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Drones for Area-Wide Larval Source Management of Malaria Mosquitoes

Wolfgang R. Mukabana [1,2,3], Guido Welter [4], Pius Ohr [5], Leka Tingitana [6], Makame H. Makame [7], Abdullah S. Ali [7] and Bart G. J. Knols [8,9,10,*]

1 School of Biological Sciences, University of Nairobi, Nairobi P.O. Box 30197-00100, Kenya; rmukabana@uonbi.ac.ke
2 Science for Health, Nairobi P.O. Box 44970-00100, Kenya
3 African Institute for Development Policy, Nairobi P.O. Box 14688-00800, Kenya
4 InGuide, Gevers Deynootweg 1108A, 2586 BX The Hague, The Netherlands; info@inguide.nu
5 UCL School of Management, One Canada Square, Canary Wharf, London E14 5AA, UK; piusohr@yahoo.de
6 Tanzania Flying Labs, Science Junction, Bagamoyo Road, Dar es Salaam, Tanzania; leka@flyinglabs.org
7 Zanzibar Malaria Elimination Programme, Zanzibar P.O. Box 236, Tanzania; makame.makame@yahoo.com (M.H.M.); info@zamep.go.tz (A.S.A.)
8 Environmental Health and Ecological Sciences Department, Ifakara Health Institute, Ifakara P.O. Box 53, Tanzania
9 Department of Environmental Science, Faculty of Science, Radboud University, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands
10 Soneva Fushi, 4th Floor Jazeera Building, Boduthakurufaanu Magu, Male 20077, Maldives
* Correspondence: bart@malariaworld.org

Abstract: Given the stagnating progress in the fight against malaria, there is an urgent need for area-wide integrated vector management strategies to complement existing intra-domiciliary tools, i.e., insecticide-treated bednets and indoor residual spraying. In this study, we describe a pilot trial using drones for aerial application of Aquatain Mosquito Formulation (AMF), a monomolecular surface film with larvicidal activity, against the African malaria mosquito *Anopheles arabiensis* in an irrigated rice agro-ecosystem in Unguja island, Zanzibar, Tanzania. Nine rice paddies were randomly assigned to three treatments: (a) control (drone spraying with water only), (b) drone spraying with 1 mL/m^2, or (c) drone spraying with 5 mL/m^2 of AMF. Compared to control paddies, AMF treatments resulted in highly significant ($p < 0.001$) reductions in the number of larvae and pupae and >90% fewer emerging adults. The residual effect of AMF treatment lasted for a minimum of 5 weeks post-treatment, with reductions in larval densities reaching 94.7% in week 5 and 99.4% in week 4 for the 1 and 5 mL/m^2 AMF treatments, respectively. These results merit a review of the WHO policy regarding larval source management (LSM), which primarily recommends its use in urban environments with 'few, fixed, and findable' breeding sites. Unmanned aerial vehicles (UAVs) can rapidly treat many permanent, temporary, or transient mosquito breeding sites over large areas at low cost, thereby significantly enhancing the role of LSM in contemporary malaria control and elimination efforts.

Keywords: drones; UAV; malaria; larval source management; larvicide; rice agro-ecosystem; Zanzibar

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1. Introduction

Despite the tremendous gains made in curbing malaria mortality and morbidity, especially in sub-Saharan Africa, between 2000 and 2015, progress has stalled since then [1,2]. The more than 1.7 billion averted cases and more than 10 million lives saved since the turn of the millennium are largely the result of successes in controlling *Anopheles* mosquitoes that transmit malaria parasites between humans. Notably, the free and large-scale distribution of long-lasting insecticidal bednets (LLINs), and, to a lesser extent, indoor residual spraying (IRS) of insecticides, together accounted for 78% of the reported success [3]. Vector control thus remains a crucial element in the fight against malaria but is increasingly plagued by resistance of mosquitoes to the chemicals used on bednets or sprayed on indoor walls [4,5]. Not only resistance to insecticides but also changes in mosquito behavior that result in avoidance of contact with insecticides through early evening and outdoor biting are increasingly observed [6–9]. Furthermore, *Anopheles arabiensis*, which is the main malaria mosquito species in Zanzibar, is primarily an outdoor biting (i.e., exophagic) mosquito [10]. This, combined with weak health delivery systems, drug resistance of *Plasmodium* parasites that cause malaria [11], and the absence of an effective vaccine resulted in an estimated 627,000 deaths and 241 million cases in 2020 [1]. As a consequence, the call for innovative tools that can target malaria mosquitoes in different (non-chemical) ways in the outdoor environment is now more widely and frequently heard [12–15].

Virtually every country that successfully eliminated malaria relied in some way on the active control of aquatic stages of the mosquito through larval source management (LSM) [16]. LSM is the management of water bodies that are potential larval habitats to prevent the development of immature mosquitoes into adults [17]. Despite huge and well-documented successes both outside and inside Africa, LSM became to be viewed as an inferior strategy at the end of the Global Malaria Eradication Programme based on models that indicated that a focus on the daily survival rate of the adult female mosquito had a much larger impact on malaria transmission intensity than larval control [18]. Nevertheless, historical campaigns that were successfully executed over sometimes extremely large areas, notably the eradication of the African malaria vector *Anopheles arabiensis* from Brazil [19,20] and Egypt [21] and the near elimination of the yellow fever mosquito *Aedes aegypti* from the whole of South America by 1962 [22], have fueled renewed enthusiasm for LSM. Although larviciding is an old tool for mosquito control and has played a prominent role in malaria elimination efforts in many countries (the USA, Europe, Australia, etc.), its wide-scale adoption for malaria control and elimination in Africa is only starting. Nevertheless, by 2017, nearly 60 countries were implementing some form of mosquito larval control, and 24 out of 46 endemic African nations apparently were doing so on a smaller or larger scale [23]. LSM, therefore, appears to be an increasing trend. Current larvicides include surface films such as mineral oils and alcohol-based surface products that suffocate larvae and pupae; synthetic organic chemicals such as organophosphates that interfere with the nervous system of larvae; microbials such as *Bacillus thuringiensis israeliensis* (Bti) and *B. sphaericus* (Bs) that kill only larvae since their toxins have to be ingested and lead to starvation; and insect-growth regulators that interfere with insect metamorphosis and prevent adult emergence from the pupal stage [24]. These products are mostly applied from the ground by hand, using knapsack sprayers or granular product formulations.

The use of unmanned aerial vehicle (UAV) technology is rapidly advancing and applied for an increasing number of purposes in health and civil applications [25–28]. It is already being used to map potential breeding sites for malaria mosquitoes using aerial imagery [29–32], for terrain reconnaissance and vector control program planning, and is even being considered for the release of sterile male mosquitoes in genetic control programs [33,34]. In this study, we present an example of architectural innovation by combining two existing and proven technologies and applying them within a different context and market. On the one hand, we used Aquatain Mosquito Formulation (hereafter AMF) [35], which has been field-tested as a larvicide in Africa [36]. On the other hand, we used a commercial drone platform (the Agras MG-1 S), normally used for precision-spraying of crops, to apply AMF on mosquito larval habitats. AMF is a commercially available surface film, with polydimethylsiloxane (PDMS) as its major constituent, with known lethal physical action against the aquatic stages of mosquitoes [36–38]. AMF obtained WHO prequalification in December 2018 and is now included as a larvicide for use by the UN and other organizations and countries [39]. The objectives of the pilot trial presented here were to assess how efficiently aerial application of AMF using drones can suppress the development of malaria mosquitoes in rice paddies and measure how long this suppression would last. Below, we describe the outcome of a trial conducted in

Zanzibar (Unguja island), where AMF was used in different concentrations and compared against a control in nine selected rice paddies.

2. Materials and Methods

2.1. Study Area

This study was carried out in the rice irrigation scheme of Cheju, an area situated in the Central/South region of Zanzibar's main island, Unguja, the United Republic of Tanzania (Figure 1). Although back in 2009 a feasibility assessment deemed the prospects of malaria elimination on the island challenging but feasible [40], recurring introductions of parasites by travelers [41] and seasonal workers from the mainland [42] have so far prevented this. Nevertheless, intense campaigns with contemporary control tools resulted in highly impressive reductions in the community prevalence of malaria to as low as 0.43% in 2015 [43]. Today, resistance to insecticides used and evasion of contact with insecticides by mosquitoes through outdoor biting have stagnated further progress and call for interventions that can target vectors outdoors and with novel tools. This is particularly so now that the more outdoor feeding *Anopheles arabiensis* has become a dominant malaria vector in Zanzibar [10,43].

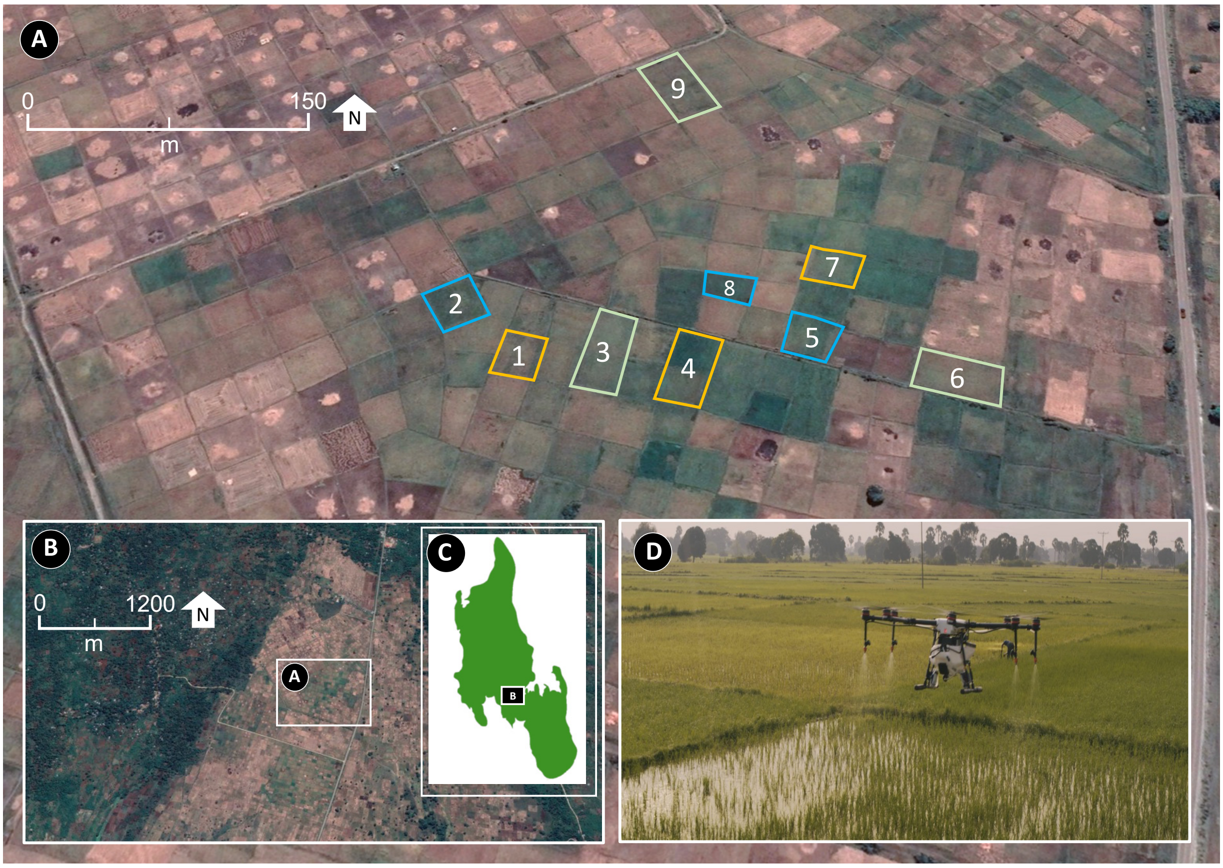

Figure 1. (**A**) Location of the 9 experimental paddies in the Cheju rice irrigation scheme. Control paddies are indicated by 3, 6, and 9; low-dose AMF (1 mL/m^2) by 2, 5, and 8; and high-dose AMF (5 mL/m^2) by 1, 4, and 7. (**B**) The location of the irrigation scheme within Unguja island, Zanzibar (**C**). (**D**) The Agras MG-1 S drone spraying AMF in a rice paddy.

2.2. Site Selection and Drone Spraying

A total of nine rice paddies were availed by local farmers. The surface area of individual paddies ranged between 768 and 2405 m^2. These paddies were randomly assigned as control fields ('C') or treatment fields ('T'). The control paddies were sprayed by drone with water only, and the treatment paddies received either a low dose (T_{low}, 1 mL/m^2) or high dose of AMF (T_{high}, 5 mL/m^2). Baseline mosquito larval/pupal densities and adult emergence counts were measured in all nine paddies for a period of five days prior to spraying. This roughly equals one full mosquito life cycle (from egg to adult) duration under the prevailing tropical climatic conditions.

An Agras MG-1 S drone (DJI, Shenzhen, China; 10 L carrying capacity) was used to spray water or AMF. Since AMF, a biodegradable, non-toxic, silicone-based liquid, is more viscous than water ('honey-like'), the original stock membrane pumps were removed from the drone and new specially fabricated mechanical pumps installed. In addition to hardware modifications, extensive software redesign was undertaken, including the rewriting of the flight controller algorithm, and the installation of custom firmware on the drone and its remote controller. The battery power of the drone lasted, depending on the payload weight, between 10 and 15 min. During the spraying exercise, the drone was provided with extra batteries that were charged by a small fuel generator kept in the field. From a working distance of 2 m above the water surface, a spraying swath of up to 5 m and an operating speed 2–5 m/s ensured that a correct and even amount of formulation was sprayed on the surface of the paddies. In this way, the amount of the liquid suspension was precisely regulated to economize operations. The drone was operated in manual mode but can also be pre-programmed to operate in automatic mode. It was able to fly above little water pockets in the rice paddies that were cornered by muddy edges or had lower water levels, which prevented the self-spreading characteristic of AMF from covering all water surfaces within those pockets. Overall, the water levels in all treated and untreated rice paddies were equally high. The capacity of the pumps (33 mL/s) limited the time that was needed to spray the calculated amount of AMF per paddy because the maximum operating speed of the drones is 8 m/s. The drone with integrated mechanical pumps sprayed each of the 1000 m^2 of the rice paddies in 30 (for T_{low}) or 150 s (for T_{high}) at its maximum spraying capacity.

2.3. Monitoring Immature and Adult Mosquitoes

Mosquito larval/pupal densities and adult emergence counts were conducted daily in all 9 paddies for a period of 46 days (from 27 October 2019 to 16 December 2019) during the intervention (post-treatment) phase. Upon arrival at a rice paddy, a standard WHO dipper ('cup') was lowered gently at an angle of 45 degrees just below the water surface to allow any larvae/pupae present in the water to flow into the dipper. Larval/pupal sampling was carried out between 9 and 11 am. The number of *Anopheles* larvae collected from each of 6 dips was summed and recorded for individual paddies. Sampling was carried out in areas around floating debris and the edges of the habitats, which are the preferred sites for mosquito larvae [17]. Collected larvae were identified morphologically using taxonomic keys [44].

Adult mosquitoes emerging from the paddies were collected using emergence traps [45]. Three emergence traps were placed in each of the 9 paddies. The traps were positioned over water in areas likely to contain anopheline larvae (e.g., at the edges of each experimental paddy or over tufts of vegetation inside the paddies). Every other day, the traps were relocated to other places inside the paddies to minimize sampling bias. The traps were constructed from conical metal frames (1 m high and 1 m in diameter at the base (0.785 m^2 surface area)) and covered with mosquito netting to reduce shading of the water, which might reduce catches. Adult mosquitoes were removed from the trap using an aspirator through a netting sleeve placed on the side of each trap. The traps were emptied daily, and specimens transported to the laboratory for identification and counting.

2.4. Data Analysis

The effect of AMF treatment on the number of larvae present inside experimental rice paddies in Cheju was modeled using generalized linear models (GLMs) with a Poisson distribution and a log link function [46]. The stage of development of the rice plants was included as a moderator variable in the GLM. All data were analyzed using version 23 of the IBM SPSS statistical package.

The percentage reduction in larval and adult mosquito densities over time was calculated using Mulla's formula [47,48]:

$$\% \text{ reduction} = 100 - (C_1/T_1 \times T_2/C_2) \times 100 \quad (1)$$

where C_1 and C_2 describe the average number of larvae in the control paddies pre- and post-treatment, and T_1 and T_2 describe the average number of larvae in the intervention group pre- and post-treatment.

3. Results

Over the entire study period, a total of 11,241 larvae, 1789 pupae, and 1278 adult mosquitoes, consisting mostly (~90%; M.H. Makame, *pers. comm.*) of the malaria mosquito *Anopheles arabiensis*, were collected from the rice paddies (see Supplementary Materials for raw data).

3.1. Impact of the Low and High Dose of AMF on Different Life Stages

The highest mean (± SE) number of larvae (73.8 ± 7.1) were collected from untreated paddies during the baseline data collection period ('untreated before', Figure 2a). Untreated paddies used as controls during the intervention period ('untreated after') also yielded large numbers of larvae (41.7 ± 3.6), albeit significantly fewer than during the 'untreated before' period. However, the mean number of larvae present in paddies treated with 1 or 5 mL/m^2 of AMF was much reduced (10.7 ± 1.8 and 5.1 ± 1.3, respectively) compared to the controls pre- and post-intervention, demonstrating a strong insecticidal effect of AMF. Statistical analyses showed highly significant differences ($p < 0.001$) between all pairwise comparisons of the four treatments (Figure 2a). The number of pupae found in paddies with the different treatments assumed exactly the same pattern as that of the larvae. Thus, the mean (±SE) number of pupae reduced progressively from the 'untreated before' (10.8 ± 1.3) followed by 'untreated after' (7.2 ± 0.8) to AMF 1 and 5 mL/m^2 (1.7 ± 0.3 and 0.9 ± 0.2, respectively; Figure 2b). Finally, significantly fewer adult mosquitoes emerged from paddies in the 'untreated before' (3.1 ± 1.9) than in the 'untreated after' (7.5 ± 0.9; $p < 0.001$). However, the mosquitoes collected in these two treatments were significantly ($p < 0.001$) higher than those collected from paddies treated with AMF at both concentrations (which did not differ; $p = 0.744$; Figure 2c). The finding that both the low and high concentration yielded similar reductions in adult mosquito emergence favors the cheaper option of AMF application at a dose of 1 mL/m^2. Results from the larval sampling for the individual fields are shown in Figure 3, which, especially for the 5 mL/m^2 treatment, show comparable numbers.

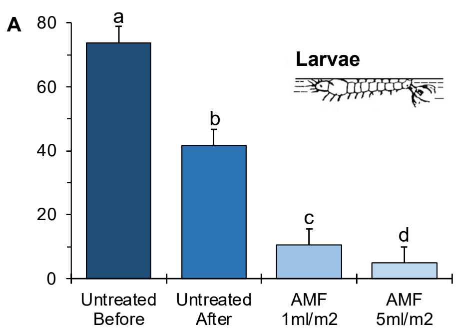

Figure 2. *Cont.*

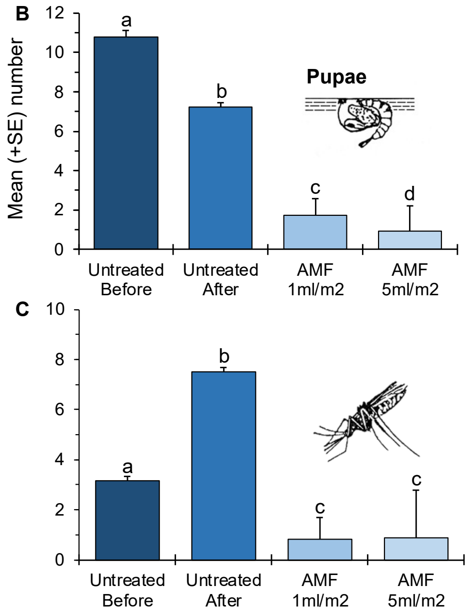

Figure 2. Mean number (± SE) of *Anopheles arabiensis* larvae (**A**), pupae (**B**), and adults (**C**) sampled from rice fields in Cheju, Zanzibar. The paddies were sprayed (by drone) with water ('untreated after') or with 1 or 5 mL/m^2 of AMF. Bars without letters in common are significantly different at $p < 0.001$.

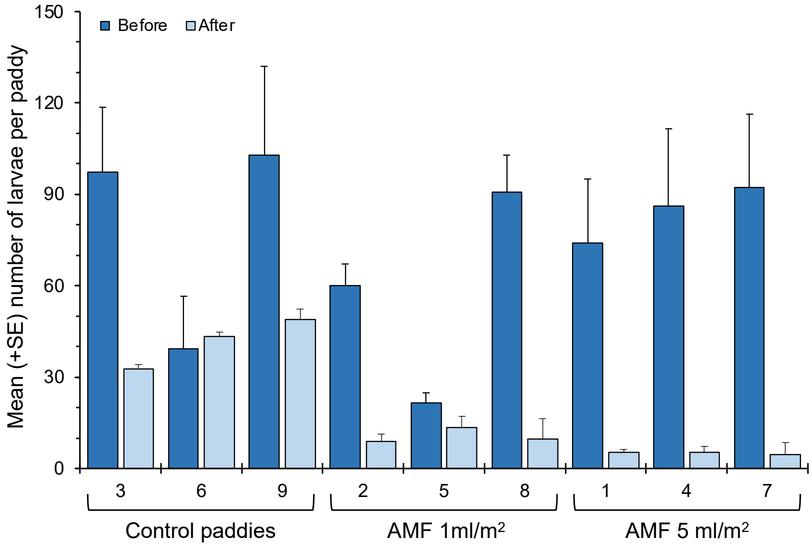

Figure 3. Mean number (± SE) of *Anopheles arabiensis* larvae collected from the 9 individual paddies during the pre-intervention ('before') and post-intervention ('after') period.

3.2. Residual Effect of AMF Treatments

The residual effect of AMF in suppressing the development of mosquito larvae in habitats was observed throughout the 7-week (day 5–day 51) period over which measurements post-treatment were carried out (Figure 4). The percent reduction in larval density, calculated using Mulla's formula, reached a ceiling of 94.7% in week 5 and 99.4% in week 4 for the 1 and 5 mL/m^2 AMF treatments, respectively (Table 1). Larval densities started increasing thereafter, signifying the onset of a diminishing performance of AMF, which biodegrades under the influence of UV light.

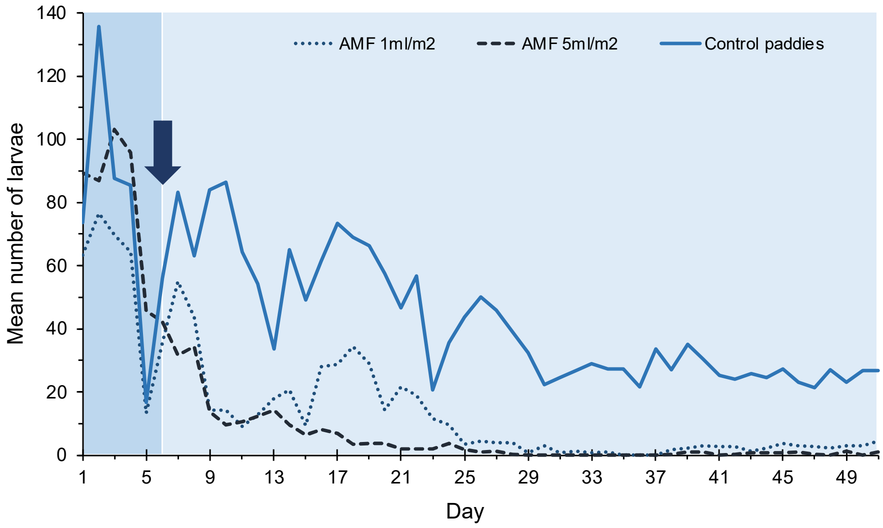

Figure 4. Reduction in the mean larval abundance in untreated rice paddies and those treated with 1 or 5 mL/m^2 of AMF. The pre-treatment period is indicated in dark blue. Thereafter, AMF was applied by drone (arrow) and observations of larval abundance continued until day 51.

Table 1. Weekly mean abundance and percent reduction of mosquito larvae in rice paddies treated with two doses of AMF (1 or 5 mL/m^2); data were pooled for the different treatments. No paddy was treated with AMF in week 1 (pre-test period). The highest reductions observed for both treatments are underlined.

Week	**Control**	**AMF**		**% Reduction**	
		1 mL/m^2	**5 mL/m^2**	**1 mL/m^2**	**5 mL/m^2**
1 (Pre)	80.0	57.0	84.0	—	—
1 (Post)	70.2	26.4	22.1	47.2	70.0
2	59.7	24.0	7.5	43.6	88.0
3	42.5	12.0	2.3	60.4	94.8
4	31.4	2.0	0.2	91.1	<u>99.4</u>
5	28.9	1.1	0.3	<u>94.7</u>	99.0
6	24.5	2.6	0.5	85.1	98.1
7	25.8	3.2	0.6	82.6	97.8

3.3. Drone Operation Speed

We treated six rice paddies with AMF: three paddies with T_{low} (measuring 1325, 1299, and 769 m^2) and three paddies with T_{high} (measuring 1082, 1820, and 1157 m^2). The operating speed of the drones was limited by two factors. One limiting factor was the capacity of the pumps, which sprayed at a maximum of 33 mL/s. The second limiting factor was the maximum load of the Agras MG-1 with the newly installed mechanical pumps. We used a safe maximum load of 8 L (instead of the maximum of 10 L this drone features). Given this, we were required to refill the tank with AMF during the treatment of the larger rice paddy of 1820 m^2 and treatment with T_{high}.

It took the drone 40 s to spray the 1325 m^2 paddy, 39 s to spray the 1299 m^2 paddy, and 23 s to spray the 769 m^2 paddy, when treating with T_{low} (see Supplementary Materials for a video of a spraying drone). The back-and-forth flight to the three paddies took 30–50 s. The flight between the treated paddies lasted 20 s. The overall operating time for the T_{low} treatment of the three paddies took 3:22 min.

For T_{high}, it took the drone 30 s to fly to the 1082 m^2 paddy, 162 s to spray it, plus 240 s of refill time; 174 s to spray the 1157 m^2 paddy plus 240 s of refill time; and 273 s to spray the 1820 m^2 paddy plus 50 s for the flight back to base. Thus, the overall operating time for the T_{high} paddies was 19:29 min. Combined, the treatment of all six paddies, totaling

7452 m^2, including the times to reach plots and return to base for intermittent refueling, took 22:51 min.

4. Discussion

In this research, we report the successful use of drones in Africa to control malaria mosquitoes in irrigated rice agro-ecosystems. The aerial application of AMF with the use of drones resulted in impacts on the different life stages comparable to original evaluations of AMF against anopheline mosquitoes in the laboratory, where a dose of 1 mL/m^2 resulted in a median lethal time to death of 0.98 (95% CI = 0.75–1.20) days for *Anopheles gambiae s. s.* [35] and a dose of 1.35 mL/m^2 (95% CI 1.09–1.75) resulted in complete larval mortality of *An. arabiensis* [37]. In an open field study in Kenya, in which AMF was poured into paddies by hand at 1 mL/m^2, a reduction in anopheline emergence of 93.2% was monitored [36]. Within 3–4 weeks after AMF application, similar reductions were observed in the current trial. Therefore, the application of AMF from the air by drone or manually on the ground does not, as expected, make a difference in terms of the impact on mosquito numbers and the different life stages. The slowly increasing numbers of larvae by week 6 indicate the necessity for retreating the rice paddies in week 6 irrespective of the amount of larvicide used per unit area and is similar to what was reported from a field study in Kenya, where a single dose of AMF at 1 mL/m^2 inhibited emergence by 85% (95% CI 82–88%) for 6 weeks [36]. Thus, not only can drones be used for applying AMF with similar impact to ground application but this impact also lasts equally long (ca. 6 weeks at a dose of 1 mL/m^2) compared to previous studies. It is noteworthy that the untreated paddies, despite having received no larvicide, also exhibited a reduction in mosquito larval densities over the eight weeks of observation, which can be explained by the fact that crop growth during the tillering stage leads to an increase in shade covering the water surface, thereby making it less attractive for gravid mosquitoes to oviposit their eggs, which they preferably do so in sunlit water bodies. Alternatively, due to the proximity between treated and control fields, lower mosquito densities due to treatments could also have affected the numbers in control paddies.

A major aim of this study was to perform cost-benefit analyses querying the effectiveness of drones as a method for applying mosquito larvicides in rice paddies. Although it was difficult to measure the application speed of AMF by hand, since this varies between individuals and paddy conditions (deep mud inhibits fast walking), we can draw some conclusions. On the assumption that a person spraying from a knapsack would cover 6 m in 10 s, we inferred that the drones as used in this study were 2–6 times faster and less costly than manual application of AMF, underscoring their superiority and suitability for this purpose. These findings could even be enhanced by using newer, more efficient model drones with a larger carrying capacity and flight times. For instance, DJI's T30 drone (DJI, Shenzhen, China; carrying capacity 30 L) with integrated mechanical pumps is almost eight times faster than hand spraying. Their edge over hand spraying also resides in the fact that drones ensure continuous and precise dosing at pre-set flight speeds. This ensures more efficient and accurate application of AMF. Furthermore, drones can easily access edges and muddy areas in rice paddies that cannot easily be accessed on foot and without causing damage to the rice crop. Finally, spraying by hand cannot be carried out similarly due to unsteady spraying rates and a varying walking speed. Hence, an application rate of at least double what was sprayed with the drone could be assumed for manual spraying. Due to the rather high purchase price of AMF, the application efficiency has a significant impact on the price comparison between the application by drones or by hand. Given the fact that the pumps installed on the drones are the limiting factor for the spray rate (in our case, 33 mL/s), the drone cannot play out its maximum possible speed. Consequently, with the installation of more powerful pumps, more area could be covered per second, which would further reduce the operating and re-fueling times, and thus the cost of drone application. For instance, an increase in the pumping speed of 50% would result in 49 mL/s and would increase the application speed of the drones in comparison to hand spraying to 9 times faster.

Although the results presented here are the outcome of an initial pilot trial and need further expansion, they do present a very attractive option to augment the limited number of tools to control malaria mosquitoes, notably outdoors. Paddies are generally well demarcated, and coordinates can easily be uploaded on the drones' operating software to enable precise and recurrent treatments over time. Some of the intricacies experienced with coverage path planning in the built environment do not apply due to the openness of and generally sparsely vegetated rice irrigation schemes [49]. Especially in large rice irrigation schemes, this can result in the treatment of very large areas over a very short period of time. Rice is the fastest growing food in Africa; harvested areas increased by over 600% from 1961 to 2019 [50,51], but this simultaneously increases the risk of mosquito-borne disease, notably malaria [52]. When rice cooperatives bundle resources, this could provide opportunities for entrepreneurs to develop drone application of anti-mosquito products on a commercial basis. As a result, it is anticipated that a reduced malaria incidence will benefit the rice farming communities and result in less absenteeism from work, in turn resulting in higher yields and income.

The World Health Organization suggests the regular application of insecticides to water bodies (larviciding) for the prevention and control of malaria in children and adults as a supplementary intervention to LLINs or IRS in areas with ongoing malaria transmission where aquatic habitats are few, fixed, and findable [53]. This mostly follows an earlier WHO report [17] and the outcome of a systematic review [54] that outlines that at present, there is still too little evidence of the true value of this vector control tool. We would argue here that with regards to rice irrigation schemes, these are both 'fixed', 'findable', and 'few' if one considers the entire scheme as one (massive) breeding site. This is surely not what was meant with 'few' but given the speed at which aerial application of larvicides can be undertaken with drones, this warrants a re-evaluation. Similarly, the use of drones for area-wide larval source management could be extended to other large open areas such as floodplains or estuaries, for which large-scale larviciding has proved too difficult in the past due to access problems [55,56]. Future studies should focus on broadening the applications of this approach for larval source management of mosquitoes, should assess its direct impact on malaria transmission intensity, and provide more details on the economics of drone use in similar settings.

5. Conclusions

Drones have previously been used for mosquito habitat surveillance. In this experimental study, we showed that commercially available drones and mosquito-killing agents can be used to effectively reduce mosquito population densities for up to 1.5 months after a single application of AMF (at 1 mL/m^2). Given the urgent need for outdoor malaria mosquito control measures, this approach warrants further development in terms of cost and application in rice irrigation schemes in Zanzibar and beyond.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/drones6070180/s1, Raw data file. Video of drone spraying of AMF in a rice paddy. For a visual impression of the trial, see: https://www.youtube.com/watch?v=Vo9Sn2kFDo4&ab_channel=DJI (accessed on 20 May 2022).

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Conflicts of Interest: The authors declare no conflict of interest.

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