analysis calculation, the estimated size needed to achieve adequate power for this study was 379 (see Sharp, 2013).

Results

The results of this study are divided into three parts and the findings will be presented in the same order: descriptive statistical analysis, inferential bivariate statistical analysis, and inferential multivariate statistical analysis. The first part will include the descriptive statistics used to describe the populations. The second part will be inferential bivariate statistical analysis, which was used to establish the bivariate association between two variables; in this case, between one independent variable and the dependent variable. The third part will be the multivariate statistical analyses used to establish association with more than two variables. In this study, multivariate statistical analysis was used to analyze the association between all the independent variables and the dependent variable.

Descriptive Analysis

The descriptive analysis for this study includes frequency distributions (the measures of central tendency) including the means and percentages of the mosquito mortalities. The primary objective here was to illustrate whether there were disparities in the resistance in each of the areas or categories for each variable presented in the data. The dataset collection was between January 1, 2013 and December 31, 2017, included information $N = 29,794$ on the culex mosquitoes with exposures to pesticides (see Table 11). The population characteristics of the mosquitoes were all females and about 7 to 10 days old. The mosquito eggs were collected from nine different locations in the county and reared in the laboratory and insectary for proper age distribution. No male mosquito was included in the study.

Table 11 summarizes the mosquito population characteristic for the study period. In 2013, the numbers of Culex mosquito used for the study was 6,004, and that represents 20.2% of the total mosquito populations. In 2014, 5,890 Culex mosquitoes were used in the study and that was about 19.8% of the total study population. In 2015, there were 5,902 Culex mosquitoes used representing 19.8% of the total populations. In 2016 about 5,985 Culex mosquitoes were utilized in the study representing 20.1%, while in 2017, there were 6,014 Culex mosquito used for the study 20.2%. In all, a total of 29,794 Culex mosquitos were used in the study (see Table 11).

Table 11

Mosquito populations used in the test each year

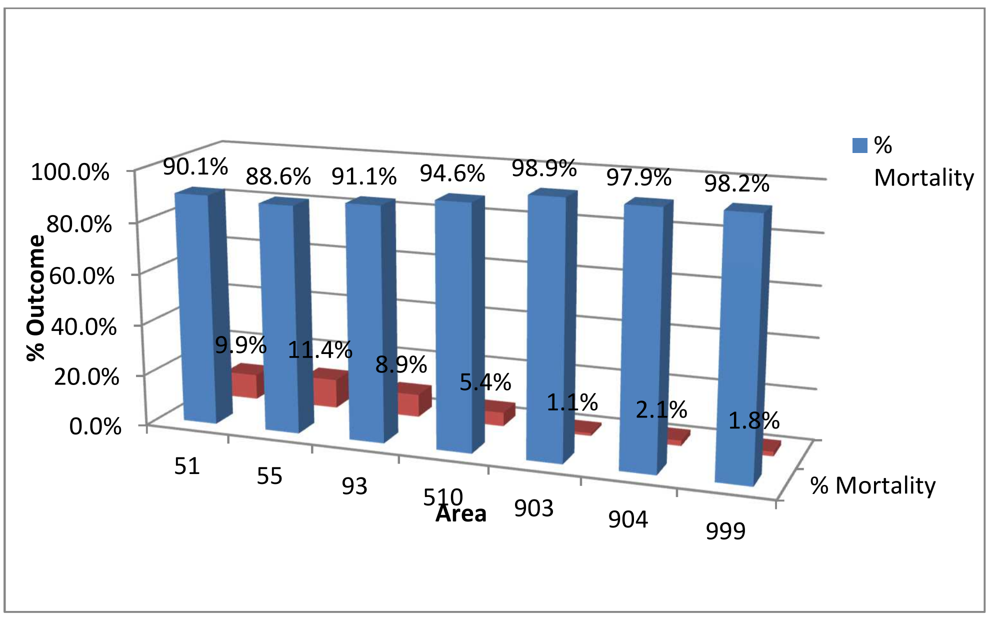
Year	Frequency	Percent	Cumulative Percent
2013	6004	20.2	20.2
2014	5890	19.8	39.9
2015	5902	19.8	59.7
2016	5984	20.1	79.8

2017	6014	20.2	100.0
Total	29794	100.0	

Note: n = 29,794

Figure 3 illustrates the mean mosquito mortality for each of the testing areas during the testing period (2013 through 2017). From Figure 1, the mean mortality for Area 51 was 90.1%, Area 55 was 88.6%, Area 93 was 91.1%, Area 510 was 94.6%, Area 903 was 98.9, Area 904 was 97.9, and Area 999 coded to represent the Sebring Colony was 98.2%. Bases on the evidence, Area 903 had the best resistance level at about 99% while Area 55 had worst resistance level at about 89%.

Figure 3: Proportion of mosquito mortality the Areas



The tests involved the application of two major classes of pesticides: Malathion and Permethrin. Table 12 shows the utilization of the Culex mosquitoes for each of the five years under investigation. The Table (12) illustrates the total number of Culex mosquitoes that were assigned to investigate each of the classes of pesticides: Permethrin and Malathion. In 2013, there were a total of 6,004 Culex mosquitoes used for the study. The percentage of the Culex mosquitoes used in the tests on both pesticides was almost equal. The tabulation shows that about 49.9% of the available population was used on Permethrin while 50.1% was used on Malathion. In 2014, the total number of Culex mosquitoes that were administered to both of the pesticides was *N* = 5,890, of which approximately 50% was each administered either the Permethrin or Malathion. Similarly, in 2015, about 50.3% of the mosquito populations available were tested on Permethrin class while 49.7 of the mosquitoes were tested on Malathion. In that year (2015) a total of *N* = 5,902 Culex mosquitoes were available for the Field Cage Tests. In 2016, out of a total of *N* = 5,984 of the Culex mosquitoes used in the tests, 50.1% of the total population was tested on Permethrin while about 49.9% was tested on Malathion. In 2017, there was a total of *N* = 6014 of Culex mosquitoes available for the test and approximately 50% of culex mosquito populations were tested on each of the pesticides.

Table 12
Two classes of pesticides used in the field cage test years (2013- 2017)

		Year					
		2013	2014	2015	2016	2017	Total
Chemical	Permethrin	2994(49.9)	2946 (50.0)	2970(50.3)	2997(50.1)	3005(50.0)	14912(50.1)
	Malathion	3010(50.1)	2944(50.0)	2932(49.7)	2987(49.9)	3009(50.0)	14882(49.9)
Total		6004(100)	5890(100)	5902(100)	5984(100)	6014(100)	29794(100)

Table 13 illustrates the efficacy of the two classes of pesticides that the department uses in the control of the mosquito populations. From the table 3 below, the efficacy of the two classes of pesticides: Permethrin and Malathion was investigated as shown by mosquito mortality. Of the total mortality in the year 2013, Permethrin was effective in killing 84.4% while Malathion killed 97.2%. In 2014, Permethrin killed 89.6% and Malathion 90.1% of the total of mosquitoes. In 2015, Permethrin killed 94.3% of mosquitoes while Malathion killed 98.3% of the mosquitoes. In 2016, Permethrin killed 97.1% of the mosquitoes while Malathion killed 99.3% of the mosquitoes. In 2017, the mortality rate for Permethrin was 83.9% and Malathion 96.6%. The total mean effectiveness of Permethrin for the 5 years under investigation was 89.9% while that Malathion was 96.3% (see Table 4). In sum, Malathion was more effective in killing the culex mosquitoes (96.3% than Malathion 89.9%).

Table 13

Distribution of yearly mortality rates (2013- 2017)

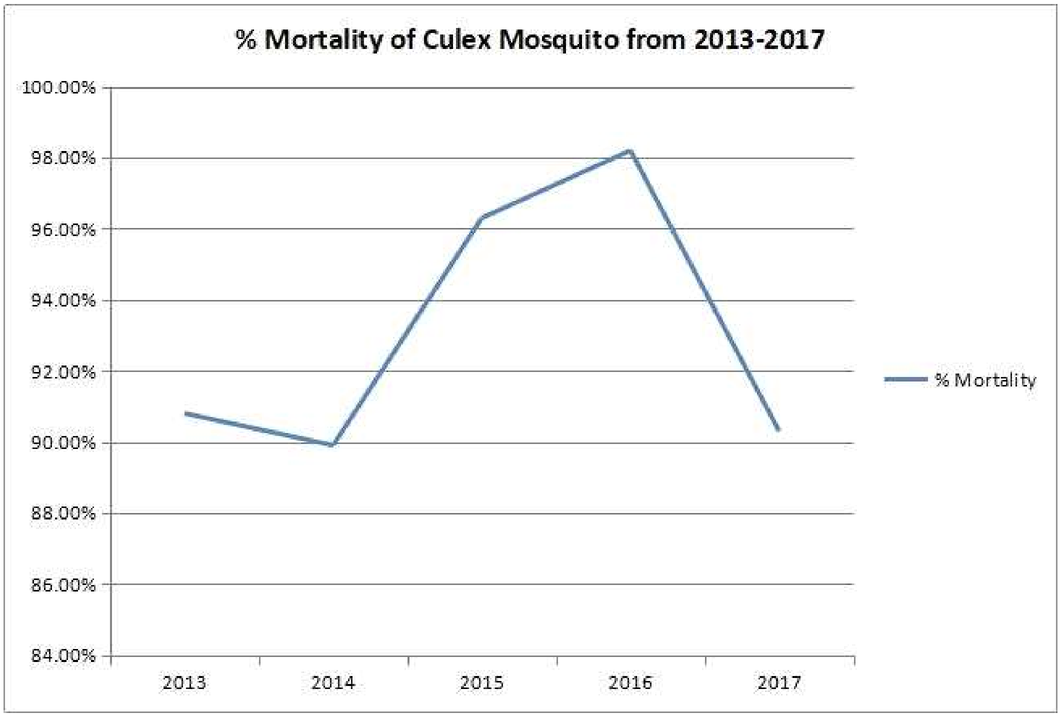
	100 Feet 2013		2014		• 2015		• 2016		• 2017	
	Perm	Mal	Perm	Mal	Perm	Mal	Perm	Mal	Perm	Mal
51	96	98.7	88.7	98.6	94.4	98.4	-	-	94.2	97.4
55	92.7	99.22	84.9	92.8	95.7	98.9			86.5	97.6
93	96.3	96.6	85.4	98.9	-	-	97.3	99.2	96.6	95.8
510	-	-	-	-	93.3	97.5	-	-	-	-
903							99.5	99.7	-	-
904	-	-	-	-	-	-	97.6	99.5	-	-
999	99.5	99.5	98.4	99.5	100	98.6	99.2	99.2	99.2	100
Total	96.1	98.5	89.4	97.4	95.8	98.6	98.4	99.4	94.1	97.7
	200 Feet									

	2013		2014		2015		2016		2017	
	Perm	Mal	Perm	Mal	Perm	Mal	Perm	Mal	Per	Mal
51	67.1	95.7	86.7	85.8	90.4	98.3	-	-	54.1	97.1
55	61.7	96	91	72	89.8	98.1	-	-	69.1	91.2
93	71.9	93.9	81.4	80.5	-	-	93.1	98.6	77.3	94.2
510	-	-	-	-	91	96.7	-	-	-	-
903	-	-	-	-	-	-	96.8	99.7	-	-
904	-	-	-	-	-	-	95.5	99.2	-	-
999	90.1	98.1	99.5	92.5	100	100	97.6	99.2	93.3	100
Total	72.7	95.9	89.8	82.7	92.8	98.3	95.7	99.2	73.6	95.6
% M	84.4	97.2	89.6	90.1	94.3	98.3	97.1	99.3	83.9	96.6

Note: n = 29,794

Combining the two pesticides (Malathion and Permethrin), Figure 4 illustrates the mean mortality of Culex mosquitoes for all areas during the period under investigation (2013 to 2017). From the graph, the mean mortality for all pesticides in 2013 was 90.8%; in 2014, the mean mortality was about 89.8%; in 2015, the mean mortality was 96.3%; 2016, the mean mortality was at 98.2%, and in 2017 the mean mortality was at 90.3%. In other words, the resistance was best in 2016 (98.2%) followed by 2015 (96.3%) and worst in 2014 (89.8%).

Figure 4: Percentage mortality after exposures to pesticides (2-13- 2017)



Inferential Statistics: Bivariate Analysis

The inferential statistical analysis utilized chi square tests to assess association between each independent variable and the dependent variable which is pesticide resistance in the Culex mosquito populations. Table 14 includes a summary of the results of the bivariate analysis using the chi square statistic with alpha set at .05.

The Culex mosquito mortality was assessed factoring in by wind speed, temperature (in Fahrenheit degrees ^{0F}), humidity (in Percentages %), test month, test time, and weather condition. Upon application of pesticide- either permethrin or Malathion, there was a statistically significant association between the resistances in Culex mosquitoes sprayed at 1-5 mph (8%) compared to wind speed at 5.1-10mph (6.6%) [x^2 (1) =14.01, p = .000]. Similarly, there was a statistically significance association between Culex mosquito's resistances at temperatures between 82^{0F}- 85^{0F} (4.2%) relative to temperatures at 85.1^{0F}-95.00^{0F} (9.8%) [$x^2(1)$ =3 46.5, *p* = .000]. Also in analyzing pesticides resistance of Culex mosquitoes at humidity of 50.00-65.00% (6.6%) against 65.10-80 0% (7.50), a statistically significant association was identified [$x^2(1)$ =7.23, *p* = .007].

In comparing Culex mosquitoes resistance to pesticides June (13%), July (3.3%), and August (8.9%), a statistically significant association was found [x^2 (2) = 702.606, *p* =. 000]. Comparing mosquitoes resistance to pesticide application from 7pm-8pm (8%) and 8:01-9:00pm (6.1%), a statistically significant association was found [x^2=40.99,*p* = .000. Similar statistically significant difference was identified [$x^2(2)$ =527.98*, p* = .000] when resistance to pesticides was compared during the clear weather (4.1%), cloudy (7.5%), and sunny weather (13.0%).

The description of findings by each research question

Research Question 1

What is the association between wind speed and pesticide resistance in culex mosquitoes? The null hypothesis is that there are no associations between wind speed and pesticide resistance in Culex mosquitoes. From the data, the wind speed varied between 3 mph and 10 mph and was coded low speed (0 to 5 mph) and high speed (5.1- 10 mph). To answer the research question that there is no association wind speed and pesticide resistance in the Culex mosquitoes, a chi-square analysis was performed to compare the mosquito mortality at various wind speeds during the original tests. The significance level was set at .05, and the results of the chi-square analysis are presented in Table 14. At low wind, about 8% of mosquitoes were alive (resistant to pesticides) while 92% of mosquitoes died (nonresistant to pesticides). On the other hand, at high wind, about 6.6% of mosquitoes were alive (resistant) while 93.4% dead (nonresistant) to pesticides. With a total number *N* = 29,794, a statistically significance association between wind speed and culex mosquito mortality was identified [$x^2(1)$ =14.01, *p* = .000]. Since the *p* value is less than our chosen significance level α = 0.05, it is possible to reject the null hypothesis and accept the alternate hypothesis that there is an association between wind speeds and pesticide resistance in Culex mosquitoes.

Research Question 2

What is the association between temperatures and pesticide resistance in Culex mosquitoes? In the data set, the temperatures varied between 65^{0F} and 95^{0F}. For the statistical analysis, the temperatures were coded low (65^0- 84^0F) and high (85^0- 95^0F). In order to test the null hypothesis that there is no statistical significance in the relationship between temperature and pesticide resistance in the Culex mosquitoes, a chi-squared analysis was performed. The statistically significant set at alpha = .05. The result is presented in the table 14 below. At low temperature, the resistance level was at 4.2% while the resistance level at high temperature was at 9.8%. Using chi-square analysis, a statistically significance association between temperature and Culex mosquito mortality was identified [$x^2(1)$ =346.5, *p* = 000]. Since the *p* value is less than our chosen significance level α = 0.05, it is reasonable to reject the null hypothesis and accept the alternate hypothesis that there is an association between wind speeds and pesticide resistance in Culex mosquitoes.

Research Question 3

What is the association between humidity and pesticide resistance in Culex mosquitoes? In the data set, the humidity levels varied between 50% and 80%. For the purpose of this statistical analysis, humidity was coded low (50%- 65%), and high humidity (65%- 80%). In order to test the null hypothesis that there is no statistical significance in the relationship between humidity and pesticide resistance, a chi-square analysis was applied and the statistically significant at alpha = .05. The result is presented in the Table 14. At low humidity, the resistance is at 6.6% while at high humidity the resistance level is at 7.5%. Using chi-squared analysis, a statistically significance association between humidity and Culex mosquito mortality was found [$x^2(1)$ =7.23, *P* = .000]. Since the *p* value is less than our chosen significance level α = 0.05, the null hypothesis can be rejected and conclude that there is enough evidence to suggest an association between humidity and pesticide resistance in Culex mosquitoes. Here, the null hypothesis is rejected because the P-value is less than α (see Table 4).

Research Question 4

What is the association between month of pesticide application and pesticide resistance in Culex mosquitoes? From the data set, the months of testing included June, July, and August. To test the hypothesis that there is no statistical significance in relationship between months of the year that pesticides are applied and pesticide resistance in Culex mosquitoes, a chi-square was conducted to test the null hypothesis. The statistically significance level was set at alpha =.05 and the results presented in Table 14. For the pesticides applied in the months of June, the resistance level was at 13%

while that of July was at 3.3%. On the other hand, the resistance level for the month of August was 8.9%. This difference produced a statistically signification association between months and Culex mosquito mortality at (x^2 (2) = 702.606, p = 000). Therefore, based on this evidence, it is possible to reject the null hypothesis in favor of the alternative hypothesis. That means there is a statistical significance in the relationship between months of the year in which pesticide was applied and pesticide resistance in Culex mosquitoes.

Research Question 5

What is the association between time of the day that pesticide is applied and the development of resistance by the Culex mosquitoes? In order to test the null hypothesis that there is no statistical significance in the relationship between the time of pesticide application and mosquito mortality, a chi-square tests was conducted and the statistically significant at alpha = .05. For the purpose of this analysis, the times of pesticide application was coded to 1 (7 pm – 8 pm), and 2 (8 pm and 10 pm).The result of the chi-square is presented in table 4 below. Based on the analysis in the early evening hours (7pm-8pm), the resistance level was 8% while the late evening hours (8pm- 10pm), the resistance level was 6.1%. The chi-squared analysis identified a statistically significance association between time and Culex mosquito mortality [x^2=40.99, p = 000] (see Table 14). Therefore, based on the evidence, the null hypothesis is rejected in favor of the alternative hypothesis that there is a statistical significance in the relationship between time of the day that pesticide is applied and pesticide resistance in Culex mosquitoes.

Research Question 6

What is the association between weather (clear, cloudy, sunny) at the time of pesticide application and pesticide resistance in Culex mosquitoes? From the data presented, the weather condition at the time of pesticide application include clear, cloudy, and sunny. To answer the question, a chi-square analysis was conducted. The statistically significant level was set at alpha .05, and the results presented in the Table 4. In the cloudy weather, the resistance was at 7.5%, in sunny weather, the resistance level was at 13.0%, and in clear weather the resistance level was at 4.1%. As illustrated in Table 4, a statistically significance association was identified between weather and Culex mosquito mortality [$x^2(2) = 527.98$, $p = .000$]. Since the p value is less than our chosen significance level $\alpha = 0.05$, the null hypothesis is rejected and accept the alternate hypothesis that there is an association between weather and pesticide resistance in culex mosquitoes.

Table 14

Chi square analysis of mosquito mortality of all the IVs (N 29,794)

RQ		**Outcome**				
	Variable	**Alive (%)**	**Dead (%)**	**Total (%)**	X^2 ***(df)***	***P***
1	Wind Speed (Mph)				14.01 (1)	0.000*
	1.00-5.00	474 (8.00)	5442 (92.00)	5916 (19.86)		
	5.10-10.00	1584 (6.60)	2294 (93.40)	23878 (80.14)		
2	Temperature ^{0F}				346.49	0.000*
	80.00-85.00^{0F}	617 (4.20)	14213	14830 (49.78)		

			(95.80)			
	85.1-95.00^{0F}	1441 (9.60)	13523 (90.40)	14964 (50.22)		
3	Humidity (%)				7.23	0.007*
	50.00-65.00	1385 (6.60)	19447 (93.40)	20832 (69.92)		
	65.10-80.00	673 (7.50)	8289 (92.50)	8962 (30.08)		
4	Test Month				702.61	0.000*
	June	772 (13.00)	5168 (87.00)	5940 (19.94)		
	July	490 (3.30)	14428 (96.70)	14918 (50.07)		
	August	796 (8.90)	8140 (91.10)	8936 (29.99)		
5	Time				40.10	0.000*
	7:00-8:00pm	961 (8.00)	10985 (92.00)	11946 (40.10)		
	8:01-9:00pm	1097 (6.10)	16751 (93.90)	17848 (59.90)		
6	Weather				527.98	0.000*
	Clear	613 (4.10)	14279 (95.90)	14892 (49.98)		
	Cloudy	673 (7.50)	8289 (92.50)	8962 (30.08)		

Sunny	772 (13.00)	5168 (87.00)	5940 (19.94)

Note: *Statistically significant at alpha = .05

Multivariate Statistical Analysis

Table 15 summarizes the logistic regression model for the independent variables of interest and Culex mosquito mortality upon application of pesticides. Using a 95% confidence interval, the overall model was statistically significant [*OR* = 13.48, $p < .05$]. Although chi-square analysis found time of pesticide application to be statistically significant, it was however insignificant using the logistic regression model [*OR* = 1.19 (95% *CI*: .99-1.45), $p = .07$]. Similarly, it was insignificant for wind speed [*OR* =.93 (95% *CI*: .81-1.07), $p = .29$] despite its significance using chi-squared analysis.

Table 15

Binomial logistic regression effects of all the IVs

Variable	Exp (B)	95% CI	p Value
Wind Speed	0.927	0.806-1.065	0.285
Temperature	2.067	1.832-2.331	0.000*
Humidity	0.322	0.261-0.396	0.000*
Test Month	0.346	0.293-0.407	0.000*
Time	1.194	0.986-1.477	0.07*
Weather	0.325	0.29-0.364	0.000*

Table 16 summarizes the logistic regression analysis for multivariate association between all the independent variables of interest and the dependent variable in the culex mosquito mortality upon application of pesticide (*N* = 29,794). Using a 95% confidence interval, the overall model was statistically significant [*OR* = 13.48, $p < .05$]. A statistically significant association between wind speed and the culex mosquito mortality was identified ($p = .285$). Also, a statistically significant association between test month and Culex mosquito mortality was identified (*OR* = 0.346, 95% *CI*: 0.293-0.407 $p = .000$).

Although there was no statistical variation in mosquito mortality between mosquitoes sprayed in June and August ($p = .05$), a statistically significance association was identified between the Culex mosquitoes sprayed in July compared to August ($p < .05$). Culex mosquitoes sprayed in July were 2.892 times more likely to die from pesticides application than mosquitoes sprayed in August [*OR* = 2.892, 95% *CI*: 2.455-3.408]. With statistical significance ($p < .05$), Culex mosquitoes sprayed at lower temperatures were 2.067 times more likely to die when compared to those sprayed at higher temperatures [*OR* = 2.067, 95% *CI*: 1.832-2.331, $p = .000$]. This implies that mosquito resistance to pesticide application is more likely at higher temperatures than in much lower temperatures.

Also, a statistically significant likelihood of pesticides resistance was found when mosquitoes were sprayed at lower humidity compared to the higher. Mosquitoes sprayed at lower humidity were 0.322 times less likely to be resistance when compared to culex species sprayed at higher humidity [*OR* = 0.32, 95% *CI*: .261-.396, $p = .000$]. When culex mosquitoes were sprayed in different times, a statistical significance variation in mosquito mortality was not identified ($p > .05$). Additionally, there was a statistically significant variation in the mortality of mosquitoes sprayed at different weather conditions [*OR* = 0.325, 95% *CI*: 0.29-0.364, *p* = .000]. Mosquitoes sprayed during the clear weather were 3.480 times less likely to be resistance when compared to those sprayed during the sunny weather [*OR* = 3.480, 95% *CI*: 3.115 – 3.887, *P* = .000]. Similarly, mosquitoes sprayed during the cloudy weather were 1.840 times less likely to be resistance when compared to mosquitoes sprayed during the sunny weather [*OR* = 1.840, 95%*CI*: 1.650-2.052, *p* = .000].

Table 16

Binomial logistic regression effects of all the IVs

Variable	**Alive**	**Dead**	**Total**	**Exp (B)**	**95% CI**	***P-Value***
Wind Speed						
1.00-5.00	474 (8.00)	5442 (92.00)	5916 (19.86)	0.927	0.806-1.065	0.285
5.10-10.00	1584 (6.60)	2294 (93.40)	23878 (80.14)		Ref	Ref
Temperature						
82.00-85.00^{0F}	617 (4.20)	14213 (95.80)	14830 (49.78)	2.067	1.832-2.331	0.000*
85.1-88.00^{0F}	1441 (9.60)	13523 (90.40)	14964 (50.22)		Ref	Ref
Humidity						
50.00-65.00	1385 (6.60)	19447 (93.40)	20832 (69.92)	0.322	0.261-0.396	0.000*
65.10-80.00	673 (7.50)	8289 (92.50)	8962 (30.08)		Ref	Ref
Test Month				0.346	0.293-0.407	0.000*
June	772 (13.00)	5168 (87.00)	5940 (19.94)	0.884	0.675-1.157	0.368
July	490 (3.30)	14428 (96.70)	14918 (50.07)	2.892	2.455-3.408	0.000*

August	796 (8.90)	8140 (91.10)	8936 (29.99)		Ref	Ref
Time						
7:00-8:00pm	961 (8.00)	10985 (92.00)	11946 (40.10)	1.194	0.986-1.477	0.070
8:01-9:00pm	1097 (6.10)	16751 (93.90)	17848 (59.90)		Ref	Ref
Weather				0.325	0.29-0.364	0.000*
Clear	613 (4.10)	14279 (95.90)	14892 (49.98)	3.48	3.115 – 3.887	0.000*
Cloudy	673 (7.50)	8289 (92.50)	8962 (30.08)	1.84	1.650-2.052	0.000*
Sunny	772 (13.00)	5168 (87.00)	5940 (19.94)		Ref	Ref

Summary

The objective of this quantitative cross-sectional correlational study was to investigate the environmental factors associated with pesticide resistance in culex mosquitoes. Both descriptive and inferential statistics were applied in the analyses. At the bivariate level, using chi-square analysis, there was a statistically significant association between the resistances in Culex mosquitoes sprayed at low wind speed (8%) compared to high wind speed (6.6%) [x^2 (1) =14.01, p = .000]. Similarly, there was a statistically significant association between Culex mosquito's resistances at low temperature 80 ^{0f}-85^{0F} (4.2%) relative to high temperatures at 85.1^{0F}-95.00^{0F} (9.8%) [x^2(1) =3 46.5, p = .000]. Also, the result shows statistically significant association at low humidity 50.00-65.00% (6.6%) against high humidity 65.10-80 0% (7.50) [x^2(1) =7.23, p = .007]. In comparing Culex mosquitoes resistance to pesticides June (13%), July (3.3%), and August (8.9%), a statistically significant association was found [x^2 (2) = 702.606, *p* = .000]. Comparing mosquitoes resistance to pesticide application from 7pm-8pm (8%) and 8:01-9:00pm (6.1%), a statistically significant association was identified [x^2=40.99, *p* = .000]. Lastly, a statistically significant association was identified [x^2(2) =527.98, *p* = .000] when resistance to pesticides was compared during the clear weather (4.1%), cloudy (7.5%), and sunny weather (13.0%).

At multivariate level, using a 95% confidence interval, the overall model was statistically significant [*OR* = 13.48, *p* = .00]. Although results from the chi-square analysis showed that time of pesticide application was statistically significant, it was however insignificant using the logistics regression model [*OR* = 1.19 (95% *CI*: .99-1.45), *p* = .07]. Similarly, it was insignificant for wind speed [*OR* = .93 (95% *CI*: .81-1.07), *p* = .29] despite its significance using chi-squared analysis.

However, in concordance with the chi-squared analysis, statistically significant was found between test month, weather, temperature, humidity, and Culex mosquito mortality. This shows that the odds of mosquito developing resistance to pesticides are 2.07 times higher when sprayed during higher temperature compared to lower temperatures [*OR* = 2.07(95% *CI*: 1.32-2.33), *p* = .000]. Also the odds of mosquito developing resistance to pesticides are 1.5 higher when sprayed during high humidity than in lower humidity [*OR* = 0.32 (95% *CI*: .26-.40), *p* = .000]. Additionally, statistically significant association was identified in weather from cloudy to sunny [*OR* = 0.33 (95% *CI*: .29-.36), *p* = .000] and similarly in test month from June to August (*OR* = 0.35 (95%

CI: .29-.41), $p = .000$]. The final Chapter includes an interpretation of findings reported in this chapter. The discussion of the implications and significance of the findings for social change is also discussed and recommendations for action and further research.

Chapter 5: Discussion, Conclusions, and Recommendations

Introduction

The purpose of this quantitative, cross-sectional, correlational study was to investigate the environmental factors associated with the resistance to pesticides by the Culex mosquito populations. I explored the association between the independent variables (i.e., wind speed, outside temperature, humidity, time of the day, month of the year, and weather condition at the time of pesticide application) and the dependent variable of pesticide resistance among Culex mosquito populations. Mosquito-borne diseases, of which malaria is one of the most well-known, are among the leading causes of human deaths globally (Velázquez-Castro et al., 2018). Mosquito vector control is an important part of public health efforts in the management of these diseases, and pesticide still remains the most effective component of the control efforts; however, the effectiveness of pesticide is now threatened by wide-spread mosquito resistance (Bustamante et al., 2016). Additionally, the use of pesticides to control these diseases is a triple-edged sword because on one hand, the pesticides can help to control the mosquito populations, but on the other hand, the mosquitoes can develop resistance that can render the pesticide ineffective and pesticides can damage the environment.

Mosquito-borne diseases are destructive. In 2015, malaria alone accounted for about 212 million illnesses and 430,000 deaths globally (WHO, 2016). About half of the world population (i.e., 3.2 billion people) living in over 100 countries remain at a high risk of malaria infection (Reid and McKenzie, 2016). In addition to the human suffering, malaria also carries a high financial burden, estimated to be at least $15 billion per year, mostly affecting poorer countries of the world (CDC, 2018). Besides, the socioeconomic burden associated with malaria, the disease presents a significant impediment to development in many African countries. Malaria remains the major cause of poverty in sub-Saharan Africa and other developing countries, and it is estimated that malaria alone has reduced the gross national product of the African continent by more than 30% (Ricci, 2012).

In recent years, mosquito-borne diseases are reemerging because of the pesticide resistance that has developed in mosquitoes. The development of pesticide resistance in mosquitoes is due to the broad and sometimes repeated applications of those pesticides to control mosquitoes and other pests (Polson et al., 2012). The decrease in the effectiveness of the pesticides have resulted in the failure of many mosquito control programs and probably resulted in the increased mosquito-borne disease transmission (Velázquez-Castro et al., 2018). Public health's improvement in pesticide resistance management as a field will involve a better understanding of the environmental factors potentially associated with the development of the resistance (Nkya et al., 2015). The management of pesticide resistance in the mosquito vector population is also essential for the public health efforts to contain the emergence and reemergence of mosquito-borne diseases such as malaria, St. Louis encephalitis, West Nile virus, and the Zika virus, among others. Hence, this study was important because of my examination of the environmental factors that could potentially affect the development of resistance to pesticides by Culex mosquitoes.

The major findings of the study indicated that the odds of the Culex mosquitoes developing resistance to pesticides were 2.1 times higher when sprayed during higher temperatures than at lower temperatures. Moreover, the Culex mosquito developed resistance to pesticides 1.5 times higher when sprayed during high humidity than in lower humidity. The likelihood of Culex mosquitoes developing resistance to pesticides in August were three times higher than in June and July.

Interpretation of Findings

Review of the Major Findings

In this study, I investigated the environmental factors associated with pesticide resistance in the Culex mosquito populations. The independent variables were wind speed, outside temperature, humidity, time of the day, month of the year, and weather condition at the time of pesticide application, while the dependent variable was pesticide resistance. Previous research has shown that pesticide resistance among the mosquito populations has resulted in the public health failure to control mosquito populations (Ranson and Lissenden, 2016).

My analyses in this research involved the use of both the chi-squared and logistic regression statistics. I used chi-square to establish bivariate statistical association between two variables, while logistic regression was used to establish the multivariate statistical association between more than two variables.

At the bivariate level, using chi-squared analysis, there was a statistically significant association between the resistances in Culex mosquitoes sprayed at low wind speed (8%) compared to high wind speed (6.6%) [x^2 (1) =14.01, p = .000]. Similarly, there was a statistically significant association between Culex mosquito resistance at low temperature 65 ^{0f}– 85^{0F} (4.2%) relative to high temperatures at 85.1^{0F}–95.00^{0F} (9.8%) [$x^2(1) = 346.5$, p = .000]. Also, the result identified a statistically significant association at low humidity 50.00%–65.00% (6.6%) against high humidity 65.10%–80.0% (7.50) [$x^2(1) = 7.23$, p = .007]. In comparing Culex mosquito resistance to pesticides in June (13%), July (3.3%), and August (8.9%), a statistically significant association was found [x^2 (2) = 702.606, p = .000] between June and August and Culex mosquito mortality. In comparing Culex mosquito resistance to pesticide application from 7pm–8pm (8%) and 8:01pm–9:00pm (6.1%), a statistically significant association was identified [x^2 = 40.99, p = .000]. Lastly, a statistically significant association was identified [$x^2(2) = 527.98$, p = .000] when resistance to pesticides was compared during the clear weather (4.1%), cloudy (7.5%), and sunny weather (13.0%).

At the multivariate level, the logistic regression analysis using a 95% confidence interval, resulted in the overall model being statistically significant [*OR* = 13.48, $p < .05$]. The results from the chi-square analysis showed that time of pesticide application was statistically significant, and it was also insignificant using the logistics regression model [*OR* = 1.19 (95% *CI*: .99–1.45), p = .07]. The association was not statistically significant for wind speed [*OR* = .93 (95% *CI*: .81–1.07), p = .29] despite its significance using chi-square analysis.

As with the chi-squared analysis, I found a statistically significant association between test month, weather, temperature, humidity, and Culex mosquito mortality. This finding showed that the odds of a mosquito developing resistance to pesticides are 2.07

times higher when sprayed during higher temperature compared to lower temperatures [*OR* = 2.07(95% *CI*: 1.32–2.33), p = .000]. Also, the odds of a mosquito developing resistance to pesticides are 0.32 higher when sprayed during high humidity than in lower humidity [*OR* = 0.32 (95% *CI*: .26–.40), p = .000]. I also identified a statistically significant association in weather from cloudy to sunny [*OR* = 0.33 (95% *CI*: .29–.36), p = .000] and similarly in test month from June to August (*OR* = 0.35 (95% *CI*: .29–.41), p = .000].

Discussion of Findings and Implications by Research Question

Research Question 1

Research Question 1 was: What is the association between wind speed and pesticide resistance in culex mosquitoes? The null hypothesis was that there are no associations between the wind speeds and pesticide resistance in Culex mosquitoes. In the data set, the wind speed varied between 3 mph and 10 mph and was coded low speed (0 to 5 mph) and high speed (5.1 to 10 mph). To answer the research question, I performed a chi-square analysis to compare the mosquito mortality at various wind speeds during the original tests. The significance level was set at .05. With a total number of 29,794 participants, I identified a statistically significant association [$x^2(1) = 14.01$, p = .000]. Since the p value was less than the chosen significance level $\alpha = 0.05$, the null hypothesis was rejected and the alternate hypothesis was accepted that there is an association between wind speeds and pesticide resistance in Culex mosquitoes.

From the chi-square analysis, I found that at low wind, the mortality rate of the Culex mosquitoes was 92%, while at high wind speed their mortality rate was 93.4%. The dependent variable for this study was pesticide resistance, which was measured by Culex mosquito mortality. High mortality means low resistance level. The implication here is that the pesticides were moderately effective (93.4%) when sprayed at a higher wind speed (5.1mph–10mph) than at a lower wind speed (0mph–5mph) with 92% mortality. This could mean that at a higher wind speed, the pesticide particles were propelled to the target Culex mosquitoes. On the other hand, a low wind speed could have had insufficient force to move the pesticide particles to reach the mosquitoes, thereby presenting a false negative result. Based on the results of this study, public health professionals and pest control practitioners should understand that a moderate wind speed is needed to circulate the pesticide particles. This result was similar to the findings in previous studies that indicated the wind speed and direction do favor the spread in the likelihood of pest control failure and should be considered when designing pest management programs (Gontijo et al., 2013; Nkya et al., 2015). Additionally, this finding supported the current policy of the public health department that conducted the original study that the ideal wind speed that the county sprays the pesticides in their mosquito control operations is between 5mph to 15mph.

Research Question 2

Research Question 2 was: What is the association between temperatures and pesticide resistance in Culex mosquitoes? In the data set, the temperature varied between 65 and 95 degrees and was coded low (65^{0F}–85^{0F}) and high (85^{0F}–95^{0F}). In order to test the null hypothesis, I performed a chi-square analysis that there was no statistical significance in the relationship between various temperatures and resistance in Culex mosquitoes. The significance level was set at .05. Using chi-square analysis, a statistical significance was found [x2(1) = 346.5, p = .000]. Since the p value was less than the chosen significance level α = 0.05, the null hypothesis was rejected, and I accepted the alternate hypothesis that there is an association between different temperatures and pesticide resistance in Culex mosquitoes.

The chi-square analysis showed that when the Culex mosquitoes were sprayed at a low temperature, the mortality rate was a 96% compared to 92% at a high temperature. This finding was equally significant using logistic regression analysis. Using logistic regression, the findings of this study indicated that the odds of a Culex mosquito developing resistance to pesticides was 2.1 times more in a higher temperature than in a lower temperature. The implication here is that the pesticides were more effective at a lower temperature than at a high temperature. The results of this study supported that school of thought.

However, previous studies linking temperature to pesticide resistance in mosquitoes have had contrasting findings. Some researchers have suggested that high temperatures increase the abundance of mosquitoes and cause the breakdown of pesticide molecules that can induce pesticide resistance in mosquitoes (Samy, Elaagip, Kenawy, Ayres, Peterson and Soliman, 2016); Nsengimana, 2018). Russell et al., (2016) argued that high temperatures cause mosquitoes to become more susceptible to pesticides. The results of previous research have indicated the need for further investigation regarding the association between temperature and pesticide resistance in mosquitoes (Johnsen, 2007; Kristan et al., 2018).

Research Question 3

Research Question 3 was: What is the association between humidity and pesticide resistance in Culex mosquitoes? In the data set, the humidity levels varied between 50% and 80%. For the purpose of this statistical analysis, I coded for low humidity (50%–65%) and high humidity (65%–80%). In order to test the null hypothesis that there is no statistical significance in the relationship between humidity and resistance in Culex mosquitoes, chi-square analysis was performed and the significance level was set at .05. Using chi-square analysis, I found a statistical significance [$x^2(1) = 7.23$, $p = .007$]. Since the p value was less than the significance level $\alpha = 0.05$, the null hypothesis was rejected, and I concluded that there was enough evidence to suggest an association between humidity and pesticide resistance in Culex mosquitoes.

The findings further showed that at low humidity, the Culex mosquito mortality rate was moderately higher at 93.4% while the mortality rate at high humidity was at 92.5%. Using logistic regression analysis, the finding here was equally significant showed that the odds of Culex mosquitoes developing resistance to pesticides was 1.5 times higher when sprayed at lower higher humidity compared to when sprayed at low humidity. Therefore, the efficacy of the pesticides was greater when the mosquitoes were sprayed at lower humidity than at a high humidity. This result is in contrast to the finding of some previous studies indicating that relative humidity had significant effects on mosquito mortality and that mosquitoes survive well at a moderately high humidity (Kristan et al., 2018). Mosquitoes survive better at a higher humidity. Mosquitoes also are more active when humidity rises; as a result, they are more active and prefer feeding during the night time when the humidity is higher (Nkya, 2015).

Research Question 4

What is the association between month of pesticide application and pesticide resistance in Culex mosquitoes? From the data set the months of testing included June, July, and August. To answer the research question, a chi-square analysis was conducted to test the null hypothesis that there is no statistical significance in the relationship between the months of pesticide application and mosquito mortality. The significance level was set at .05. This difference was statistically significant at (x2 (2) = 702.606, *p* = .000). Therefore, based on this evidence, the null hypothesis was rejected in favor of the alternative hypothesis. That means there is a statistical significance in the relationship between months of the year in which pesticide was applied and pesticide resistance in Culex mosquitoes. Also, the results demonstrate that in June, the pesticides were effective in killing 87% of the Culex mosquitoes. In July, the Culex mosquito mortality rate was 97%. In August, the Culex mosquito mortality rate was at 91%. As shown in the result, the pesticides were more effective in July (97%) than June and August.

The findings indicate that pesticide resistance of Culex mosquitoes was three times more likely to develop in August than June and July. This finding is similar to a widely held notion that the development of mosquito resistance to pesticides is due to the broad and repeated applications of those pesticides to control mosquitoes in the first place (Polson et al., 2012). The losses of the pesticide efficacy have resulted in operational failure of mosquito control (Velázquez-Castro et al., 2018). It could be that after broad and repeated application of pesticides to control mosquitoes in June and July, in August, the mosquitoes have already developed resistance to the pesticides. This may help to explain why the odd of Culex mosquitoes developing resistance to pesticides was three times in August than in June and July.

More research is needed on this topic. First, the original field cage tests were conducted only in the four summer months (June, July, August, and September). No data were available to establish the response of the Culex mosquitoes in the remaining eight months of the year (October through May). Studies suggest that during the four summer months, the intensive use of pesticides to control the mosquitoes is hypothesized to decrease the pest susceptibility from spring to winter months (Khoa et al., 2018).

Moreover, extending the study period to all year round could determine what happens to the levels of pesticide resistance in the Culex populations during the winter months where no active mosquito control management program is practiced. This is the period when entomologists believe that susceptibility is bred back into the mosquito populations (Smartt et al., 2016). Such a study could provide a better overview of the status of pesticide resistance in the Culex mosquito populations than testing only during the summer months (June through September).

Research Question 5

What is the association between time of the day that pesticide is applied and the development of resistance by the Culex mosquitoes? In order to test the null hypothesis, that there is no statistical significance in the relationship between the time of pesticide application and mosquito mortality, a chi square test was conducted. For the purpose of this analysis, the times of pesticide applications were coded: 1 (7 pm–8 pm) and 2 (8 pm–10 pm). The Chi-squared analysis identifies statistical significance [x 2 = 40.99, *p* = .000]. Therefore, the null hypothesis was rejected in favor of the alternative hypothesis that there is a statistical significance in the relationship between time of the day that pesticide is applied and pesticide resistance in Culex mosquitoes.

Based on the analysis, in the early evening hours, the Culex mosquito mortality rate was at 92% while the late evening hours, the mortality rate was at 94%. The result implies that the pesticides were more effective when sprayed during the late evening hours 94% compared to 92% at early evening hours. Hence, less resistance to the Culex mosquitoes when the pesticides were sprayed in the late evening hours. This is similar to previous studies that showed the Culex mosquitoes as active during the evening time and early night time biters (Samy et al., 2016). Other researchers have been more specific in time. Nsengimana (2018) and Russell et al. (2016) found that about 76% of the mosquito bites occurred just about 9pm. This may help explain why the mortality rate was higher in the 9pm hours. On the whole, mosquitoes are more active and prefer feeding during the early to late evening time (Nkya, 2015).

Research Question 6

What association between weather (clear, cloudy, sunny) at the time of pesticide application and pesticide resistance in Culex mosquitoes? From the data presented, the weather condition at the time of pesticide application include clear, cloudy, and sunny. To answer the question, a chi-square analysis was conducted. The significant level was set at .05. A statistical significance was identified [x (2) = 527.98, *p* = .000]. Since the p value is less than the significance level α = 0.05, the null hypothesis was rejected and accepted the alternate hypothesis that there is an association between weather and pesticide resistance in Culex mosquitoes. The findings further shows that at cloudy weather, the Culex mosquito mortality rate was at 92%, at sunny weather; at cloudy weather, the mortality rate was at 87%, and at clear weather, the mortality rate was at 96%. This finding suggests that the pesticides were more effective in killing the Culex mosquitoes when sprayed during clear weather 96%. This may imply that at clear weather, the pesticide particles were able to move freely in the air reaching the mosquito target.

The public health practitioners should conduct more research to either support or provide contrasting evidence to the result of this study. However, it is generally noted that the weather conditions have significant effects on pesticide applications and the potential for wastage through drift and breakdown (Kristen et al., 2018; Samy et al., 2016). Also, studies of the mosquito populations have revealed that increases in environmental conditions such as temperature and humidity are more likely to accelerate mosquito development, increase vector abundance, and lead to emergence of mosquito-borne diseases (Polson et al., 2016). Maybe these factors are more likely to prevail in clear weather condition.

Limitations of the Study

This study involved the analyses of an achieved secondary data. As such, the limitations incumbent in using secondary data do apply. First, the purpose and collection method of the original data was not influenced by the current research. Secondly, because this study involved the analysis of archived secondary data, I had no control over the original data collection. Other limitations to this study are related to external validity examined below.

External Validity

Threats to external validity occur when a researcher makes incorrect generalizations from the sample data to other populations or situations that could compromises the ability and confidence in stating whether the research results could be generalized to other populations (Creswell, 2009). The focus of this study and the data used are specific to the Culex mosquito populations and the two classes of pesticides: Malathion and Pyrethrin. The findings of this study cannot be generalized to any mosquito species other than the Culex mosquitoes. Even within the Culex mosquito populations, the findings of this study cannot be generalized beyond the Southern part of US and the four summer months (June, July, August, and September). Though most countries in sub-Sahara Africa and most other developing countries face similar and even worse mosquito problems, the results of this study cannot be generalized to other mosquito endemic regions. The response of the Culex mosquitoes was not tested during the winter months. This is the period when the entomologist hypothesized that susceptibility is bred back into the mosquito populations (Smart et al., 2016). The result cannot be generalized to other mosquito species and other classes of pesticides than those used in this study and analyses.

Recommendations

Future research is needed to help determine the significance of the results presented in this study. The county from where the mosquitoes were sampled utilized two classes of pesticides- Pyrethrin and Malathion. To help in determining what effect the resistance management program initiated by the county had on the results, studies should be conducted in another county that perform regular mosquito control program with same classes of pesticides. That can possibly provide the data that might support or refute the theory that the pesticide rotation was suppressing the development of resistance in the Culex mosquito populations in the county that conducted the original study (Johnsen, 2007).

Another modification to this research study would be the extension the testing period from four months to the twelve months of the year. Currently the study is conducted only in the summer months (June, July, August, and September) in the Southern part of United States. The Field Cage Tests should be conducted all year round (January through December). Extending the study period to all year round could determine what happens to the levels of pesticide resistance in the Culex populations during the winter months where no active mosquito control management program is practiced. This is the period when the entomologist hypothesized that susceptibility is bred back into the mosquito populations (Smartt et al., 2016). Such a study could provide a better overview of the status of pesticide resistance in the Culex mosquito populations than testing only during the summer months (June through September).

Although the findings of this study cannot be generalized to other mosquito species and locations, some areas with mosquito species such an Anopheles that transmits malaria may benefit from the conclusions of this study. One of the limitations of this study is that only one mosquito specie (Culex) was investigated. It would be of public health significance to conduct same research on Anopheles mosquito, the primary vector of malaria. Also, same study should be conducted on Aedes mosquitoes that vector the Zika virus. Therefore, there is a need to conduct more studies to establish the effect of environmental factors: temperature, humidity, and wind speed, time of the day, and months on the development and distribution of pesticide resistance among different mosquito species. Such research is needed because so far studies investigating the association between temperature and pesticide resistance have contracting results. Some studies suggest that high temperatures can induce pesticide resistance in mosquitoes. In the contrast, other studies observe that high temperatures can cause mosquitoes to become more susceptible to pesticide (Johnsen, 2007). Additionally, the findings of this study showed that the overall mosquito mortality rate in 2013 was 91%, and the mosquito mortality rate was 89% in 2014. In 2015, 2016, and 2017 the mosquito mortality rates were 97%, 98%, and 91% respectively. Further research is needed to find out the reason why the mosquito mortality rates were abnormally low in 2013, 2014, 2017.

Implications for Social change

A potential positive social change impact of this study could be a reduction in the numbers of nuisance and disease-carrying mosquitoes thereby creating healthier environments and possibly reduce the incidence of mosquito-borne diseases. Over time, with the repeated and broad application of pesticides in an area, resistance can develop in the target mosquito populations. Hence, if the development of pesticide resistance by the mosquito populations can be minimized, that would represent a positive social change. Furthermore, understanding the environmental factors associated with the development of resistance by mosquitoes could increase public health knowledge about mosquito vector control strategies. That could promote wise use of pesticide. For instance, if high wind speed, high humidity, and high temperature can adversely affect pesticides application, then applying pesticide at time and conditions more conducive to minimizing the adverse effects of resistance development is a wise use of pesticide and an important positive social change.

Summary

In this study, I investigated the environmental factors (wind speed, humidity, temperature, time, months, and weather) associated with pesticide resistance in the Culex mosquito populations. The analyses involved the utilization of both the chi-square and logistic regression. Chi-square was used to establish bivariate statistical association between two variables while logistic regression was used for the establishment of the association between more than two variables.

The findings showed that the odds of the Culex mosquitoes developing resistance to pesticides were higher when sprayed during higher temperature than at lower temperatures. Furthermore, the result demonstrated that the odds of Culex mosquito developing resistance to pesticides were higher when sprayed during high humidity than in lower humidity. Also, the odd of Culex mosquitoes developing resistance to pesticides in August were higher than June and July. Therefore, there is a need for researchers to conduct more studies on the effect of temperature, humidity, and months on the development and distribution of pesticide resistance among different mosquito species. This is necessary because studies linking temperature to pesticide resistance in mosquitoes is contrasting. Some studies suggest that high temperatures induce pesticide resistance in mosquitoes. In contrast, other studies note that high temperatures cause mosquitoes to become more susceptible to pesticide (Johnsen, 2007). Until whenever the scientists will come up with more definite information about the effects of temperature, humidity, and months on the development of pesticide resistance by mosquitoes, public health practitioners and mosquito control professionals should practice wise use of pesticides. That means minimizing pesticide application during high temperature and high humidity as well as in the month of August

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