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Evaluation of autodissemination technique using novaluron against anopheles arabiensis under semi-field conditions in south-eastern Tanzania

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EVALUATION OF AUTODISSEMINATION TECHNIQUE USING NOVALURON AGAINST *ANOPHELES ARABIENSIS* UNDER SEMI-FIELD CONDITIONS IN SOUTH-EASTERN TANZANIA

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

This study assessed the susceptibility of immature stages of *An. arabiensis, An. gambiae* and *An. funestus* to novaluron and the autodissemination technique using *An. arabiensis*. Susceptibility bioassays using technical grade novaluron (98% active ingredient) were performed inside the semi-field system using first instar larvae of the mosquito test species. A total of 1500 larvae were exposed to novaluron within three replicates of control and treatment assays. Concentration ranges of 0.01 mg/l to 2 mg/l of novaluron were tested to establish lethal concentration (LC) sufficient to kills 50%, 90% and 99% of the exposed larvae (LC_{50}, LC_{90} and LC_{99}) by using log-dose response analysis. The autodissemination experiment exposed 2500 mated female blood fed *An. arabiensis* mosquitoes (aged 6-7 days) to both contaminated and uncontaminated clay pots. In two each chambers in the semi-field tunnel cage; an artificial breeding habitats were provided in each chamber to assess the autodissemination. The successful autodissemination and contamination was assessed by comparing larval mortality from treated and untreated chambers. *An. gambiae* were highly susceptible to novaluron followed by *An. arabiensis* and then *An. funestus*. Lethal concentrations, LC_{50}, LC_{90} and LC_{99} (95%CI) in mg/l for *An. gambiae* were 0.018 (0.016-0.020), 0.546 (0.374-0.719) and 2.001 (1.986-3.206) respectively. For *An. arabiensis* were 0.032 (0.027-0.038), 0.332 (0.168-0.496) and 2.013 (1.997-4.491); and for *An. funestus* were 0.02561 (0.02140-0.0299), 1 (0.4657-1.5347) and 5.580 (4.687-8.496). High larval mortality was recorded at high concentration (2mg/L) for all species, with 80% mortality within 3 days post exposure. Similarly, low larval mortality was observed at low concentration (0.1 mg/L) (for all species) with 80% mortality within 9 days post exposure. There were no evidence of autodissemination following adults' exposure to novaluron. The results showed no significant difference between treatment and control cups when *An. arabiensis* larvae were exposed to the water samples from the breeding habitats. The study demonstrates the efficacy of novaluron against immature stages of susceptible and resistant *Anopheles* mosquito species. The findings present a promising candidate IGR for rotation to counteract the insecticide resistance development. Moreover, these results warrant further evaluation of novaluron for autodissemination by vector species for its inclusion in rotation to prevent evolution of resistance in both chemistries.

Keywords: Insect growth regulator, novaluron, autodissemination, *An. arabiensis, An. gambiae, An. funestus,* Tanzania.

DECLARATION

I, **Amos Ngonzi Justinian**, hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that, this research titled "Evaluating efficacy of autodissemination technique with novaluron against *Anopheles arabiensis* under semi-field conditions in southeastern Tanzania" is my work under the guidance of supervisors and has never been or intending to be submitted for a degree award in any other university.

July 23/2022

_____   _____

Amos Ngonzi Justinian   Date

STATEMENT OF COPYRIGHT

CERTIFICATION

The undersigned certify that they have read and hereby confirm that the dissertation entitled "Evaluating efficacy of autodissemination technique with novaluron against *Anopheles arabiensis* under semi-field conditions in south-eastern Tanzania" submitted by Amos Ngonzi Justinian to Nelson Mandela African Institution of Science and Technology, Tanzania in fulfillment of the requirements for the award of Master of Science in Public Health Research is a trustworthy work done under supervision.

July 24/2022

Adj. Prof. Dickson Lwetoijera   Date

Supervisor

July 24/2022

Dr. Sr. John-Marry Vianney   Date

Co- Supervisor

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ACKNOWLEDGEMENT

DEDICATION

I dedicate this work to my family, parents, mentors and supervisors. Also, I dedicate this work to IHI- Training unity and entire IHI community and Nelson Mandela African Institution of Science and Technology (NM-AIST).

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LIST OF ABBREVIATIONS

IGRs   Insect Growth Regulators

IHI   Ifakara Health Institute

IRB   Institutional Review Board

IRS   Indoor Residual Spray

IVCC   Innovative Vector Control Consortium

JH   Juvenile Hormone

LC   Lethal Concentration

LLIN   Long Lasting Insecticide Nets

LSM   Larval Source Management

NIMR   National Institute for Medical Research

PPF   Pyriproxyfen

SFS   Semi-field system

USD   United States Dollar

WHO   World Health Organization

CHAPTER ONE

INTRODUCTION

1.1. Background of the problem

Outdoor and indoor malaria transmissions have profoundly led to the present malaria morbidity and mortality. In the year 2020 alone, there were 241 million malaria cases and 627 000 deaths globally (WHO, 2021). Disproportionately, countries in sub-Saharan Africa, including Tanzania, have continued to accounting 95% of total cases and 602 000 deaths (WHO, 2021). Additionally, malaria is considered to be a major economic burden in Africa, whereby the continent lost 12 billion USD in the year 2000 (Kidd, 2003). It has been demonstrated that a 10% global decrease in malaria incidence can result up to a 0.3% average increase in income per capita, with high malaria endemic areas benefiting most (Gallup & Sachs, 2001; Sarma *et al*., 2019). With this potential economic impact, effective malaria prevention strategies are essential to overcome this burden.

Different strategies for malaria prevention using vector control tools, primarily the long-lasting insecticide mosquito nets (LLINs) and indoor residual sprays (IRS) are strongly recommended by the Word Health Organization (WHO, 2021). Across sub-Saharan Africa where >90% of the disease burden is concentrated, both LLINs and IRS have significantly suppressed malaria vectors, especially, those that bite and rest indoors (Agossa *et al*., 2018; Bhatt *et al*., 2015). These control measures have contributed to nearly 40% to 57% reduction of clinical disease incidences (Bhatt *et al*., 2015). However, rapid increase in insecticide resistance and observed higher outdoor biting and resting patterns of malaria vectors could potentially jeopardize the impact of the available primarily malaria vector control interventions towards malaria elimination efforts (Hemingway, 2017). These challenges demonstrate the prompt need of alternative malaria vector control measures which can complement the existing malaria vector interventions.

One promising option that has been widely used and proven effective is larval source management (LSM) which entails the use of chemical and biological agents in controlling malaria vectors (Walker & Lynch, 2007; WHO, 2014). This technique works by reducing vector densities in mosquito breeding habitats either through killing effect of mosquito immature stages or inhibiting the emergence of adult mosquitoes (WHO, 2014). However, despite the demonstrated success and

recent renewed momentum on the use of larviciding for mosquito control across Africa (Chaki *et al*., 2009; Stanton *et al*., 2021), high operational costs and poor coverage of targeted breeding habitats have remained to be its greatest challenge (Chaki *et al*., 2009; Geissbühler *et al*., 2009; Stanton *et al*., 2021).

Of importance, mosquito assisted larviciding commonly known as autodissemination with insect growth regulators (IGRs) such as pyriproxyfen (PPF) and novaluron has proven its effectiveness in terms of accurately targeting aquatic habitats with larvicide (Devine et al., 2009; Lwetoijera *et al., 2014a;* Mbare *et al., 2014;* Swale *et al., 2018)*. By definition, autodissemination is a management method in which insects such as mosquitoes get exposed and pick biological or chemical insecticide such as IGRs while foraging or resting, and transfers lethal concentrations horizontally to the oviposition sites which results in reduction of adult mosquitoes densities (Devine *et al*., 2009; Gaugler *et al*., 2012).

Novaluron is an IGR that has recently been tested using the autodissemination technique against different mosquito species (Swale *et al*., 2018). It works by inhibiting the chitin synthesis process on larval stages of mosquitoes through contact and ingestion of a benzoylphenyl urea, and the larvae succumb to death as the results of abnormal endocuticle deposition (Arredondo-Jiménez & Valdez-Delgado, 2006; Swale *et al.,* 2018; Tunaz & Uygun, 2004) In addition, novaluron is safe to the environment including mammals, birds, aquatic animals and non-targeted insects (Tunaz & Uygun, 2004).

Recently, novaluron has shown reduction of different adult mosquito species density through killing immature mosquitoes at their larval stage. Novaluron has demonstrated efficacy against immature *Anopheles quadrimaculatus, Aedes aegypti* and *Culex quinquefasciatus* in the laboratory and field settings (Mulla *et al*., 2003; Su *et al*., 2014; Swale *et al*., 2018)*. Further, have been shown to inhibit the emergence of adult *An. quadrimaculatus* by up to 22% (Swale *et al*., 2018).

Despite the benefit that novaluron can offer in the control of other mosquito borne diseases, paucity of evidence exists on their application in the control of the main malaria vectors in rural area of south-eastern Tanzania. This study evaluated the susceptibility of the main malaria vectors in south-eastern Tanzania, *Anopheles arabiensis*, *Anopheles gambiae* and *Anopheles funestus,* to varying doses of novaluron. Additionally, the study assessed the potential of *Anopheles arabiensis*

to autodisseminate novaluron to the breeding habitats and prevent adult emergence under semi-field settings.

1.2. Problem statement

Despite of various efforts and approaches used to control malaria in endemic areas, malaria transmission persist. Primary vector control tools namely LLINs and IRS have played a significant role in preventing indoor malaria transmissions. However, development and behavioral resistance against LLINs and IRS that target indoor malaria mosquitoes is increasing (Hemingway, 2017). Human behaviour that exposes the community to mosquito bites and poor compliance to LLINs usage are among the drivers of malaria transmission and on-going malaria transmission (Gryseels *et al*., 2015; Moshi *et al*., 2017). Therefore, there is a need for a complementary tool to fill this gap such as the use of autodissemination technique with pyriproxyfen, with proven easy transferability and high efficacy at the breeding habitats. Though scanty evidence exists that pyriproxyfen is metabolized in *An. gambiae* by the same mechanism as pyrethroids (Yunta *et al*., 2016), there is still no evidence of pronounced resistance to PPF in any mosquito species, but possibility for its development should not discounted. To maintain PPF efficacy there is a need of another larvicide such as novaluron that would work in rotation with PPF. But similar evidence is yet to be demonstrated against *An. arabiensis*. Therefore, this study evaluated the susceptibility of major malaria vectors in Tanzania to novaluron and documented the ability of *An. arabiensis* to autodisseminate novaluron to its breeding habitats under controlled settings.

1.3. Research rationale

Malaria control programs require effective tools, which can reduce or eliminate malaria vector populations in endemic areas. Outdoor malaria transmission that significantly contributes the on-going malaria transmission cannot be effectively managed by current tools LLINs and IRS, hence there is a need for novel tools that can complement primarily control strategies tool. Therefore, this study demonstrated the ability of the adult *An. arabiensis* to autodisseminate the novaluron compound and the susceptibility status of *An. Arabiensis, An. gambiae* and *An. funestus* larvae to novaluron.

1.4. Research objectives

1.4.1. General objective

The overall aim of this study was to assess the efficacy of novaluron autodissemination technique by *An. arabiensis* under semi-field settings

1.4.2. Specific objectives

The study intended to achieve the following objectives:

(i)   Assessing the susceptibility status of *An. arabiensis, An. gambiae* and *An. funestus* to novaluron chitin synthesis inhibitor.

(ii)   Assessing the potential of *An. arabiensis* to autodisseminate novaluron to the breeding habitats and prevent adult emergence under semi-field settings.

1.5. Research questions

(i)   Can *An. arabiensis* autodisseminate novaluron from contamination stations to the breeding habitat under the semi-field condition?

(ii)   What is the susceptibility status of novaluron in *An. arabiensis* mosquitoes?

1.6. Hypothesis

The following hypothesis were tested

(i)   *An. arabiensis* can autodisseminate the novaluron to oviposition sites and prevent emergence of adult mosquitoes

(ii)   *An. arabiensis, An. gambiae* and *An. funestus* are susceptible to standard concentration of novaluron-chitin synthesis inhibitor

1.7. Significance of the study

Larval source management (LSM) with larviciding can be used as a supplementary strategy to control adult mosquitoes in Africa (Tusting *et al*., 2013; WHO, 2014). Therefore, this study provides information with potential to improve malaria control using novaluron in autodissemination technique. Furthermore, the study has the potential to contribute into country's larviciding strategy guidelines whereby autodissemination technique can be deployed to cover

habitats that cannot be detected hence remain untreated by ground community owned resource person (CORP).

1.8. Delineation of the study

This study evaluated the susceptibility of the main malaria vectors in south-eastern Tanzania, *Anopheles arabiensis*, *Anopheles gambiae* and *Anopheles funestus,* to varying doses of novaluron. Additionally, the study assessed the potential of *Anopheles arabiensis* to autodisseminate novaluron to the breeding habitats and prevent adult emergence under semi-field settings. This study had the following limitations; under laboratory settings no attempt was made to test for persistence of novaluron in the test cups beyond single larval exposure. While low susceptibility of *An. funestus* to novaluron was attributed to its high insecticide resistance status, no actual experiments that were carried to ascertain this assertion. Therefore, these limitations add on the list of future studies towards development of novaluron as the potential larvicide for malaria vector control.

CHAPTER TWO

LITERATURE REVIEW

1.1. Malaria burden in Africa

Malaria has risks to pregnant women, fetus and early childhood (Guyatt & Snow, 2001; WHO, 2021). It is reported that *plasmodium* infections cause severe diseases and death to mothers before or after giving birth (WHO, 2021). Among 11.6 million pregnancies, 819 000 children were born with low birth weight from women exposed to malaria infections during pregnancy (WHO, 2021). These infections are significant contributors of stillbirth and preterm birth, and also, lead to poor fetal growth and low birth weight (Guyatt & Snow, 2001; WHO, 2021). In Tanzania, the burden of malaria in pregnant women has remained the problem, causing maternal, fetus, and neonatal health effects such as spontaneous abortion, maternal anemia, stillbirth, premature birth, low birth weight, and maternal death despite 78% ITNs coverage in 2018 (Kitojo *et al*., 2019; Mikomangwa *et al*., 2020; Mlugu *et al*., 2020). Therefore, there is the need for complementary interventions to accelerate malaria control and elimination efforts.

1.2. Malaria vectors in Africa

Most of malaria vectors in Africa are *Anopheles* species namely *Anopheles gambiae* complex (*An. arabiensis*, *An. gambiae*, *An. quadriannulatus*, *An. melas*, *An. merus*, *An. bwambae*, *An. amharicus*, *An. fontenillel* and *Anopheles coluzzii*) and *Anopheles funestus* group (*An. funestus s.s, An. parensis, An. vaneeden, An. rivulorun, An. brucei, An. aruni, An. confuses, An. leesoni* and *An. fuscivenosus)* (Coetzee, 2020; Sinka *et al*., 2012)*.* The major malaria vectors found in Tanzania are *An. funestus*, *An. gambiae* and *An. arabiensis*. Following a significant decline of *An. gambiae* to undetectable number in Tanzania (Lwetoijera *et al.,* 2014b), the remaining major malaria vectors that contribute to malaria cases in the country are *An. funestus* and *An. arabiensis*. *An. arabiensis* is abundantly compared to *An. funestus* which is occurring in fewer number, but contribute a major risk than *An. Arabiensis* (Kaindoa *et al.,* 2017; Lwetoijera *et al.,* 2014a)*. An. funestus* contributes major risk because they are predominantly anthrophilic (prefer blood from human than other species) and endophilic (Muturi *et al.*, 2009; Spielman, 1970). Additionally, *An. funestus* are resistant to some of the commonly-used pyrethroids insecticides and their superior daily survival probabilities as reflected in the higher parity rates compared to other malaria

transmitting mosquitoes (Kaindoa *et al.,* 2017; Lwetoijera *et al.*, 2014a; Morgan *et al.*, 2010; Riveron *et al.,* 2016).

1.3. Malaria control and its associated challenges

The mainstay malaria vector control tools, Long lasting insecticide nets (LLINs) and indoor residual sprays (IRS) target indoor biting mosquitoes (WHO, 2021). Long lasting insecticide nets and indoor residual sprays have contributed up to 66% malaria reduction globally (Disch, 2011). Among others, insecticide resistance, outdoor biting, and poor compliance to LLINs usage have remained to be major challenges in malaria control which necessitates the need for alternative outdoor based tools to complement the existing indoor based interventions (Hemingway, 2017; IVCC, 2020; Riveron *et al*., 2018).

Intervention such as attractive targeted sugar baits (ATSB) that kills mosquitoes upon ingestion, eave tubes that prevent mosquito entry into houses, genetically modified organisms (GMO) such as male mosquitoes that spreads germs to wild female mosquitoes and larvae that hatch from eggs of affected females do not survive to adulthood, and house improvements prevent mosquitoes from entering inside the house (Knols *et al*., 2016; Müller *et al*., 2010; Sternberg *et al*., 2016) represent some of the alternative transformative tools for controlling mosquitoes while outside and inside the house. However, studies have demonstrated the use of Insect Growth Regulators (IGRs) that target both indoor and outdoor malaria mosquito by suppressing mosquito population at their immature stages (Devine *et al.,* 2009; Lwetoijera *et al.,* 2014a; Swale *et al.,* 2018).

1.4. Challenge on LLINs and IRS

The main threats to LLINs and IRS is the widespread mosquito resistance to the pyrethroid insecticides via metabolic, physiological and cuticular mechanisms (Hemingway, 2017; Riveron *et al*., 2018; WHO, 2012). This increase in mosquito insecticide resistance is an inherent characteristic that involve changes in mosquito genes (Hemingway *et al*., 2004) due to extensive exposure to insecticides. These changes are associated with the altering of insecticide active target site to prevent insecticide from binding, insecticide reduced penetration and metabolic resistance (Hemingway, 2017; Riveron *et al*., 2018; WHO, 2012). Second is the change in mosquito feeding behavior to avoid insecticide treated nets and IRS, by feeding outdoors and in early and late hours

of the day (Russell *et al*., 2013; Sougoufara *et al*., 2014). Third is the increase in human-outdoor activities at time when mosquitoes are active (Russell *et al*., 2013), and poor compliance on the usage of LLINs and IRS expose the community to mosquito bite (IVCC, 2020; Russell *et al*., 2013). Therefore, these challenges need a supplementary measure for malaria vector control such as the larval source management (LSM) with the aim of reducing the number of mosquito larvae and pupae.

1.4.1. Larviciding for mosquito control

Larviciding refers to the application of biological and chemical insecticides to water containers or water bodies (oviposition sites of mosquitoes) to kill the aquatic immature stages of mosquitoes (larval or pupa stages) therein (Tusting *et al*., 2013). The use of larviciding as a method is growing in sub-Sahara Africa as a supplement of LLINs and IRS that are challenged with the increase of mosquitoes' resistance, behavioral avoidance and outdoor malaria transmission (Hemingway, 2017; WHO, 2014). Larviciding is environmentally acceptable since it has minimal or no effect on non-target invertebrate and vertebrate populations, and can be applied both in rural and urban areas (Fillinger & Lindsay, 2011; Maheu-Giroux & Castro, 2013; WHO, 2014).

The use of microbial larvicides such as *Bacillus sphaericus* (*Bs*) and *Bacillus thuringiensis, var. israeliensis* (*Bti*) has recently demonstrated and impact in Tanzania against malaria mosquitoes (Mazigo *et al*., 2019), in reducing adult mosquito population (Dambach & Becker, 2019; Mazigo *et al*., 2019). Use of *Bti* is however challenging since it is quickly broken down in the environment, and therefore requires frequent applications (Mazigo *et al*., 2019; WHO, 2014), another challenge it is difficult to locate the breeding habitats during the use of microbial larvicides (Chaki *et al*., 2009).

Using geographical information system (GIS) and unmanned aerial vehicles (UAV) has potential to identify and treat mosquito breeding habitats with larvicide and render them unable to produce adult mosquitoes (Stanton *et al*., 2021) and It's application has been demonstrated in Malawi and Zanzibar, to generate orthophotos of mosquito larval habitats that are suitable for larviciding activities (Hardy *et al*., 2017; Stanton *et al*., 2021). However, this technique when applied to larviciding activities is time-consuming, requires a high-specific computer and a large capacity for

data storage and people skilled in both image capture and processing causing a challenge in its applicability (Stanton *et al*., 2021; UNICEF, 2020).

On the other hand, autodissemination technique with Insect Growth Regulator (IGRs) such as PPF has recently demonstrated an impact against mosquitoes (Devine *et al.,* 2009; Lwetoijera *et al.*, 2014a). Pyriproxyfen (PPF) has proven its effectiveness in terms of accurately targeting aquatic habitats with larvicide and delivering the desired outcome (Devine *et al.*, 2009; Lwetoijera *et al.,* 2014a; Mbare *et al.,* 2014). Existing studies have demonstrated the effectiveness of this technique with PPF under controlled conditions against *An. arabiensis* and *An. gambiae* in reducing the emergence of adult mosquitoes (Lwetoijera *et al.,* 2019, 2014a; Mbare *et al.,* 2014). Although there is no sound evidence of PPF resistance by malaria vectors, its future wide application will require an additional IGR such as novaluron to maintain its efficacy.

1.5. Insect growth regulators (IGRs)

IGRs are chemicals that break the life cycle of an insect by interfering with its growth and development (Swale *et al*., 2018). IGR can be divided into two groups which juvenile hormone mimics such as pyriproxyfen and chitin synthesis inhibitors such as lufenuron, triflumuron and novaluron (WHO, 2014). The main advantages of IGRs is that they are effective in low dosages, have long-lasting residual impact, low toxicity to mammals and birds, are effective where mosquitoes have established resistance and safe when used in drinking water (Tunaz & Uygun, 2004; Wang *et al.,* 2005; WHO, 2007, 2008, 2014) In addition, IGRs such as PPF, novaluron and triflumuron have played a significant role in autodissemination technique to suppress the emergence of adult mosquitoes (Devine *et al.*, 2009; Lwetoijera *et al.,* 2014a; Swale *et al.,* 2018).

1.6. Autodissemination technique with PPF and novaluron

Autodissemination technique is the method that co-opt mosquitoes' behaviors when exposed to a contaminated device (Devine *et al.,* 2009; Lwetoijera *et al.,* 2014a). Mosquitoes pick the insecticide from the contaminated devices and transfer it to the resting, feeding or breeding sites (Devine *et al.,* 2009; Lwetoijera *et al.,* 2014a). The transferability process of insecticide can either be horizontally (from contaminated adults to larvae/pupae in breeding habitats) or vertically (i.e., inability of contaminated mosquitoes to lay eggs or males to form sperm) depending on the insecticides mode of action (Dell Chism & Apperson, 2003; Mbare *et al*., 2014).

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Recently, autodissemination technique has been shown using IGRs against different mosquito species. Devine *et al.,* (2009) and Lwetoijera *et al.,* (2014) demonstrated the autodissemination method with PPF against *An. gambiae* and *An. arabiensis* respectively (Devine *et al.,* 2009; Lwetoijera *et al.,* 2014a), and in the findings, PPF showed a significant effect by preventing emergence of adult mosquitoes (Devine *et al.,* 2009; Lwetoijera *et al.,* 2014a). Moreover, novaluron, a chitin synthesis inhibitor has demonstrated impact against different mosquito species in their larval stage (Arredondo-Jiménez & Valdez-Delgado, 2006; Mulla *et al.*, 2003; Swale *et al.*, 2018). With evidence reported on its efficacy, novaluron worked against immature *Aedes aegypti* and *Culex quinquefasciatus, An. albimanus, An. quadrimaculatus* and *An. pseudopunctipennis* in the laboratory and field settings and reduced the pupation rate of all the mosquito species (Mulla *et al., 2003;* Su *et al., 2014;* Swale *et al., 2018)*. Swale *et al* and others demonstrated novaluron as being an effective autodissemination agent in transferability compared to pyriproxyfen when *An. quadrimaculatus* was the main vehicle and caused 22% adult reduction emergence (Swale *et al.*, 2018).

Therefore, the integration of autodissemination with multiple larvicides such as PPF and novaluron that has a different mode of action in realm of existing LLINs and IRS has potential to complement vector control efforts through insecticide resistance management (Choi *et al.*, 2019; Hemingway, 2017; IVCC, 2019). Targeting mosquito at its habitats has advantage of controlling mosquitoes that are resistant and/or susceptible and prefer biting indoors and outdoors as well (Devine *et al.,* 2009; Lwetoijera *et al.,* 2014a; Swale *et al.,* 2018). Interestingly, the technique requires minimal human intervention and is a cost-efficient strategy that provides a promising way for controlling malaria vectors in Tanzania.

1.7. Mechanism of novaluron

Novaluron is a benzoylphenylurea derivative which by contact/diffusion and ingestion inhibits the biochemical process responsible for the formation of chitin synthase (the enzyme that synthesizes chitin in insects) (Santorum *et al.*, 2019; WHO, 2014). Novaluron mainly affects larval stages of mosquitoes and causes death by abnormal endocuticular deposition and interrupting ecdysis resulting to adult mosquito emergence reduction (Swale *et al.*, 2018). At cellular level this is achieved when novaluron inhibits N-acetylglucosamine incorporation into the insect chitin, which

in turn disrupts the transportation of the protein involved in the chitin polymerization. Field studies demonstrated the success of novaluron in inhibiting chitin synthesis of *Culex* and *Aedes* mosquitoes (Mulla *et al*., 2003; Su *et al*., 2014). Similarly, semi-field based-autodissemination with novaluron reduced the adult *Anopheles*' mosquitoes emergence of *An. albimanus, An. pseudopunctipennis* and *An. quadrimaculatus* (Mulla *et al*., 2003; Su *et al*., 2014).

1.8. Effect of novaluron on non-targeted species

Mosquito aquatic habitats are occupied with different organisms such as nematodes, fish, insects such as cladocerans, beetles, dragonflies and countless other micro-organisms all of which contribute to the balance of this aquatic ecosystem, including roles such as mosquito predators (Arredondo-Jiménez & Valdez-Delgado, 2006; Cutler *et al*., 2006). Novaluron being selective in its mode of action, has a reduced hazard to the environment including mammals, birds, aquatic animals and is beneficial and non-target insects (Tunaz & Uygun, 2004). The recommended dosage for novaluron ranges from 0.01 mg/l to 0.05 mg/l in the container breeding habitat. The range of the dosage varies because it depends on the species of mosquitoes, pattern of rainfall and type of larval habitat (WHO, 2014).

1.9. Potential of novaluron impact on malaria transmissions

Novaluron impact on malaria transmission is achieved through reduction of adult mosquitoes densities (through inhibiting adult mosquito emergence), a significant element of vectorial capacity and malaria transmission (Swale *et al.*, 2018). In contaminated oviposition sites, novaluron acts on and kills 1st and 2nd larval stage of mosquitoes, hence prevent pupation (Mulla *et al*., 2003; Swale *et al*., 2018). Pupal emergence inhibition has been observed in *An. quadrimaculatus* (Swale *et al*., 2018)*, An. pseudopunctipennis* (Arredondo-Jiménez & Valdez-Delgado, 2006) as well as *Culex* and *Aedes* mosquitoes (Mulla *et al*., 2003; Su *et al*., 2014). The combination of autodissemination with novaluron and WHO recommended tools LLINs and IRS may potentially provide human protection from malaria transmission through reduced mosquito densities and survival of adult mosquito populations (Hemingway, 2017; IVCC, 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study site

This study was conducted in a semi-field system (SFS) and semi-field tunnel cage in Ifakara Health Institute between May to October, 2021. The SFS (Fig. 1) and SF-Tunnel cage (Fig. 2) are located at Kining'ina village (8.11417°S, 36.67484°E) in Ifakara, Kilombero district, southeastern Tanzania. Detailed description and dimensions of the SFS and SF-Tunnel cage have been described elsewhere (Ferguson *et al.,* 2008; Lwetoijera *et al.,* 2014a).

Figure 1: **Semi-field system used in experiments A); Chambers inside the semi-field B); Mosquito rearing insectary inside the semi-field system C)**
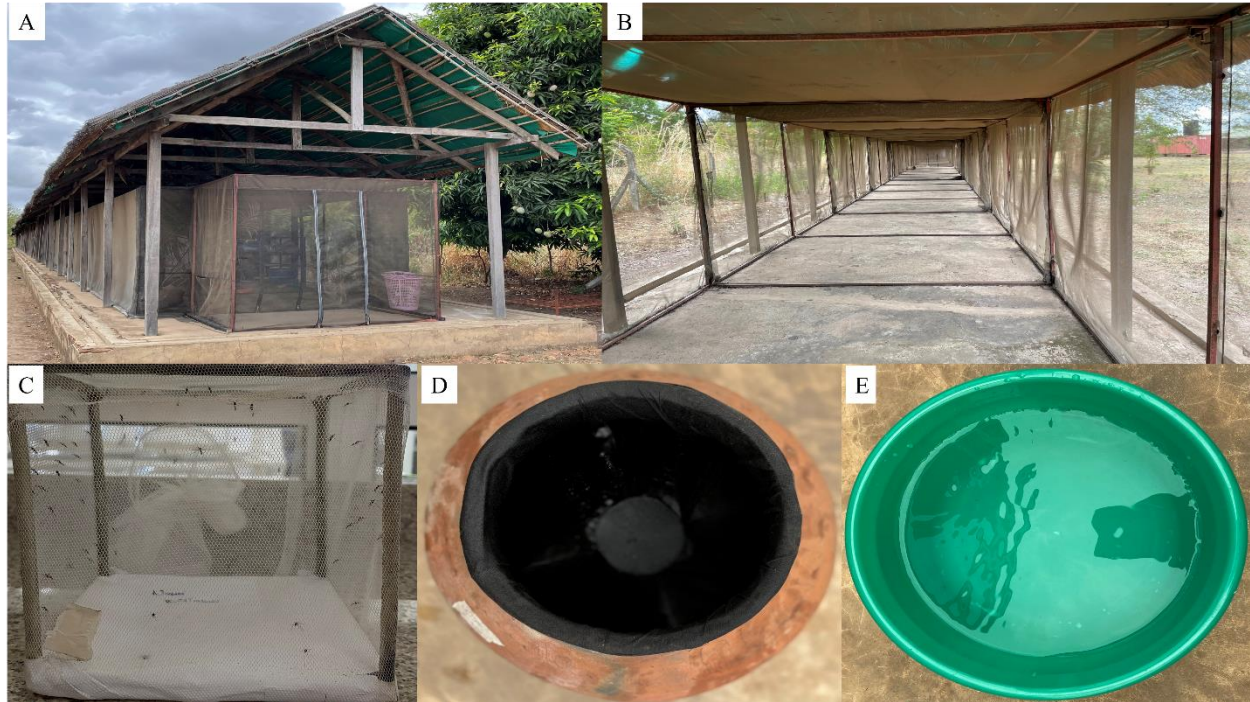
Figure 2: The semi-field tunnel cage used for experiment A); Adjoining chambers inside the semi-field tunnel cage B); Blood fed mosquitoes inside the cage C; Novaluron treated clay resting pot inside the chamber D); Plastic basin within a chamber to provide the artificial breeding habitat E*)*

3.2. Mosquitoes

The study used insectary reared mosquitoes from the established colonies of *An. arabiensis*, *An. gambiae* and *An. funestus* (FUTAZ). Details of colonies rearing and maintenance are also provided elsewhere (Agumba *et al*., 2019; Ferguson *et al*., 2008; Ng'habi *et al*., 2015; Ngowo *et al*., 2021). All bioassays used first instar larvae owing to its high susceptibility to novaluron (Swale *et al*., 2018). For autodissemination, the experiment used gravid and blood fed females *An. arabiensis* aged between 3 – 9 days from Kining'ina mosquito rearing insectary.

3.3. Study design

The study had two experiments, first was assessing the susceptibility status of *An. arabiensis larvae, An. gambiae larvae* and *An. funestus larvae* to novaluron, an insect growth regulator (IGR).

Second, assessing the potential of *An. arabiensis* to autodisseminate novaluron to the breeding habitats and prevent adult emergence under semi-field settings, whereby the expected outcomes were high larval mortality at the treated aquatic habitats, confirming the technique.

3.4. Assessing the susceptibility status of *An. arabiensis* to novaluron-chitin synthetic inhibitor

3.4.1. Novaluron and preparation of test concentrations

The technical grade novaluron was in a powder formulation with 98% active ingredient (AI) from technical materials; (Jiaozuo Huisell Chem, Ltd, China). The test concentrations were prepared using standardized procedures (WHOPES, 2005). Mass of novaluron of; 0.01 mg, 0.05 mg, 0.1 mg and 2.0 mg were measured using electronic beam balance and dissolved in 1000 mls of tap water to prepare the concentrations; 0.01 mg/L, 0.05 mg/L, 0.1 mg/L and 2 mg/L respectively. Aliquots of 200 mls of each prepared concentration was placed in plastic cup (four replicates) for bioassays plus four control plastic cups containing tap water alone.

3.4.2. Laboratory Susceptibility test

The bioassays had control and treatment cups containing test concentration and mosquito larvae. The expected outcome was larval mortality in the treatment cups compared to the control cups to confirm lethal concentrations that were required to kill 50%, 90% and 99% of exposed larvae. Twenty-five (25) first instar larvae per replicate were exposed to novaluron concentrations; 0.01 mg/L, 0.05 mg/L, 0.1 mg/L and 2.0 mg/L. The set-up was repeated three times on different days to counter confounders in the assay. Larvae were fed at 1-day interval with Tetramin® fish food throughout the course of the assay. The larval mortality was monitored on 24 hours intervals until all larvae were dead or pupated. Dead larvae were counted and removed from the plastic cups.

3.4.3. Effect of novaluron on pupation rate

The effect of novaluron on larval mortality was recorded to determine the percentage inhibition of pupation (PI%). Moribund and dead larvae and pupae that did not completely separated from the larvae case, were considered as affected by novaluron. The data from all replicates were combined

to calculate the mean of affected larvae. The PI% of *Anopheline* larvae caused by novaluron was calculated using the formula.

$$PI\% = 100 - \left(\frac{T \times 100}{C}\right) \text{ Whereby;}$$

$$T = Percentage\ pupation\ in\ treated\ cups$$
$$C = Percentage\ pupation\ in\ control\ cups$$

3.5. Assessing the potential of *An. arabiensis* to autodisseminate novaluron to the breeding habitats and prevent adult emergence under semi-field settings

Two experimental phases of three replicates each were performed to assess the ability of *An. arabiensis* to autodisseminate novaluron to the provided artificial breeding containers following exposure. Each replicate had a total of 5000 female blood-fed mosquitoes equally divided between control and treatment chambers. In the first experimental phase clay pots of 10L capacity for mosquito exposure were prepared by lining its inside with the black cloth that has been dampen with water and treated with 0.3 g of novaluron powder using a paint brush. The control clay pot was left untreated. Following treatment, clay pot was left for 24 hours to dry inside the chamber. To maximize the mosquito contamination, the blood-fed mosquitoes were held in a treated clay pot by covering a pot with a net for 24 hours, and then released in chambers in which artificial breeding habitat made of 1.5 L plastic basin, filled 1.5 L of water, and kept 3 m from the clay pots were provided (Fig. 2). The set up was the same for the control chamber except that clay pot was not treated. On a daily basis, breeding habitats were visually examined to assess the oviposition event via presence of eggs or larvae. The experiment was repeated three times over a period of 1 month. The successful autodissemination and contamination events were assessed by comparing the larvae mortality results in the treated and untreated chambers. To avoid contamination during the experiment, treated and control chambers were spaced 10 meters and were not rotated but fixed for experiment.

In the second experimental phase, procedures for experiment one we adopted, except that the inside of the clay pot was treated by spraying with novaluron solution, made by dissolving 0.3 g of novaluron powder in 1 L of tape water. This assay was run from 2nd -17th September 2021 a total of sixteen (16) days. Sprayed clay pot was left to dry for 24 hours inside the chamber, then 2500

blood-fed adult female *An. arabiensis* were introduced inside the clay pot for 24 hours to allow forced contamination, and then released into the chambers within breeding containers. The set up was the same for the control chamber except that clay pot was not sprayed. The experiment was repeated three times per concentration.

3.5.1. Bioassay to assess the contamination of artificial breeding habitats with novaluron

Four days post-exposure, an aliquot of 200 ml water samples were collected from each habitat both in a control and treatment chambers and transferred to separate 250 ml plastic cups. Twenty-five (25) 1st instar *An. arabiensis* larvae were taken from the insectary placed in a plastic cup containing aliquoted water samples. Larvae were fed at 1-day interval with Tetramin® fish food throughout the course of the experiment as per WHO standard mosquito rearing procedures. The larval mortality was monitored at 24 hours interval until all larvae were dead or emerged to adult. Dead larvae were counted and removed from the plastic cups (Fig. 3).

Figure 3: Preparation of subsample aliquots of novaluron from treated and untreated basins A); counting and removing dead larvae B)

3.5.2. To investigate the effect of novaluron exposure on mosquito fecundity

The blood fed female *An. arabiensis* were exposed to novaluron following experimental set up procedures described in Section 3.5. After 24 hrs exposure, a subsample of 100 mosquitoes were

aspirated from the clay pot and maintained on 10% glucose *ad libitum* in the netted cage both for control and treatment (Fig. 4). In each cage, a small (200 mls) plastic container was provided for egg laying, and number of laid eggs both from the control and treated chamber were counted and recorded.
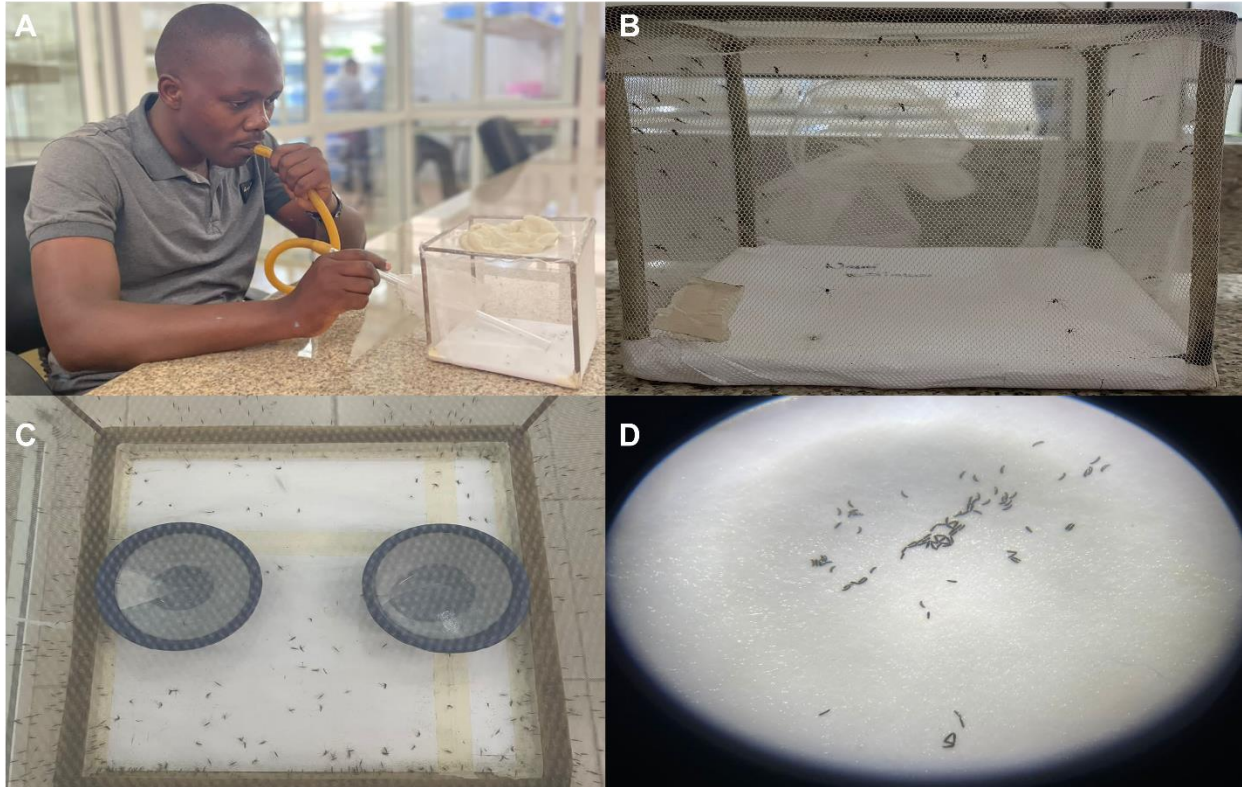
Figure 4: Introducing 100 adult mosquitoes inside the cage A); adult mosquitoes inside the cage B); Small plastic containers for laying eggs C); Laid eggs by adult mosquitoes D)

3.6. Statistical analysis

Data were analyzed using R software (Rv-4.1.1) (R Core Team, 2021) and excel. Generalized linear mixed models were used to assess the proportion of dead larvae for each concentration (Bates *et al*., 2015). The proportion of dead larvae were modeled as a response variable and concentration were considered as fixed effect while replicates and days were included as a random term to account for the pseudo replicates and unexplained variation between days. We also tested for lethal concentration to determine LC_{50} LC_{90} and LC_{99} using log-dose response analysis from

drc package (Ritz & Streibig, 2012). The curve was used to determine the desired concentration of novaluron. The diagnostic concentration (DC) was established from the lethal concentrations that killed up 99% of the exposed *Anopheles* larvae, and it was defined as the two times of LC_{99}. Additionally, Tukey honest significance differences (Tukey HSD) was used to assess the pairwise difference between concentration levels. Risk ratio and their corresponding 95% CI were reported, whereby, the statistical significance was considered when p-values ≤ 0.05. Notably, all figures were created using ggplot2 package (Sarkar, 2014; Wickham & Grolemund, 2016).

3.7. Ethical approval

Prior to field work the research proposal was presented to the Nelson Mandela Institution of Science and Technology and Ifakara Health Institute for approval. Further, the ethical approval for the study was granted by Institutional Review Board of Ifakara Health Institute (IHI/IRB/No: 20-2021).

3.8. Funding

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Results

4.1.1. Laboratory susceptibility test

High larval mortality in the treatment chamber was recorded with high concentrations of novaluron, and low concentrations were associated with delayed mortality. *An. gambiae* larvae were more susceptible with LC_{50} and LC_{90} being 0.0179 mg/L and 0.332 mg/L respectively, while LC_{50} and LC_{90} for *An. arabiensis* and *An. funestus* was 0.02561 mg/L and 0.5460 mg/L; and 0.0323 mg/L and 1.000 mg/L respectively (Table 1). Larval mortality in the respective control ranged from 7% to 15% depending on mosquito larvae species exposed (Fig. 5). Notably, the laboratory susceptibility test yielded the diagnostic concentrations for all three-target species (Table 1). The temperature during the assay ranged between 24 – 27 °C, 80% ± 10% relative humidity and the photoperiod of 12L: 12D.

The results on the comparison of different concentrations of novaluron on the larval mortality are summarized in Table 2. In three *Anopheles* species, *An. gambiae* was highly susceptible [RR = 1.0842, 95%CI: (0.385, 2.819), $p < 0.005$], followed by *An. arabiensis* [RR = 10.237, 95%CI: (9.204, 11.357), $p < 0.001$], and *An. funestus* [RR = 11.41, 95%CI: (10.145 12.839), $p < 0.001$]. However, the Pair-wise comparison test using Tukey HSD showed that, there is significant difference between control and 0.1 mg/L for *An. arabiensis* (z = 42.83, p < 0.001), *An. gambiae* (z = 43.87, p < 0.001) and *An. funestus* (z = 40.53, p < 0.001) (Fig. 6).

Table 1: Susceptibility status of 1st instars of malaria vector species to novaluron

Species	LC50 (mg/L)	95%CI	LC90 (mg/L)	95%CI	LC99 (mg/L)	95%CI	Diagnostic Conc. (mg/L)
An. gambiae	0.018	0.016,0.020	0.332	0.168,0.496	2.001	1.986,3.206	4.002
An. arabiensis	0.026	0.027,0.038	0.546	0.374,0.719	2.013	1.997,4.491	4.026
An. funestus	0.032	0.021,0.03	1.000	0.467,1.535	5.580	4.687,8.496	11.160
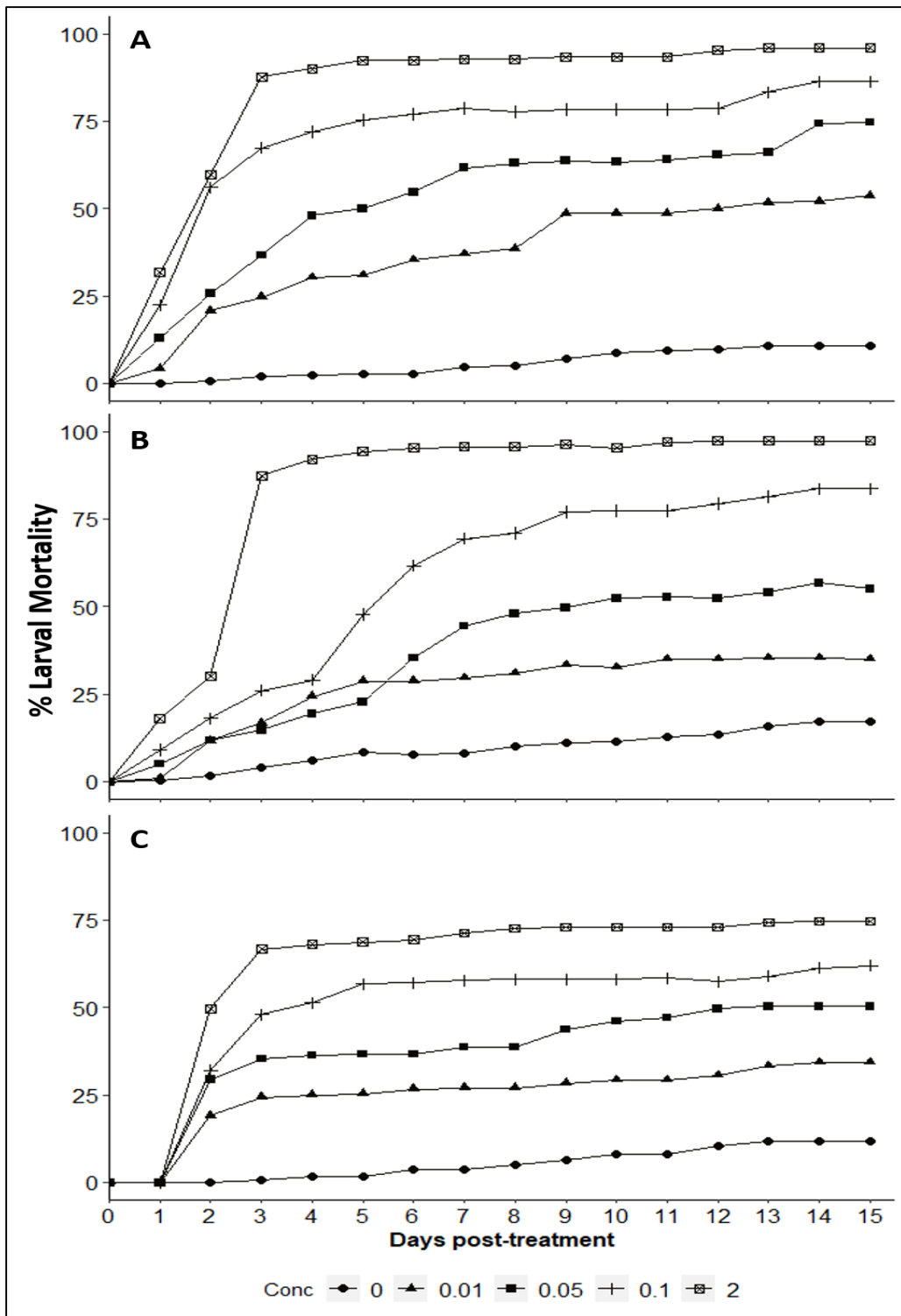
Figure 5: Cumulative mortality of *An. arabiensis* (A), *An. gambiae* (C) and *An. funestus* (B) larvae when 1st instar larvae were treated with novaluron

Table 2: Larvae mortality of *An. gambiae*, *An. arabiensis* and *An. funestus* at different concentrations of novaluron

Species	Conc. (mg/L)	Predicted Mean (95%CI)	RR (95% CI)	P-value
	0.00	0.318 (0.149,0.675)	1	
	0.01	0.595 (0.280,1.265)	0.518 (0.384,1.349)	0.177
An. gambiae	0.05	1.293 (0.609,2.745)	0.257 (0.384,0.668)	0.504
	0.10	2.957 (1.391,6.284)	1.084 (0.385,2.819)	0.004
	2.00	7.786 (3.656,16.582)	2.052 (0.386,5.321)	< 0.001
	0.00	0.144 (0.116,0.178)	1	
	0.01	0.369 (0.299,0.454)	2.567 (2.300,2.865)	< 0.001
An. arabiensis	0.05	0.600 (0.488,0.737)	4.174 (3.751,4.644)	< 0.001
	0.10	1.471 (1.197,1.808)	10.237 (9.204,11.357)	< 0.001
	2.00	6.121 (4.939,7.588)	42.604 (37.718,48.122)	< 0.001
	0.00	0.096 (0.044,0.211)	1	
	0.01	0.319 (0.145,0.699)	3.325 (2.947,3.752)	0.004
An. funestus	0.05	0.622 (0.284,1.362)	6.487 (5.767,7.298)	< 0.001
	0.10	1.094 (0.500,2.396)	11.41 (10.145,12.839)	< 0.001
	2.00	2.067 (0.944,4.528)	21.56 (19.119,24.306)	0.070

CI = confidence interval, RR = risk ratio. Control used as reference RR = 1, the predicted means were derived from generalized linear model which is the average of larvae dead in each concentration.
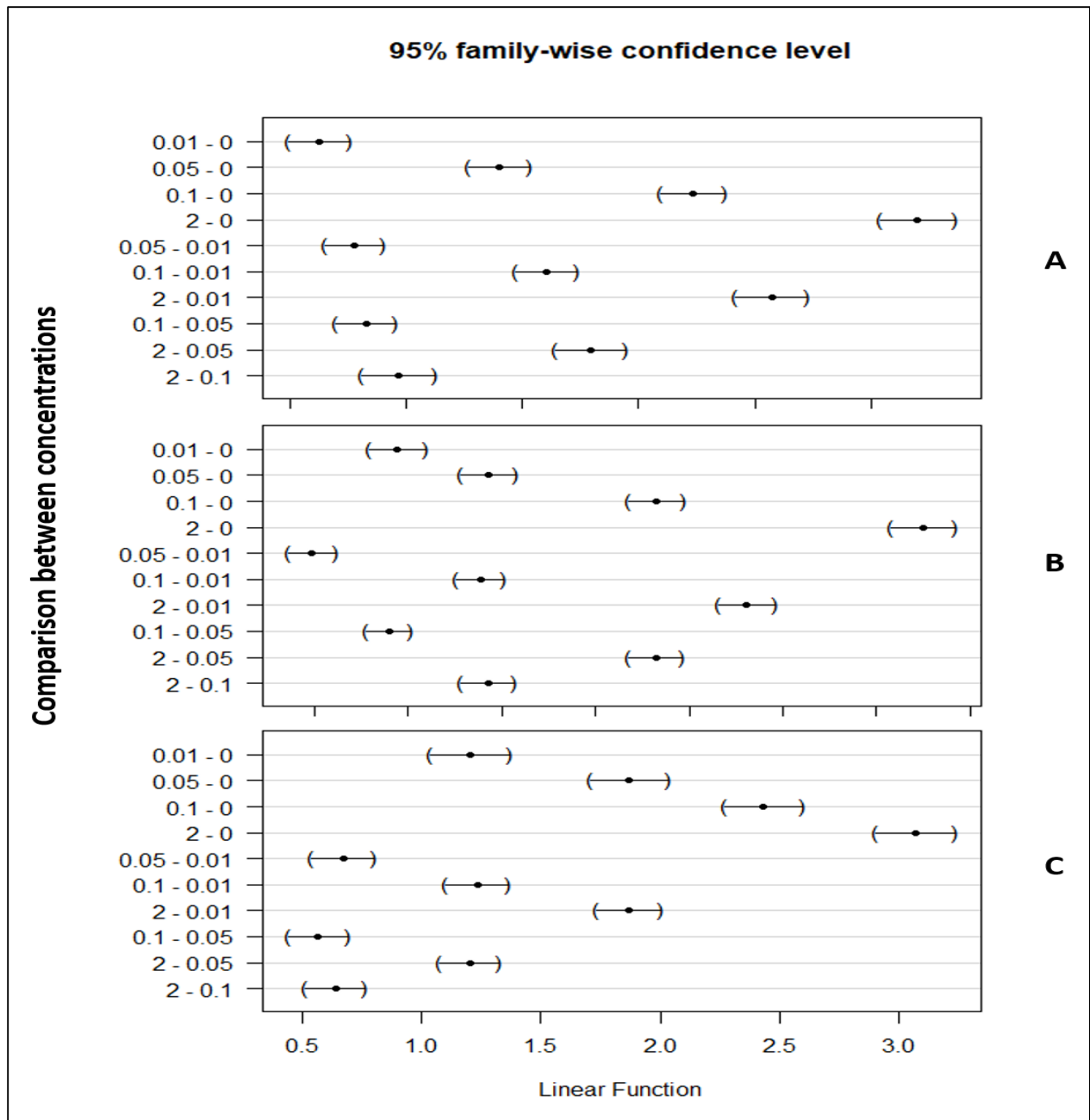
Figure 6: Results of pair-wise post hoc comparison using Tukey honestly significance tests (Tukey HSD). Similarities and differences between Larvae mortality at different concentrations; *(A) An. gambiae, (B) An. arabiensis and (C) An. funestus*

4.1.2. Effect of novaluron on pupation rate

The results demonstrated high percentage inhibition of pupation with increase in concentration. Highest PI% was recorded at 2 mg/L compared to other low concentrations across all three *Anopheles* species with PI% of 90.5%, 86.4% and 81.0% for *An. gambiae*, *An. arabiensis* and *An. funestus* respectively (Fig. 7).
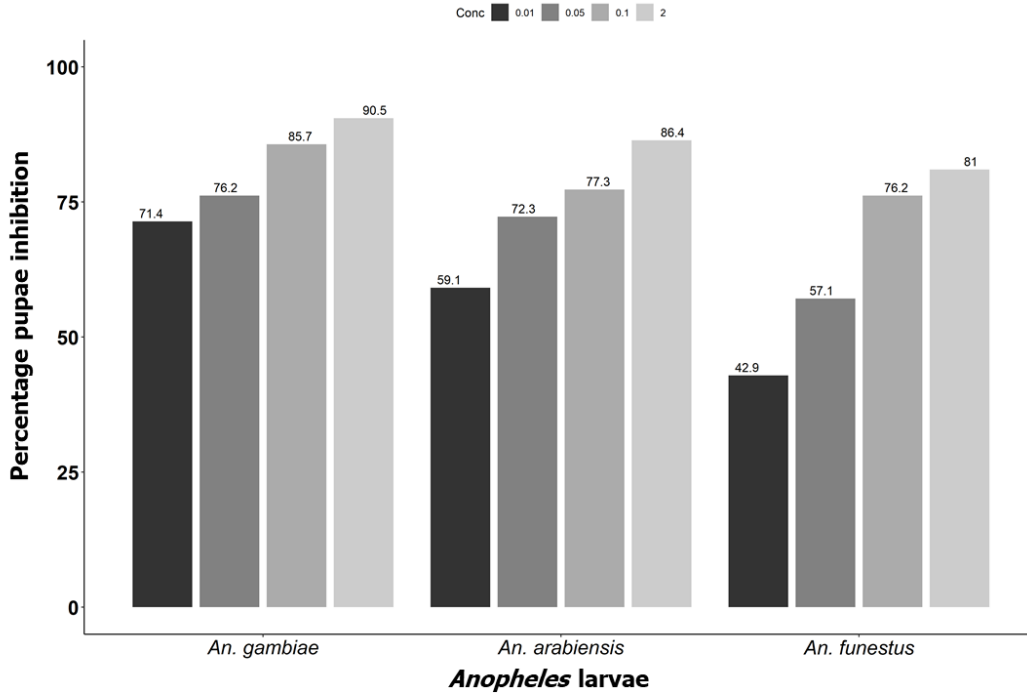
Figure 7: **Percentage inhibition of pupation of different malaria vectors at different test concentration of novaluron 15 days post-treatment**

4.1.3. Proof of novaluron autodissemination with *An. arabiensis*

In the first experimental phase, when novaluron was directly dusted on the clay pot, exposed mosquitoes could not autodisseminate the compound to the provided artificial breeding habitats. This was due to adult mosquito mortality that was observed from the contaminated clay pot before were released.

In the second experimental phase, that involved spraying the clay pots with novaluron, there were was no proof of autodissemination activities. Unexpectedly, no eggs were laid in the treatment chamber (Fig. 8).
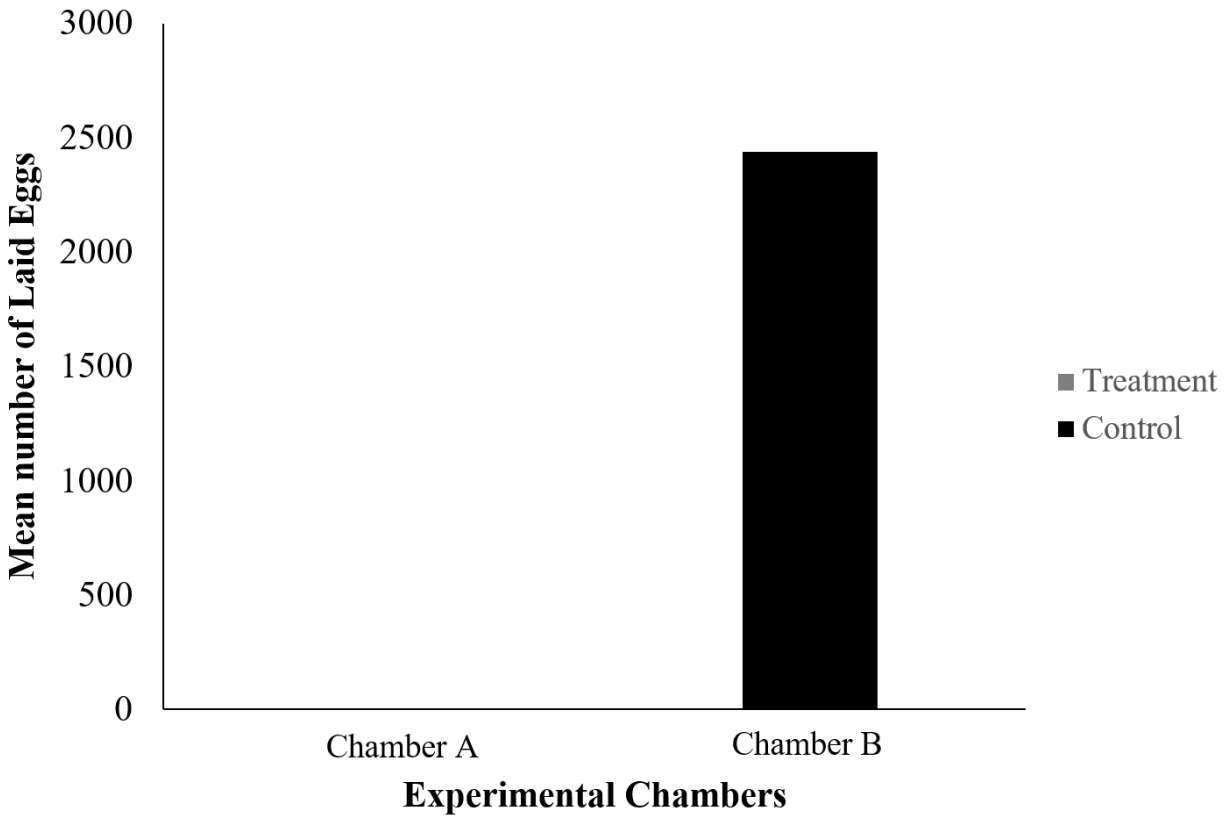
Figure 8: Comparison of mean number of laid eggs from experimental chamber

4.1.4. Confirming the contamination of artificial breeding habitats with novaluron

The mean larval mortality between treated and untreated cups were the similar (Fig. 9). These findings suggested that either there was no novaluron that was disseminated to artificial breeding habitats by exposed mosquitoes, or the amount that was disseminated was so tiny to cause noticeable mortality effect (Fig. 9).
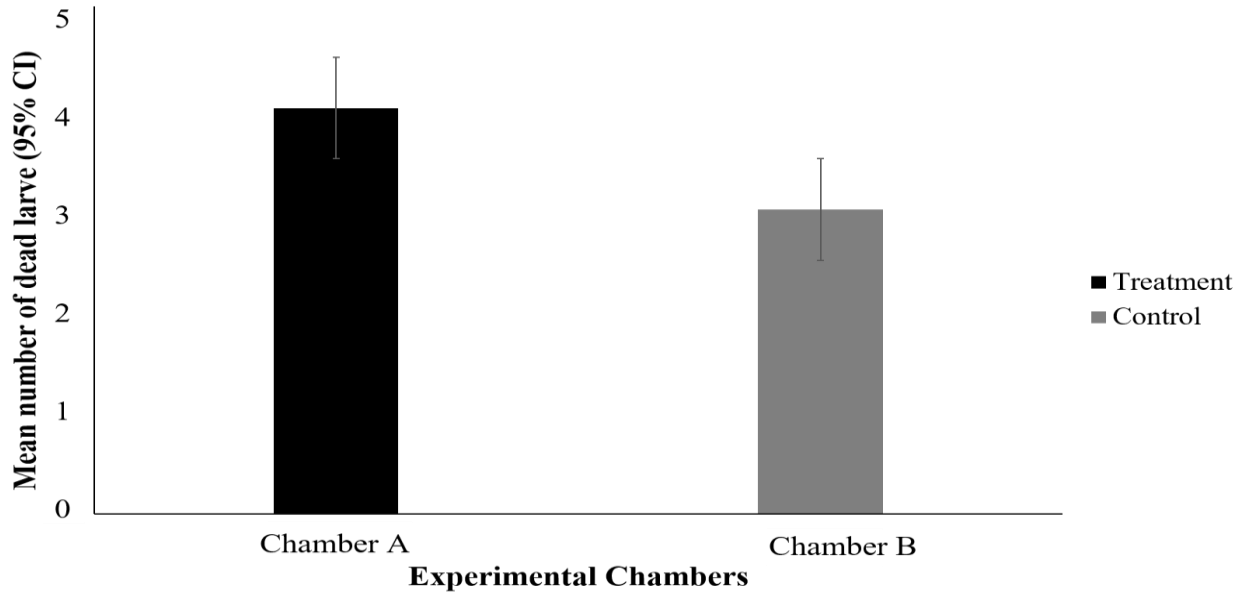
Figure 9: Mean larvae mortality of *An. arabiensis* 15 days post-exposure

4.1.5. Effect of novaluron exposure on mosquito fecundity

The mean number of eggs collected within 6 days of experimental monitoring were 104 and 44 from the control and treatment chambers respectively (Fig. 10).
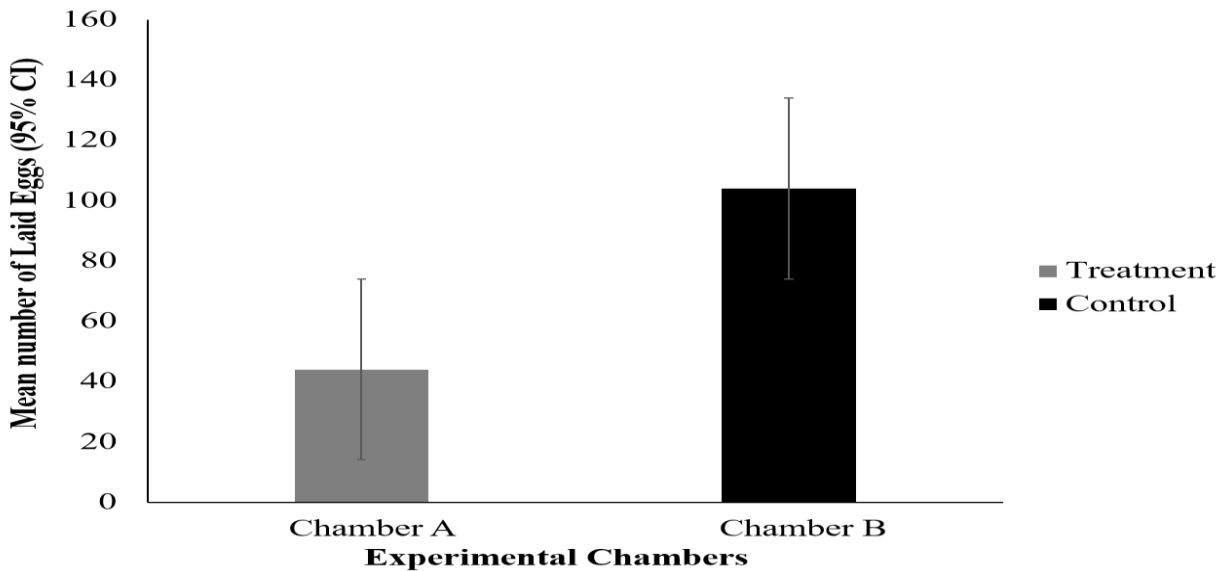
Figure 10: Mean number of eggs laid by mosquitoes exposed to novaluron in a treatment, and control chambers

4.2. Discussion

This study demonstrated up to 80% and 90% larval mortality and pupae inhibition of the exposed larvae of *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles funestus* to novaluron under controlled settings. These findings corroborate other previous reports that demonstrated the control of *Anopheline*, *Adenine* and *Culicine* mosquitoes using novaluron under laboratory and field settings (Arredondo-Jiménez & Valdez-Delgado, 2006; Mulla *et al*., 1974; Swale *et al*., 2018).

Lethal concentrations sufficient to kills 50% and 90% of the exposed mosquito larvae were different across three tested species; all achieved within 15 days post-exposure. This highlights delayed developmental duration of exposed larvae as an impact of novaluron (Arredondo-Jiménez & Valdez-Delgado, 2006; Clements, 2011; Farnesi *et al*., 2012). Over 50% mortality of all *Anopheles* larvae were observed in between 2nd and 3rd day post-exposure at maximum test concentration of novaluron (2 mg/L). Despite of the development of the exposed larvae to 3rd instar, none was able to reach 4th instar or pupae stage. Previous studies assessing the effect of novaluron to mosquito larvae have also reported slow and extended larval growth and delayed mortality post-exposure time (Arredondo-Jiménez & Valdez-Delgado, 2006; Mulla *et al.*, 2003; Swale *et al.,* 2018). This delayed mortality is expected to reduce pressures on mosquitoes to develop resistance to the novaluron, and offer a more sustainable insecticide for vector control (Farnesi *et al*., 2012; Mulla *et al*., 2003).

In comparison, *An. gambiae* was more susceptible to novaluron followed by *An. arabiensis* and lastly *An. funestus.* Lethal concentrations of novaluron required to kill 50%, 90% and 99% of *An. funestus* larvae was one to two and half times higher than that for *An. gambiae* and *An. arabiensis.* In addition, the diagnostic concentration for *An. funestus* (11.160 mg/L) was three times higher than that of *An. gambiae* (4.002 mg/L) and *An. arabiensis* (4.026 mg/L). Although not investigated under this study, the probable cause for reduced susceptibility might be a high level of pyrethroids resistance in *An. funestus* documented in other studies in the same study location (Kaindoa *et al.,* 2017; Lwetoijera *et al*., 2013). Another study, has also highlighted possibility of cross-resistance between pyrethroids and insect-growth regulators within *Anopheles* population, which might be applicable in this case (Yunta *et al.*, 2016). The difference of lethal and diagnostics concentrations

recorded under different studies might be explained by physiological difference with test species (Arredondo-Jiménez & Valdez-Delgado, 2006; Swale *et al*., 2018).

There is increasing evidence that the use of IGRs of different modes of action against mosquitoes can counteract and/or delay the development of insecticide resistance to their use (Tusting *et al.*, 2013; WHO, 2014). These findings point out the potential of novaluron in reducing the density of adult mosquito population that would emerged from the breeding habitats. Novaluron therefore present, an additional insecticide that may be applied in rotation with other IGRs, such as pyriproxyfen to manage insecticide resistance and reduce adult mosquito population at their larval habitats. In addition, WHO approval on the use of novaluron in drinking water signals its safety to human and animals, and warrant its testing using conventional larviciding or autodissemination techniques in different settings (WHO, 2007).

On the other hand, this study indicated that exposed malaria vectors cannot autodisseminate novaluron from the contamination station clay pots to the provided artificial breeding habitats. The failure for exposed mosquitoes to allow autodissemination of novaluron might be due to large particle size of novaluron that is thought to be groomed off when were carried by mosquitoes from the contaminated clay pot to the artificial breeding habitat. It is suggested that the smaller the IGR particle size the easier the mosquito loading and retention for autodissemination to occur (Gaugler *et al*., 2012). The second reason might be the effect of novaluron on adult fitness especially in fecundity and fertility. Studies have shown that novaluron exposure time to mosquitoes reduces fecundity and fertility (Bouaziz *et al.*, 2017; Djeghader *et al*., 2014; Harris *et al*., 2013).

Moreover, the findings demonstrated the effect on exposed female adult mosquitoes to novaluron that affected *An. arabiensis* fecundity by reduced the number of laid eggs and its viability. This results corroborates with a previous study that demonstrated the effect of novaluron on the production of female mosquitoes such as *Culex pipiens* (Djeghader *et al*., 2014). The reduction in number of laid eggs is expected to reduce mosquito adult population and hence reduction of malaria transmission.

This study had a number of limitations; under laboratory settings no attempt was made to test for persistence of novaluron in the test cups beyond single larval exposure. While low susceptibility

of *An. funestus* to novaluron was attributed to its high insecticide resistance status, no actual experiments that were carried to ascertain this assertion, and this represent another study limitation. Therefore, these limitations add on the list of future studies towards development of novaluron as the potential larvicide for malaria vector control.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

Overall, this study conclude that major malaria mosquitoes found southern-eastern Tanzania are susceptible to novaluron at low concentration. The findings present a promising candidate IGR for rotation to counteract the insecticide resistance development. Moreover, these results warrant further evaluation of novaluron for autodissemination by vector species for its inclusion in rotation to prevent evolution of resistance in both chemistries.

5.2. Recommendations

This study recommends assessment of novaluron effect to non-targeted species found in the same breeding habitat with malaria transmitting mosquitoes. It also suggests further tests of autodissemination technique using optimized formulation of novaluron in terms of particle sizes against *An. gambiae*, *An. arabiensis* and *An. funestus* for its inclusion in rotation to prevent evolution of insecticide resistance.

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RESEARCH OUTPUTS

Output 1: Paper published in Pan African Medical Journal

Ngonzi AJ, Muyaga LL, Ngowo H, Urio N, Vianney JM, Lwetoijera DW. (2022). Susceptibility status of major malaria vectors to novaluron, an insect growth regulator South-Eastern Tanzania. DOI: 10.11604/pamj.2022.41.273.33793

Output 2: Co-author in paper published in *Parasite & Vectors* journal

Naomi H Urio, Polius G Pinda, Amos J Ngonzi, Letus L Muyaga, Betwel J Msugupakulya, Marceline Finda, Godfrey S Matanila, Winifrida Mponzi, Halfan S Ngowo, Najat F Kahamba, Theresia E Nkya, Fredros O Okumu. (2022). Effects of agricultural pesticides on the susceptibility and fitness of malaria vectors in rural south-eastern Tanzania. DOI: 10.1186/s13071-022-05318-3

Susceptibility status of major malaria vectors to novaluron, an Insect Growth Regulator South-eastern Tanzania

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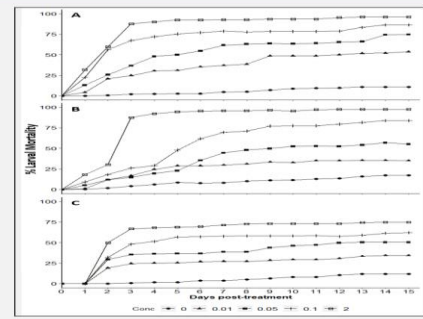IFAKARA HEALTH INSTITUTE
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Introduction

- Application of insect growth regulator (IGR) such as pyriproxyfen has shown a promising result in controlling malaria transmitting
- This presents a promising candidate IGR for rotation with pyriproxyfen to prevent a chance of resistance development
- Therefore, the study assessed the susceptibility of immature stages of *Anopheles arabiensis, Anopheles gambiae* and *Anopheles funestus* to novaluron.

Methods: Experimental Set-Up

- *Figure 1*: Semi-field system (SFS) and mosquito insectary (**A**); Susceptibility test inside the SFS (**B**)

Methods: Concentration used

- Concentration were tested to establish lethal concentration (LC) sufficient to kills 50%, 90% and 99% of the exposed larvae by using log-dose response analysis.
- Importantly, the LC of 99% x 2 provided the diagnostic concentration (DC) to be used in field experiments

Results: Larval Susceptibility and Pupae inhibition

Species	LC 50 (mg/l)	LC 90 (mg/l)	LC 99 (mg/l)
An. gambiae	0.018	0.332	2.001
An. arabiensis	0.026	0.546	2.013
An. funestus	0.032	1.000	5.580

- *Figure 2*: Percentage inhibition of pupation of different malaria vectors at different test concentration of novaluron 15 days post-treatment

Results: Larvae mortality

Figure 3: Cumulative mortality of *An. arabiensis* (A), *An. gambiae* (C) and *An. funestus* (B) larvae when 1st instar larvae were treated with novaluron-chitin synthetic

Conclusion

- Major malaria vectors found in Kilombero, Tanzania are susceptible to novaluron at low concentration.
- Therefore, these results warrant further semi-and field testing of novaluron using the autodissemination technique against *An. gambiae, An. arabiensis* and *An. funestus*

More Information

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