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Effect of pyriproxyfen on development and survival of *Anopheles gambiae* sensu stricto under forested and deforested areas

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Abstract

Background: The land cover changes in the form of deforestation are known for their impact on enhancing *Anopheline* life-history traits. In most cases, these traits depend on environmental parameters such as temperature and light. Pyriproxyfen is an insect growth regulator juvenile hormone (JH) designed to inhibit adult mosquito emergence. It is an effective biolarvicide in controlling immature stages of malaria vectors and many other insects. Despite the known efficiency of pyriproxyfen in malaria vector control, but the understanding of its performance under deforested or afforested areas is not clear. The present study aimed at evaluating the impact of pyriproxyfen on development and survivorship of *Anopheles gambiae s.s.* in forested and deforested areas. Tests of two dosages (0.03 ppm.ai. and 0.3 ppm.ai.) of pyriproxyfen were done in standardized semi-field conditions under ambient temperature and light in netting screened environment. The plastic artificial microcosms containing 1 kg of soil and 2000 mL of dechlorinated water were used. First instars larvae were distributed in densities of 20, 40, and 60 in six replicates each, to form a total of 18 microcosms per each land cover type. Larvae mortality, development, and survival time were monitored and recorded after every 24 h until pupation. Emerging adults from pupa were recorded, and their sexes were identified.

Results: Pyriproxyfen increased *Anopheles gambiae* larvae mortality rates and larvae developmental time of first instar larvae to pupal at densities of 20, 40 and 60 larvae in forested area ($p < 0.05$). Also, the larvae survival time was found to be longer in forested area compared to deforested area ($p < 0.05$) except at the density of 20 larvae. Pyriproxyfen reduced larvae pupation rates by 88% in forested area and it had 100% adult emergence inhibition regardless of land cover type and pyriproxyfen concentration, except at the density of 60 larvae exposed to 0.03 ppm, where adult emergence rate of 6.7% was detected.

Conclusion: These findings suggest that, the efficacy of pyriproxyfen against *Anopheles gambiae* s.s. larvae and pupae is dependent on land cover types and its larvicidal effect can be increased by presence of trees canopy covers. Therefore, reviving forestry schemes and community afforestation programmes could have a positive impact on mosquito larval control by using larvicides. Also, increasing land canopy cover can be opted as a way to discourage the development and survival of potential malaria vectors.

Keywords: Sumilarv, Tanzania, Canopy, Survivorship, Mortality, Larval developmental time, Vector control, Mosquito

Background

Malaria vector control tools have played major roles in vector control worldwide to witness the declined population of both vectors and disease incidences (WHO, 2019, 2020). The main interventions that have been widely used are Long-lasting insecticidal nets (LLINs), Indoor residual spray (IRS), availability of reliable diagnostic

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tools such as rapid diagnostic test (RDT), and appropriate treatments with anti-malarial drugs such as Artemisinin Combination Therapy (ACT) (WHO, 2019, 2020). The land cover changes that have occurred and are still occurring in malaria-endemic regions could alter mosquito vectorial capacity, leading to increased malaria transmission (Afrane et al., 2006; El-Sayed & Kamel, 2020; Kweka et al., 2016; Munga et al., 2009; Zhou et al., 2007). There is a growing deforestation rate due to demands for more lands for cultivation, grazing, building spaces and resources, and settlements due to increased population in malaria-endemic regions (Hawkes et al., 2019; Kweka et al., 2016; Lindsay & Martens, 1998). For instance, in Africa, an average of 8% of forest areas is cleared for aforementioned activities annually (Forest Resource Assessment Project, 1996; Romijn et al., 2015). The clearing of forests increase the availability of productive mosquito habitats exposed to sunlight leading to rapid development of *Anopheles gambiae* mosquitoes and shorten their life cycle (Afrane et al., 2005; Munga et al., 2009). The exposure of breeding sites to sunlight increases the temperature of the habitats and enhances the microbial community growth which act as food for mosquito larvae. (Alfano et al., 2019; Gilbreath et al., 2013; McCrae, 1984; Wang et al., 2018). The increase in habitats temperature is directly proportional to increase in larvae development rates which shorten the developmental circle (Christiansen-Jucht et al., 2014; Chu et al., 2020; Clements, 1992; Ndo et al., 2018).

Larvicides such as pyriproxyfen, methoprene, Dimilin, *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus(Bs)*have shown to be effective in reducing larva density and malaria transmission when applied alone (Derua et al., 2019; Fillinger et al., 2008; Geissbühler et al., 2009; Kweka et al., 2019; Majambere et al., 2007; Msangi et al., 2011) and even more effective when applied in combination with principal interventions such as LLINs, IRS or house improvement (Fillinger et al., 2009; McCann et al., 2017). Pyriproxyfen a juvenile hormone analogue is designed to interfere with the normal development of malaria vector larvae. It has been applied in many countries in Africa including Ethiopia, Kenya (Mbare et al., 2013) and Tanzania (Kweka et al., 2019) and it has shown to be effective in inhibiting adult emergence, compared to other larvicides (Zogo et al., 2019). However, the application on pyriproxyfen can be challenged by various factors including habitats washing particularly during heavy rainy season and environmental parameters such as temperature and light (Antonio-Nkondjio et al., 2018). Also, habitat vegetation cover and shading have been found to have a great influence on the mosquito larval density, abundance and species composition (Kweka et al., 2015; Munga et al., 2009; Wamae et al., 2010). Despite the well reported impact of forest shading on mosquito larvae development and adult productivity (Kweka et al., 2012; Munga et al., 2009; Wang et al., 2016; Zhong et al., 2016), its effect on larvicides efficacy is not well understood. There is a lack of adequate information on how different land covers changes, particularly deforestation influence the residual efficacy of larvicides against malaria vectors. Therefore, this study evaluated the effect of pyriproxyfen on development and survival of *Anopheles gambiae* s.s. under forested and deforested areas.

Methods

Study area

This study was conducted at the Tropical Pesticide Research Institute (TPRI) premises, in the division of livestock and human disease vector control (LHDV) in Arusha, Tanzania. The region is found at the foot of mount Meru 1444 m above sea level. The average annual temperature and rainfall of the region is 19 °C and 1103 mm respectively. The study was conducted from October 2019 to March 2020. Two sites were selected purposely, site A and site B found at 3° 19′ 45.1″ S 36° 37′ 31.1″ E and 3° 19′ 48.7″ S 36° 37′ 29.7″ E respectively. The study sites were found in two different land cover types. Site A, was a forested area and site B, was a deforested area found in the TPRI premises. For this study forested area was defined as an area whose trees canopy cover makes up to more than 80%, while the deforested area was defined as an area whose trees canopy cover was 10% or less (Keenan et al. 2015). The visual estimation of vegetation cover was done based on the Daubenmire cover class method (Asrat et al. 2018). The deforested area was an open space that received direct sunlight, while the forested area received less sunlight due to being closed by trees canopy cover.

Preparation of larvicide

Sumilarv® 0.5G were obtained from manufacturer (Sumitomo Chemical Company, Japan). Sumilarv is a larval control agent based on the insect growth regulator (IGR), pyriproxyfen. It is commercially available as a granular (G) formulation containing 5.1 g/kg pure pyriproxyfen (Kweka et al. 2019). In the present study, preparation of a test solution followed manufacturer's instruction and procedures from previous studies and guidelines (Kweka et al., 2019; Mbare et al., 2013; WHO, 2005). A stock solution was prepared by grinding five (5) grams of the granular formulation of Sumilarv® 0.5G into a fine powder. The grinded powder was added into 500 mL of dechlorinated tap water to make a stock solution of 10,000 ppm of Sumilarv® 0.5G. The obtained solution was placed in a beaker whose top was then covered with aluminium foil.

The mixture was further shaken for 30 min before use to get homogenous solution. The serial dilutions were made immediately after shaking using dechlorinated water to produce the test concentrations. The test concentrations of 0.03 ppm was chosen to be used in the present study based on the previous field and laboratory studies done by Mbare et al. (2013) and Kweka et al (2011) which reported 100% inhibition of adult mosquito emergence. The other concentration of 0.3 ppm was opted as the higher dosage for semi-field experiments in case of environmental effect on the efficacy of pyriproxyfen.

Larval bioassay

Anopheles gambiae sensu stricto mosquitoes from a susceptible laboratory strain were used in this study. Eggs from *A. gambiae* mosquitoes were hatched in enamel trays to obtain first instar larvae. The hatched first instar larvae were distributed into 36 plastic artificial microcosms (diameter: 21 cm and depth: 15 cm) in densities of 20, 40 and 60 larvae (12 per each density). The 18 artificial microcosms (6 per each density) were then dug into the ground to ensure that they mimic the natural mosquito habitats closely, in each land cover type (forested and deforested area) parallel to their control for each experiment. Tests were done under natural ambient climate and light conditions in a netting-screened environment with natural photo phase of 12 h dark and 12 h light cycles in both land with high trees coverage and land without trees (deforested land) (Mbare et al., 2013). The netting material prevented other gravid mosquitoes from breeding in the installed microcosms. Also, prevented predators from consuming larvae in these artificial habitats. The data for temperature and light intensity were recorded using Hobo data logger then they were transferred into the computer for further processes.

Developmental and survival studies

Semi-field experiments involved placing the artificial microcosms in each experimental site at a distance of1m apart in three rows to avoid cross-contamination between microcosms of different pyriproxyfen concentration. Each artificial microcosm was filled with 2000 mL of treated de-chlorinated water and one (1) kilogram of soil collected from nearby fields to mimic natural mosquito habitat. Larvae developmental time and survivorship in both treated and control (in dechlorinated water) was recorded in every 24 h. Development observation of *Anopheles gambiae* larvae was based on structural changes of larvae from one instar to the next (L1–L2–L3–L4). In each microcosm, surviving larvae were counted using pipette and white plate. Monitoring of larvae development in microcosms was done daily until the emergence of first pupae, then monitoring was conducted twice per day at 8:00 am, and 5:00 pm to ensure that no pupae was left to emerge into an adult. Emerged pupae were placed into 300 mL plastic cups containing 100 mL of dechlorinated water, covered by netting materials to prevent emerging adults from escaping. Separate pipettes were used to collect pupae from treated (for each concentration) and control microcosms to avoid cross-contamination. The emerged adult mosquitoes were removed from the plastic cups using an aspirator, anaesthetized by using chloroform and then their sex was identified based on their mouthpart structures (Ahmed & Ahmed, 2011; Coetzee et al., 2000). The above procedures were repeated in triplicates. Overall, two experimental groups were set up; (i) larvae reared in the forested area and exposed to pyriproxyfen (0.03 ppm or 0.3 ppm doses), and (ii) Larvae reared in the deforested area and exposed to pyriproxyfen (0.03 ppm or 0.3 ppm doses). The control groups for each larvae density were reared in both forested and deforested areas without treatment of pyriproxyfen (treated with dechlorinated tap water and soil).

Data analysis

Data were entered in Microsoft excel 2019(Microsoft, WA, USA), then transferred to IBM SPSS Statistics version 26.0 (IMB Corp., Armonk, NY, USA). The mean larval development time and mortality rates were compared using a paired sampled *t*-test. Kaplan–Meier survival analysis was performed to determine larval survivorship. Survivorship trends compared using Wilcoxon rank test to determine statistical significance. The results significance level was considered below 5%.

Results

Temperature and light intensity variation in mosquito larvae habitats in a forested and deforested area

Results showed a significant difference in temperature between larvae habitats in forested and deforested environments, with the deforested setting demonstrating significantly higher temperature and light than in forested microcosms ($p < 0.05$) (Fig. 1a and b).

Impact of pyriproxyfen on larvae mortality rates in forested and deforested areas

Larvae exposed to pyriproxyfen demonstrated higher mortality rates when compared to untreated larvae at all the three densities (20, 40 and 60 larvae). Treated larvae habitats in forested area had significantly higher mortality rates ($p < 0.05$) when compared to other habitats in deforested area. Mortality rates of larvae increased relative to increasing in pyriproxyfen concentration from 0.03 to 0.3 ppm in both land cover types (Fig. 3a and b). Also, higher mortality rates were recorded in forested

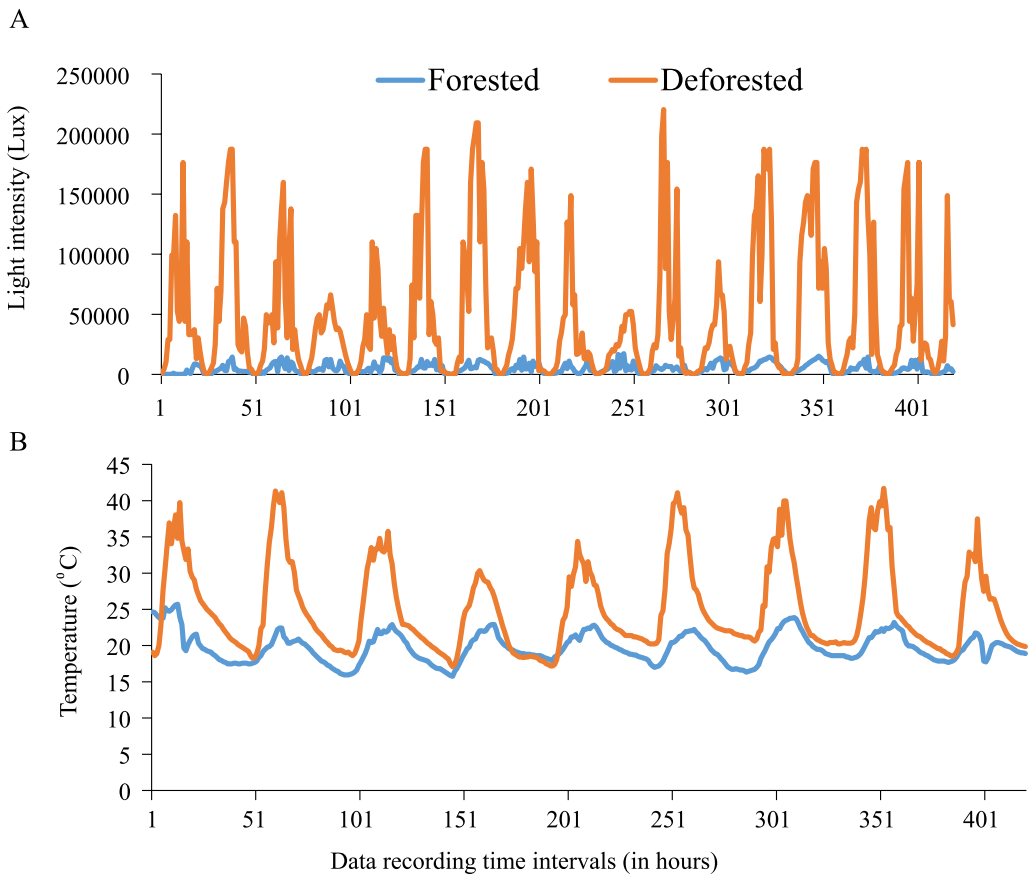

Fig. 1 The variation of **A** light intensity and **B** temperature in larvae habitats in forested and deforested areas

area at all densities when untreated habitats were compared ($p < 0.05$) (Figs. 2d and 3).

Impact of pyriproxyfen on larvae developmental time in a forested and deforested area

Larvae exposed to pyriproxyfen had short development time compared to untreated larvae (Fig. 4a and b). The developmental time between first instar and third instar larvae was almost the same in between larvae exposed to pyriproxyfen and those exposed to dechlorinated water only in both land cover types. A significantly longer developmental time in forested area was observed in treated larvae compared to deforested area ($p < 0.05$). Similar observations were made when control habitats in both forested and deforested areas were compared. Pyriproxyfen in higher concentration (0.3 ppm) decreased larvae developmental time at all densities compared to 0.03 ppm in both forested and deforested areas (Fig. 5a, b). Generally, larvae reared in forested habitats required longer time to develop to the adult stage than those larvae reared in deforested habitats at all treatments ($p < 0.05$) (Fig. 4d).

Impact of Pyriproxyfen on larvae survivorship under the forested and deforested area

The survival time of larvae was shorter in habitats exposed to pyriproxyfen when compared to control groups (Figs. 6 and 7). However, the mean proportion of surviving larvae at all larvae densities was significantly longer in deforested area than the forested area ($p < 0.05$) except at the density of 20 where insignificant difference in survival time were observed ($p = 0.183$). Similar survival trends were witnessed when larvae were exposed to 0.3 ppm of pyriproxyfen (Fig. 7a, b and c). The proportions of surviving larvae in treated microcosms in both land cover types began to drop after day 3 with low proportions of surviving larvae recorded in day 15 onward from forested habitats at both pyriproxyfen concentrations (Figs. 6 and 7).

Effects of pyriproxyfen on adult emergence in the forested and deforested area

A smaller number of pupae were recorded in forested area compared to deforested area at all densities for both 0.3 ppm and 0.03 ppm of pyriproxyfen

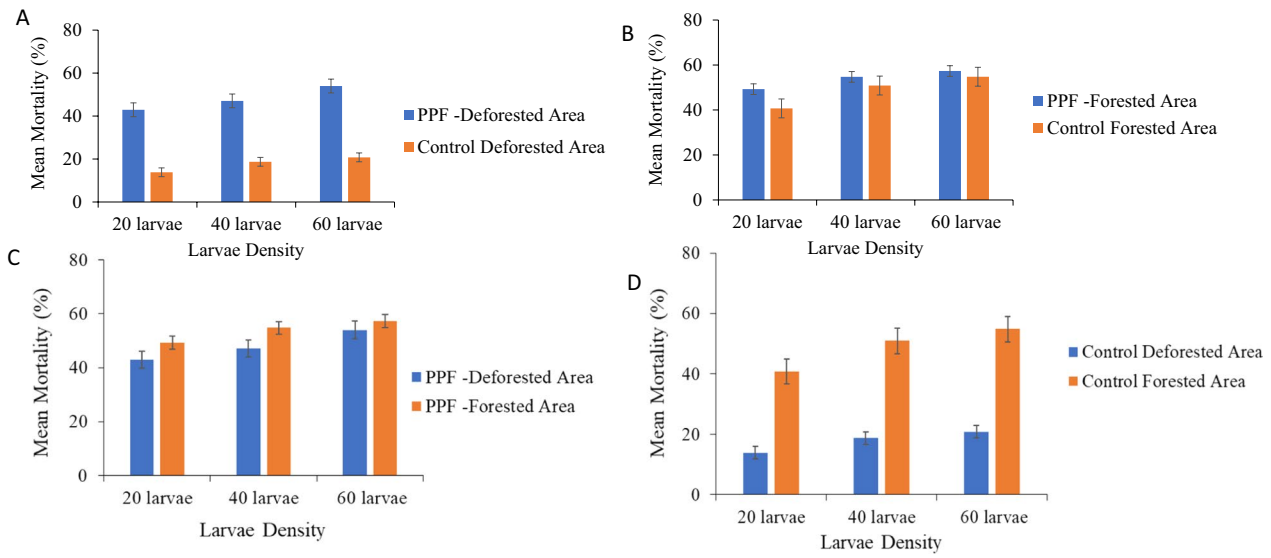

Fig. 2 The impact of Pyriproxyfen at 0.3 ppm on *An. gambiae* s.s. mortality rates between forested and deforested area **A**–**C** at densities of 20, 40 and 60 larvae and Comparison of larvae mortality in control **D** (experiments were repeated 6 times in 3 rounds)

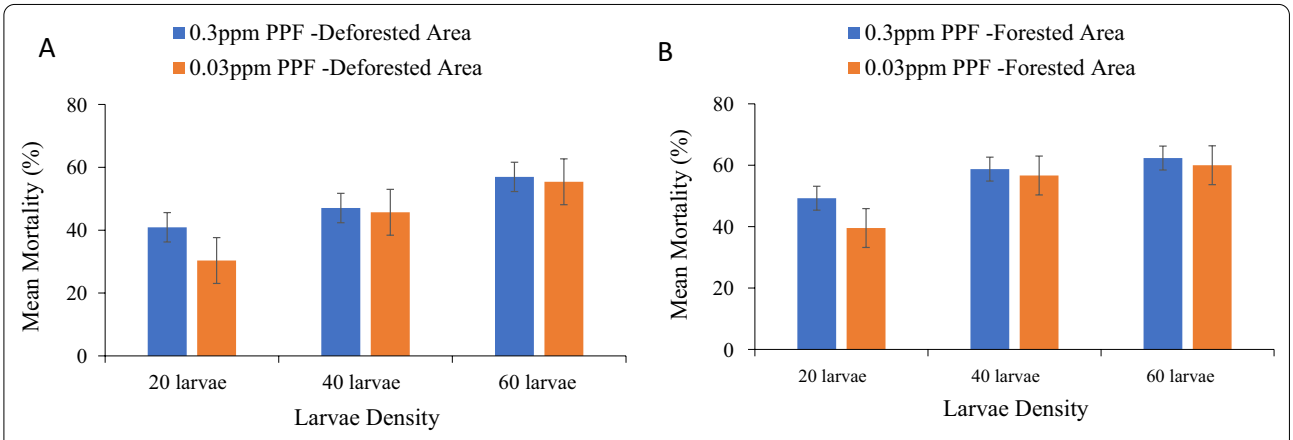

Fig. 3 Effect of changing PPF concentration from 0.03 to 0.3 ppm (**A** and **B**) on *An. gambiae* larvae mortality rates at 20, 40 and 60 larvae (Experiments repeated in 6 times and 3 rounds)

(Table 1 and 2). Pyriproxyfen treatment in larvae habitats in forested area reduced pupation rates comparative to deforested area (Tables 1 and 2). Pupation rates decreased with an increase in pyriproxyfen concentration from 0.03 to 0.3 ppm (Tables 1 and 2). In all treatments, pupation was initiated beginning from day 7 onwards in the deforested area, this increased exponentially and by day 18, 90% of larvae were either emerged into adults or dead (Figs. 6 and 7). In the forested area, it was until day 18 and took an average of 28 days for all larvae to emerge into adults. There was no adult emergence (100% adult emergence inhibition) in larvae habitats exposed to pyriproxyfen at 0.3 ppm except for 0.03 ppm at 60 larvae densities in both forested and deforested area, where small number of pupae were able to emerge into adult mosquitoes (Tables 1 and 2). However, adult emergence rates were less than 5% and many of the mosquitoes had incomplete emergence. Incomplete adult emergence was characterized by some mosquitoes having their legs and tarsi attached in the pupal exuviae. Also, there were many emerging male mosquitoes in forested area while many adult emerging mosquitoes in deforested area were females (Tables 1 and 2).

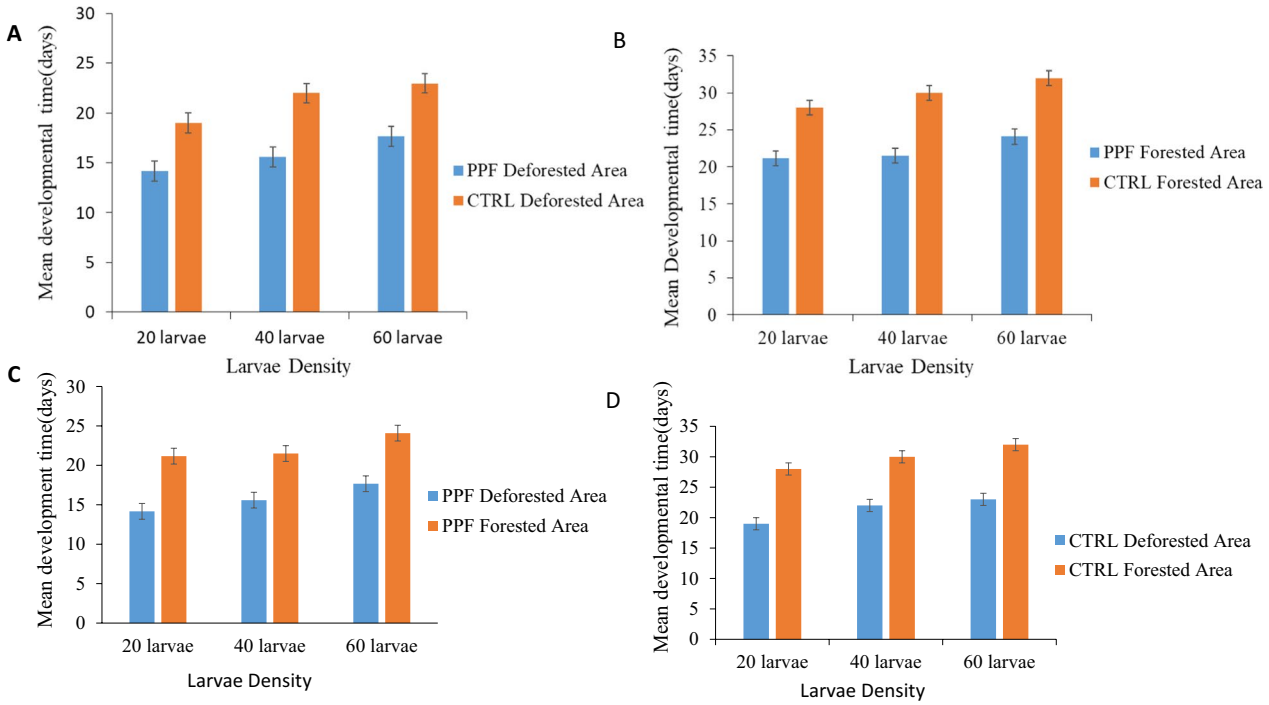

Fig. 4 The impact of pyriproxyfen at 0.03 ppm on *An. gambiae* s.s. development time between forested and deforested area **A**–**C** and the comparison of larvae development in control **D** (experiments repeated 6 times in 3 rounds)

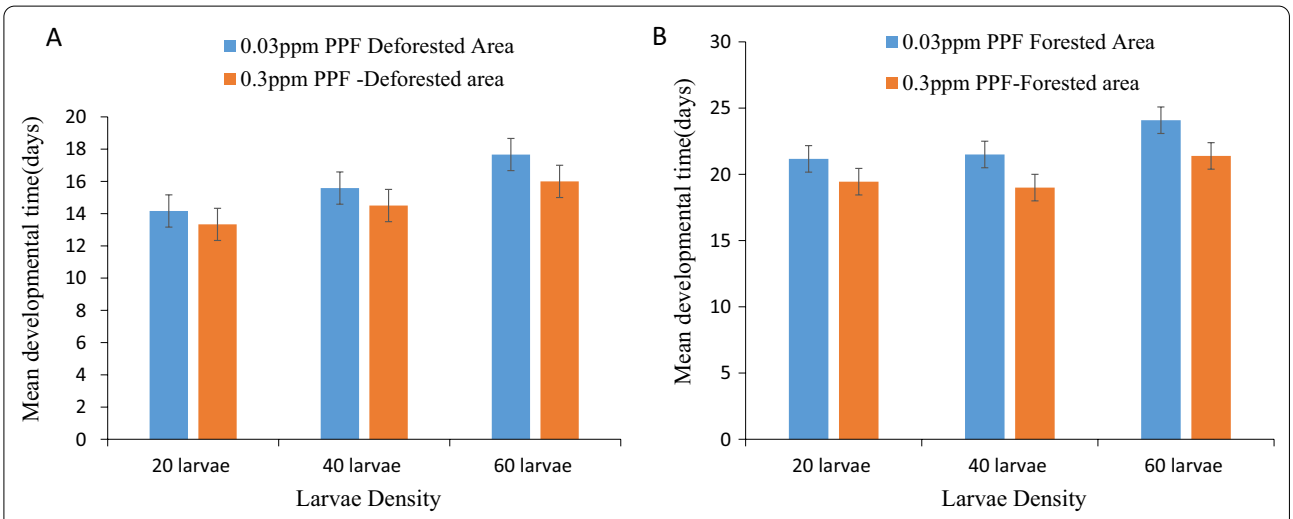

Fig. 5 The effect of increasing PPF concentration from 0.03 to 0.3 ppm (**A** and **B**) on *An. gambiae* s.s. developmental time at densities of 20, 40 and 60 larvae. Experiments were repeated 6 times and in 3 rounds

Discussion

This study was intended to evaluate the bio-efficacy of pyriproxyfen on *A. gambiae* in forested and deforested areas. Larvae mortality experiments have demonstrated that large proportions of *A. gambiae* first instar larvae to pupae exposed to pyriproxyfen in both forested and deforested area died before attaining adult stage. Larvae mortality rates were dependent on pyriproxyfen exposure and land cover types. These findings are consistent with previous studies that reported high mortality rates of *Aedes aegypti* caused by exposure to pyriproxyfen in the laboratory (de Moura et al. 2021). Also, in the present

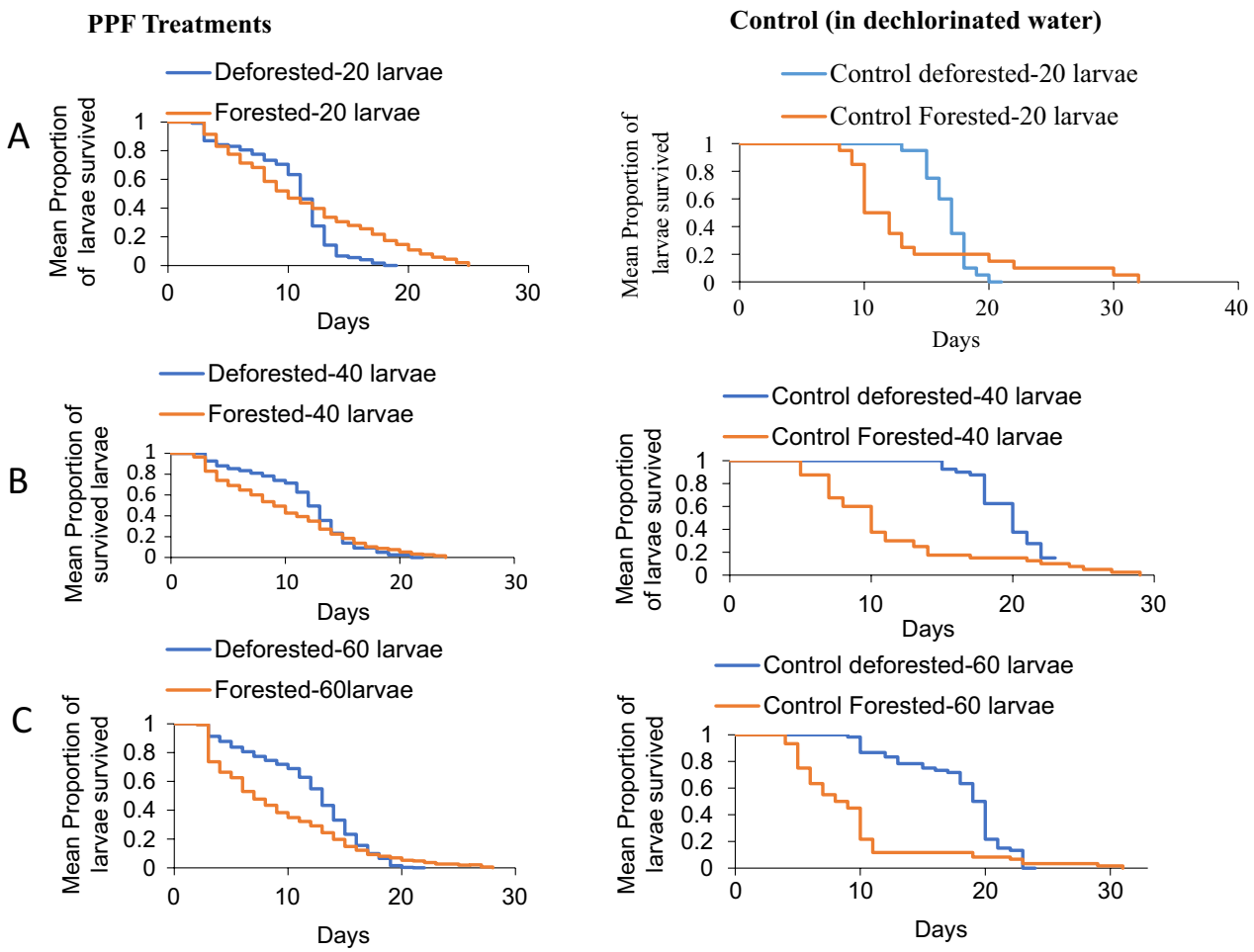

Fig. 6 The impact of pyriproxyfen at 0.03 ppm on *An. gambiae* s.s. larvae survival time in forested and deforested area at density of 20, 40 and 60 larvae (**A**, **B** and **C** respectively). Experiments were done 6 times in 3 rounds

study, high mortality rates were detected in forested area where the recorded temperature and sunlight intensity were low. These observations suggest that low temperature and light intensity in the forested area might have given conducive conditions for the larvicidal activity of pyriproxyfen against *A. gambiae* compared to defrosted area where there was direct sunlight radiation. The present observations can be supported by findings from other studies that have shown pyriproxyfen to be a highly susceptible biolarvicide to photo degradation in water with photolysis half-lives less than 20 days in river water (Sullivan and Goh 2008, Kodaka et al. 2011).

In the present study, despite pyriproxyfen demonstrating high activity on larvae, low ambient temperature and light intensity in forested area might have maintained the activity of pyriproxyfen against immature *A. gambiae* mosquitoes. Studies on the impact of environmental factors to biolarvicides have demonstrated loss of activity of biolarvicides under field condition due to degradation effect of solar gamma radiations and ultraviolet (UV) light composed of UV-A (320–400 nm) and UV-B (280–320 nm) (Manasherob et al. 2002). Observations made in the present study suggest that the bioefficacy of pyriproxyfen against mosquito vectors may be directly or indirectly dependent on surrounding temperature and light. Tree canopy covers in the forested area, might have played a significant role in increasing the efficacy of pyriproxyfen against immature *Anopheles gambiae* mosquitoes.

The observed longer developmental time of larval to pupal stage for larvae exposed to pyriproxyfen in the forested area, is an indication that the efficacy of pyriproxyfen was positively influenced by low temperature and light intensity. Other previous studies have shown that in low light pyriproxyfen is resistant to photolysis and therefore its larvicidal activity against larvae is maintained (Sullivan and Goh 2008). In addition to that, other previous studies in western Kenya highlands, found higher

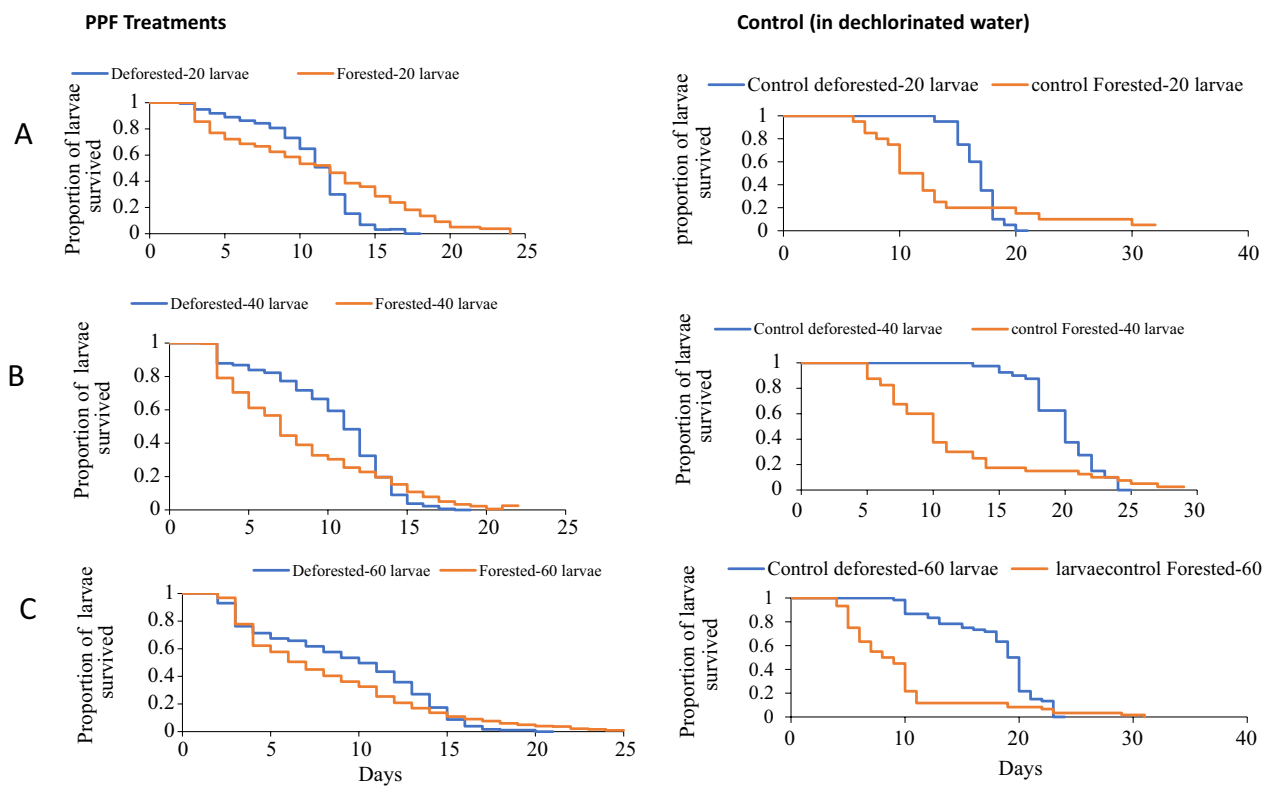

Fig. 7 The impact of pyriproxyfen at 0.3 ppm on *An. gambiae* s.s. larvae survival time in forested and deforested area at density of 20, 40 and 60 larvae (**A**, **B** and **C** respectively). Experiments were done 6 times in 3 rounds

Table 1 The effects of pyriproxyfen at 0.03 ppm and 0.3 ppm on adult *A. gambiae* s.s. emergence in the deforested Area (experiments were done 6 times in 3 rounds)

Density	PPF Conc. (ppm)	Pupa collected (%)	Adult emergence (%)			%EI
			Female	Male	Total	
20	0.03	60	0	0	0	100
	0.3	50	0	0	0	100
	Control	95	50	40	90	10
40	0.03	57.5	0	0	0	100
	0.3	47.5	0	0	0	100
	Control	80	47.5	30	77.5	22.5
60	0.03	55	3.3	1.7	5	95
	0.3	40	0	0	0	100
	Control	683	46.7	36.7	83.4	16.6

EI% is Emergence inhibition percentage

mosquito development rates in deforested area (Afrane et al., 2005, 2006; Munga et al., 2009), similar to what was observed in the present study. In the present study exposed larvae to larvicides had shorter survival time and therefore shorter developmental time, due to larvicidal actity of pyriproxyfen compared to those exposed to dechlorinated water only. Also, in this study exposed fourth instar larvae to pyriproxyfen ended up forming a larval-pupal intermediate stage characterized by remains of larval skin on the portion of the abdomen leading to inhibition of adult emergence. These observations are in agreement with what was observed by Kweka et al. (2019)

Table 2 The effect of pyriproxyfen at 0.03 ppm and 0.3 ppm on adult *An. gambiae* s.s. emergence in the forested area (experiments were done 6 times in 3 rounds)

Density	PPF Conc. (ppm)	Pupa collected (%)	Adult emergence (%)			%EI
			Female	Male	Total	
20	0.03	12	0	0	0	100
	0.3	5	0	0	0	100
	Control	15	0	10	10	25
40	0.03	7.5	0	0	0	100
	0.3	5	0	0	0	100
	Control	9	2.5	5	7.5	17.5
60	0.03	6.7	1.7	5	6.7	93.3
	0.3	3.3	0	0	0	100
	Control	8	3.3	5	8.3	8.3

EI% is Emergence inhibition percentage

in paddy field experiments in Northern Tanzania, confirming pyriproxyfen to be a stage specific biolarvicide. Knowing the specific temperature and light ranges that would affect its larvicidal activity against mosquito larvae could help to have effective use of pyriproxyfen in a variety of environmental settings.

In the present study, when concentration of pyriproxyfen was changed from 0.03 to 0.3 ppm led to no significant effect on larvae developmental time particularly during the first and second instar. This is due to larvicidal nature of pyriproxyfen, targeting late larval and pupal stages (Kweka et al., 2019). Pyriproxyfen does not have direct effect on early larval stages. Rashed and Mulla (1989) showed that age structures of mosquito larvae can have an impact on the efficacy of the larvicides used. In the present study increase in age structure seem to have increased susceptibility level of larvae to pyriproxyfen. Studies by Kweka et al. (2011) demonstrated that larvae feeding rates increased with increase in body structure and age, suggesting that third and fourth instars are likely to be the most susceptible stages to pyriproxyfen (WHO, 2005). During these stages the risk of larvae to contact pyriproxyfen more often in treated habitats increases (Mbare et al., 2013). Increase in concentration from 0.03 to 0.3 ppm had little impact on larvae developmental time, this suggests that pyriproxyfen can work better even at small dosages. Use of higher concentrations beyond the recommended may have a negative impact on non-target organisms.

The present study observed shorter survival time in deforested area, which might have been caused by high temperature and light intensity. Findings from other observations made in previous studies demonstrated that temperature and light intensity play a key role in influencing the survivorship of larvae and adult mosquitoes (Afrane et al., 2005, 2006; Bayoh & Lindsay, 2004; Kweka et al., 2011; Munga et al., 2009). High temperature increase larvae metabolic and feeding rates (Bayoh & Lindsay, 2004). This may have a positive influence on contact chances of larvae to pyriproxyfen leading to reduced larvae survival chances. On the other hand, longer survival time observed in a small proportion of surviving larvae in forested area might be caused by growth retarding effect of pyriproxyfen (Mbare et al., 2013). In this study slowed larvae development rates observed in forested area reduced larvae ability to attain a full actively feeding stage on time which would allow them to frequently contact the larvicides and therefore influencing their survivorship (Kweka et al., 2011). The prolonged the survival time of larvae exposed to pyriproxyfen might have led into reduced larvicidal effect of pyriproxyfen and therefore affecting its efficacy. These observations suggest that for efficient use of pyriproxyfen in forested area, regular re-application is needed.

The observed pupation process of *A. gambiae* in the present study was negatively influenced by larvicidal activity of pyriproxyfen. Emerged pupae from larvae exposed to pyriproxyfen demonstrated the highest mortality rates. Although many pupae emerged from the deforested area, few of them were able to completely emerge into the adult stage. These observations are similar what was observed by de Moura et al. (2021). Many of the mosquito adults had incomplete emergence with their legs and tarsi remaining attached to pupal exuviae similar to what was reported in other studies (Germain et al. 1977). Findings from this study have shown that about 40% of larvae died at the larval stage and over 90% died at pupal stage. Many of the collected pupae did not emerge into the adult stage. In other studies, done in Tanzania (Kweka et al., 2019) and Kenya (Mbare et al.,

2013) where pyriproxyfen was found to inhibit over 90% of adult mosquitoes in the laboratory and field using *An. Arabiensis*. The adult emergence inhibition percentage observed in the present study was higher in the forested area compared to the deforested area. These findings signify that the bio-efficacy of pyriproxyfen against *A. gambiae* larvae were more effective in inhibiting adult mosquito emergence in forested area.

In addition to that, the present study recorded more male adult mosquitoes emerging from habitats found in the forested area while more adult female mosquitoes emerged from habitats found in the deforested area. These findings are similar with observation made in paddy field by Kweka et al (2011). Therefore, it can be projected that communities living in deforested zones are likely to be exposed to female *Anopheles* mosquitoes that transmit malaria (Derua et al., 2019). However, in this study, emerging adult mosquitoes from treated habitats in both forested and deforested area were physiologically weak, small in body size and less active with fading body colour compared to those emerged from control microcosms, similar to what was reported by Moura et al. (2021). These results suggest that even though some mosquitoes may survive pyriproxyfen exposure during their immature development, few of them will be able to survive longer in the adult stage. This would mean that the larvicidal effect of pyriproxyfen in contaminated immature mosquitoes lasts longer and are extended to the adult stage.

Limitation of the study

This study was conducted from October 2019 to March 2020. These were rainy seasons characterized by short periods of high temperature and sunlight intensity. It is possible to have different outcomes if the same study could be conducted in May to August due to these periods having cool and hot conditions. Also, the study design did not allow to measure the precise temperature ranges that could have influenced the efficacy of pyriproxyfen activity. Future studies should investigate the efficacy of pyriproxyfen under varied temperature and light intensity, so as to know the exact temperature and light intensity which can interfere with the activity of pyriproxyfen in local environments.

Conclusion

The present study was designed to understand the impact of pyriproxyfen on *A. gambiae* mortality rates, developmental and survival time in the forested and deforested area. The efficacy of the two concentrations (0.03 ppm and 0.3 ppm) of pyriproxyfen against *A. gambiae* larvae has been found to be dependent on temperature and light intensity in the study areas. *A. gambiae* larvae have shown higher susceptibility levels to pyriproxyfen in forested than in deforested area, where high intensity of sunlight and temperature seem to have reduced its larvicidal activity against the exposed *A. gambiae* larvae. Pyriproxyfen has demonstrated high efficacy in inducing high larvae and pupae mortality rates and increasing the larvae developmental and survival time in forested area. Despite the interaction of other environmental factors, this study recommends pyriproxyfen to be the best additional tool for LSM to be used in malaria vector control in both deforested and forested endemic areas. Its high ability in inhibiting adult emergence in both forested and deforested areas provide promising results towards global elimination of malaria disease. The use of pyriproxyfen to target immature mosquitoes in combination with other tools such as IRS and LLINs would lead to reduction in the abundance of adult mosquitoes and malaria transmission. Also, reviving forestry schemes and community afforestation programmes could have an additional impact on mosquito larvae control by increasing land canopy cover to limit the availability of mosquito breeding habitats.

Abbreviations

ACT: Artemisinin Combination Therapy; IRS: Indoor residual spray; LLINs: Long lasting insectidal nets; RDT: Rapid diagnostic test; WHO: World Health Organization.

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Authors' contributions

BN: Conceptualization, Methodology, Investigation, Writing-original draft. PSC and WK: Investigation and data curation. RY: Investigation and data curation. EJK: Conceptualization, Methodology, Investigation, Writing-original draft and review. All authors have read and approved the manuscript.

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Availability of data and materials

All data gathered have been analysed and presented within this manuscript.

Declarations

Ethics approval and consent to participate

The permission was granted by postgraduate committee. The consent to participate was not applicable in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Afrane, Y. A., Lawson, B. W., Githeko, A. K., & Yan, G. (2005). Effects of microclimatic changes caused by land use and land cover on duration of gonotrophic cycles of *Anopheles gambiae* (Diptera: Culicidae) in western Kenya highlands. *Journal of Medical Entomology, 42*, 974–980.

Afrane, Y. A., Zhou, G., Lawson, B. W., Githeko, A. K., & Yan, G. (2006). Effects of microclimatic changes caused by deforestation on the survivorship and reproductive fitness of *Anopheles gambiae* in western Kenya highlands. *The American Journal of Tropical Medicine Hygiene, 74*, 772–778.

Ahmed, A., & Ahmed, M. (2011). Morphological identification of malaria vectors within Anopheles species in parts of Kano State, Nigeria. *Bayero Journal of Pure Applied Sciences, 4*, 160–163.

Alfano, N., Tagliapietra, V., Rosso, F., Manica, M., Arnoldi, D., Pindo, M., & Rizzoli, A. (2019). Changes in microbiota across developmental stages of *Aedes koreicus*, an invasive mosquito vector in Europe: Indications for microbiota-based control strategies. *Frontiers in Microbiology, 10*, 2832.

Antonio-Nkondjio, C., Sandjo, N. N., Awono-Ambene, P., & Wondji, C. S. (2018). Implementing a larviciding efficacy or effectiveness control intervention against malaria vectors: Key parameters for success. *Parasites and Vectors, 11*, 57.

Asrat, Z., Taddese, H., & Orka, H. (2018). Estimation of forest area and canopy cover based on visual interpretation of satellite images in Ethiopia. *Land, 7*, 92.

Bayoh, M. N., & Lindsay, S. W. (2004). Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. *Medical Veterinary Entomology, 18*, 174–179.

Christiansen-Jucht, C., Parham, P. E., Saddler, A., Koella, J. C., & Basáñez, M.-G. (2014). Temperature during larval development and adult maintenance influences the survival of *Anopheles gambiae* ss. *Parasites and Vectors, 7*, 489.

Chu, V. M., Sallum, M. A. M., Moore, T. E., Emerson, K. J., Schlichting, C. D., & Conn, J. E. J. (2020). Evidence for family-level variation of phenotypic traits in response to temperature of Brazilian Nyssorhynchus darlingi. *Parasites and Vectors, 13*, 55.

Clements, A. N. (1992). *The biology of mosquitoes: Development, nutrition and reproduction* (Vol. 1). Chapman & Hall.

Coetzee, M., Craig, M., & Le Sueur, D. (2000). Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology Today, 16*, 74–77.

de Moura, R.L., de Oliveira, M.E., de Carvalho, W.F., Rodrigues, M.M., Dos Santos, M.S., Edvan, R.L., Abdalla, A.L., Moreira, M.Z., da Silva, E.M., 2021. Goat performance on pure Andropogon gayanus pastures or associated with legumes. *Tropical Animal Health and Production, 53*, 1–12.

Derua, Y. A., Kweka, E. J., Kisinza, W. N., Githeko, A. K., & Mosha, F. W. (2019). Bacterial larvicides used for malaria vector control in sub-Saharan Africa: Review of their effectiveness and operational feasibility. *Parasites Vectors, 12*, 426.

El-Sayed, A., & Kamel, M. (2020). Climatic changes and their role in emergence and re-emergence of diseases. *Environmental Science Pollution Research International, 27*, 22336–22352.

Fillinger, U., Ndenga, B., Githeko, A., & Lindsay, S. W. (2009). Integrated malaria vector control with microbial larvicides and insecticide-treated nets in western Kenya: A controlled trial. *Bulletin of the World Health Organization, 87*, 655–665.

Fillinger, U., Kannady, K., William, G., Vanek, M. J., Dongus, S., Nyika, D., Geissbühler, Y., Chaki, P. P., Govella, N. J., & Mathenge, E. M. (2008). A tool box for operational mosquito larval control: Preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania. *Malaria Journal, 7*, 20.

Forest Resource Assessment Project. (1996). Forest resources assessment 1990: Survey of tropical forest cover and study of change processes. In: Food and Agriculture Organization of the United Nations. Forestry Department, Geneva.

Geissbühler, Y., Kannady, K., Chaki, P. P., Emidi, B., Govella, N. J., Mayagaya, V., Kiama, M., Mtasiwa, D., Mshinda, H., & Lindsay, S. W. (2009). Microbial larvicide application by a large-scale, community-based program reduces malaria infection prevalence in urban Dar es Salaam, Tanzania. *PLOS ONE, 4*, e5107.

Germain, M., Hervé, J.-P., & Geoffroy, B. (1977). Variation du taux de survie des femelles d'Aedes africanus (Theobald) dans une galerie forestière du sud de l'Empire Centrafricain. *Cah. ORSTOM, ser. Entomol. Med. Parasitol, 15*, 203–208.

Gilbreath, T. M., Kweka, E. J., Afrane, Y. A., Githeko, A. K., & Yan, G. (2013). Evaluating larval mosquito resource partitioning in western Kenya using stable isotopes of carbon and nitrogen. *Parasites and Vectors, 6*, 1–7.

Hawkes, F. M., Manin, B. O., Cooper, A., Daim, S., Jelip, J., Husin, T., & Chua, T. H. (2019). Vector compositions change across forested to deforested ecotones in emerging areas of zoonotic malaria transmission in Malaysia. *Scientific Reports, 9*, 13312.

Keenan, R.J., Reams, G.A.,Achard, F., de Freitas, J.V., Grainger, A., Lindquist, E., 2015. Dynamics of global forest area: Results from the FAOGlobal Forest Resources Assessment 2015. *Forest Ecology and Management, 352*, 9–20.

Kweka, E. J., Kimaro, E. E., & Munga, S. (2016). Effect of deforestation and land use changes on mosquito productivity and development in Western Kenya Highlands: Implication for malaria risk. *Frontiers in Public Health, 4*, 238.

Kweka, E. J., Munga, S., Himeidan, Y., Githeko, A. K., & Yan, G. (2015). Assessment of mosquito larval productivity among different land use types for targeted malaria vector control in the western Kenya highlands. *Parasites and Vectors, 8*, 356.

Kweka, E. J., Zhou, G., Lee, M.-C., Gilbreath, T. M., Mosha, F., Munga, S., Githeko, A. K., & Yan, G. (2011). Evaluation of two methods of estimating larval habitat productivity in western Kenya highlands. *Parasites and Vectors, 4*, 110.

Kweka, E. J., Mahande, A. M., Msangi, S., Sayumwe, S., Ouma, J. O., Temba, V., Lyaruu, L. J., & Himeidan, Y. E. (2019). Biological activity of Sumilarv 0.5 G against *Anopheles gambiae* sensu stricto and Anopheles arabiensis in Northern Tanzania. *East Africa Science, 1*, 35–42.

Kweka, E. J., Zhou, G., Beilhe, L. B., Dixit, A., Afrane, Y., Gilbreath, T. M., Munga, S., Nyindo, M., Githeko, A. K., & Yan, G. (2012). Effects of co-habitation between *Anopheles gambiae* ss and *Culex quinquefasciatus* aquatic stages on life history traits. *Parasites Vectors, 5*, 1–9.

Lindsay, S., & Martens, W. (1998). Malaria in the African highlands: Past, present and future. *Bulletin of the World Health Organization, 76*, 33.

Majambere, S., Lindsay, S. W., Green, C., Kandeh, B., & Fillinger, U. (2007). Microbial larvicides for malaria control in the Gambia. *Malaria Journal, 6*, 76.

Manasherob, R., Ben-Dov, E., Xiaoqiang, W., Boussiba, S., & Zaritsky, A. (2002). Protection from UV-B damage of mosquito larvicidal toxins from Bacillus thuringiensis subsp. israelensis expressed in Anabaena PCC 7120. *Current Microbiology, 45*, 217–220.

Mbare, O., Lindsay, S. W., & Fillinger, U. (2013). Dose–response tests and semi-field evaluation of lethal and sub-lethal effects of slow release pyriproxyfen granules (Sumilarv® 0.5 G) for the control of the malaria vectors *Anopheles gambiae* sensu lato. *Malaria journal, 12*, 94.

McCann, R. S., van den Berg, H., Diggle, P. J., van Vugt, M., Terlouw, D. J., Phiri, K. S., Di Pasquale, A., Maire, N., Gowelo, S., & Mburu, M. M. (2017). Assessment of the effect of larval source management and house improvement on malaria transmission when added to standard malaria control strategies in southern Malawi: Study protocol for a cluster-randomised controlled trial. *BMC Infectious Diseases, 17*, 639.

McCrae, A. J. (1984). Oviposition by African malaria vector mosquitoes: II. Effects of site tone, water type and conspecific immatures on target selection by freshwater *Anopheles gambiae* Giles, sensu lato. *Annals of Tropical Medicine Parasitology, 78*, 307–318.

Moura, R. L., Abieri, M. L., Castro, G. M., Carlos-Júnior, L. A., Chiroque-Solano, P. M., Fernandes, N. C., Teixeira, C. D., Ribeiro, F. V., Salomon, P. S., Freitas, M. O. (2021). Tropical rhodolith beds are a major and belittled reef fish habitat. *Scientific Reports, 11*, 1–10.

Msangi, S., Lyatuu, E., & Kweka, E. J. (2011). Field and laboratory evaluation of bioefficacy of an insect growth regulator (Dimilin) as a larvicide against mosquito and housefly larvae. *Journal of Tropical Medicine, 2011*, 394541.
Munga, S., Yakob, L., Mushinzimana, E., Zhou, G., Ouna, T., Minakawa, N., Githeko, A., & Yan, G. (2009). Land use and land cover changes and spatiotemporal dynamics of anopheline larval habitats during a four-year period in a highland community of Africa. *The American Journal of Tropical Medicine Hygiene, 81*, 1079–1084.
Ndo, C., Poumachu, Y., Metitsi, D., Awono-Ambene, H. P., Tchuinkam, T., Gilles, J. L. R., & Bourtzis, K. (2018). Isolation and characterization of a temperature-sensitive lethal strain of Anopheles arabiensis for SIT-based application. *Parasites and Vectors, 11*, 97–105.
Rashed, S. S., & Mulla, M. S. (1989). Factors influencing ingestion ofpariculate materials by mosquito larvae (Diptera: Culicidae). *Journal of Medical Entomology, 26*, 210–216.
Romijn, E., Lantican, C. B., Herold, M., Lindquist, E., Ochieng, R., Wijaya, A., Murdiyarso, D., & Verchot, L. (2015). Assessing change in national forest monitoring capacities of 99 tropical countries. *Forest Ecology Management, 352*, 109–123.
Sullivan, J. J. & Goh, K. S. (2008). Environmental fate and properties of pyriproxyfen. *Journal of Pesticide Science, 33*, 339–350.
Wamae, P. M., Githeko, A. K., Menya, D. M., & Takken, W. (2010). Shading by Napier grass reduces Malaria vector larvae in Natural habitats in Western Kenya highlands. *EcoHealth, 7*, 485–497.
Wang, X., Zhou, G., Zhong, D., Wang, X., Wang, Y., Yang, Z., Cui, L., & Yan, G. (2016). Life-table studies revealed significant effects of deforestation on the development and survivorship of Anopheles minimus larvae. *Parasites and Vectors, 9*, 323.
Wang, X., Liu, T., Wu, Y., Zhong, D., Zhou, G., Su, X., Xu, J., Sotero, C. F., Sadruddin, A. A., & Wu, K. (2018). Bacterial microbiota assemblage in *Aedes albopictus* mosquitoes and its impacts on larval development. *Molecular Ecology, 27*, 2972–2985.
WHO. 2005. Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization.
WHO. (2019). *World malaria report 2019*. World Health Organisation.
WHO. (2020). *World malaria report 2020: 20 years of global progress and challenges*. World Health Organization.
Zhong, D., Wang, X., Xu, T., Zhou, G., Wang, Y., Lee, M. C., Hartsel, J. A., Cui, L., Zheng, B., & Yan, G. (2016). Effects of microclimate condition changes due to land use and land cover changes on the survivorship of malaria vectors in China-Myanmar border region. *PLOS ONE, 11*, e0155301.
Zhou, G., Munga, S., Minakawa, N., Githeko, A. K., & Yan, G. (2007). Spatial relationship between adult malaria vector abundance and environmental factors in western Kenya highlands. *The American Journal of Tropical Medicine Hygiene, 77*, 29–35.
Zogo, B., Tchiekoi, B. N. C., Koffi, A. A., Dahounto, A., Alou, L. P. A., Dabiré, R. K., Baba-Moussa, L., Moiroux, N., & Pennetier, C. (2019). Impact of sunlight exposure on the residual efficacy of biolarvicides Bacillus thuringiensis israelensis and Bacillus sphaericus against the main malaria vector *Anopheles gambiae*. *Malaria Journal, 18*, 55.

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