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Maize pollen diet enhances malaria mosquito longevity and infectivity to Plasmodium parasites in Ethiopia

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Abstract

Although larval diet quality may affect adult mosquito fitness, its impact on parasite development is scarce. The effects of ultraviolet-sterilized *Zea mays*, *Typha latifolia* and *Prosopis juliflora* plant pollen diets on larval development, pupation rate, adult survival, and parasite infectivity were investigated. Four treatment groups and two control groups were used for each pollen diet, and each experimental tray had 25 larvae. Control larvae fed on Tetramin fish food. Female *An. arabiensis* were starved overnight and exposed to infectious blood using a membrane-feeding technique. The Kaplan-Meier curves and log-rank test were used for analysis. The *Z. mays* pollen diet increased malaria mosquito survival and pupation rate (91.3%) and adult emergence (85%). *Zea mays* and Tetramin fish food had comparable adulthood development times. Adults who emerged from larvae fed *Z. mays* pollen had the longest average wing length (3.72mm) and were more permissive to *P. vivax* (45%) and *P. falciparum* (27.5%). They also survived longer after feeding on infectious blood and had the highest number of *P. vivax* oocysts. *Z. mays* pollen improved larval development, survival, and adult infectivity to *Plasmodium*. Our findings suggest that malaria transmission in *Z. mays* growing villages should be monitored.

Background

Plasmodium falciparum and *P. vivax* are the two dominant human malaria parasites in Ethiopia, and the principal vector is *Anopheles arabiensis.* Some mosquito species are efficient and highly permissive to the parasite to complete the cycle and predominantly bite humans to increasing the parasite transmissibility[1]. There is also a link between the intensity of malaria transmission and the biology of the mosquito vectors. For instance, the quality of larval food may have an impact on adult life histories, including adult survival, the number of emerging adults, and the development of parasites[2]. Female *Anopheles* mosquitoes usually select breeding habitats suitable for the survival and development of immature stages. The pollen (fine to a coarse powdery substance comprising pollen grain) from cultivated and wild grasses supplement the natural food for *Anopheles* larvae in the breeding habitats and might support the development and increase malaria transmission[3,4]. For example, maize cultivation is mostly claimed to increase malaria transmission[5]. The odour from the maize pollen also has lured gravid malaria mosquitoes to the oviposition site[6]. Moreover, nutrition during the larval stage determines the size and metabolic reserves of adult mosquitoes [4,7].

There are, however, controversial results with regard to the plant-vector interaction. For example, a study by Muller and his colleagues[8] reported higher risk of malaria infection during the flowering season of an invasive plant species *Prosopis juliflora*. When the flowering branches of this plant were removed, the age structure of the malaria mosquitoes was changed, and the number of older malaria mosquitoes was substantially reduced. This might be related with the sugar food sources of flowering plants for adults as an energy source, which might have an impact on the age of the malaria mosquitoes. The pollen of some plants is more nutritious than others. However, several plants may inhibit disease transmission by affecting vectors in their breeding habitats because of the presence of toxic constituents. For example,

Cyperus papyrus and *Phragmites australis* contains chemicals that can kill the larval stages in breeding habitats[9]. Some plants have very attractive flowers for mosquitoes and some others have toxic sugar baits[10].

Existing knowledge on the interaction of malaria vectors and plants with respect to parasite transmission is scarce. *Zea mays* (a maize specimen) is the most common crop in the lowlands of Gamo zone where malaria is also endemic[11]. The crop is cultivated all year round. The shores of the Lake Abaya and Chamo are predominantly covered by *T. latifolia.* The shores of the two lakes are also ideal breeding sites for the primary and secondary malaria vectors[11,12]. The shrub *P. juliflora* is also a very common plant species in the region. While there is little evidence about the interaction between malaria vectors and plants, effective control requires a contextual understanding of the bio-ecology of malaria vector-plant interaction. However, in Ethiopia, there is limited understanding of the impact of pollen on early stages and its long-term effect on adult fitness and susceptibility to the parasite. In light of these factors, the purpose of this study was to demonstrate whether pollen from *T. latifolia*, *Z. mays* and *P. juliflora* enhances *An. arabiensis* larval development and survivorship, pupation rate, adult size, longevity of malaria vector, survival and infection to *Plasmodium* parasites.

Materials And Methods

Study area description

Anopheles larval sampling was made from the potential breeding habitats in the Arba Minch area, located in the southern Ethiopian Rift Valley system. The climate is hot and humid which is favourable for mosquito breeding and malaria transmission. The average maximum temperature is 30°C, while the average minimum is 17°C. The rainfall distribution is bimodal and mostly occurring in March to May and September to November. The annual rainfall average is around 900 mm. The major cash crops cultivated in the area include bananas, maize and mango using both rain and irrigation schemes. In the rainy seasons, the water pools in ditches along the roads, farmlands, tire track pooled water, and marshy areas are the potential breeding sites for *Anopheles* mosquitoes.

Malaria is a common health problem in the area[13,11] and is more prevalent after the end of the rainy season due to the potential breeding sites[11]. *Plasmodium falciparum* and *P. vivax* are the two common species[14,15].

Study design

This experimental study was conducted to assess the effect of pollen of some selected plants on the larval survival, pupation rate, adult emergence, vector longevity and infectivity to malaria parasites. Newly emerged larvae were provided with equal quantity of pollen from *T. latifolia*, *Z. mays*, *P. juliflora* and standard larval food Tetramin fish food (Cichlid Sticks; Tetra, Maidenhead Aquatics, Leicester, UK). The

emerged adults were supplied with10% sugar solution until ready for the infection experiments[16]. Then, 2–3 days old female *Anopheles* were starved overnight and provided with *P. vivax* and *P. falciparum* parasite infected blood though membrane feeding (Fig. 1).

Study plants

These plants (Fig. 2) were selected based on the scientific evidences documented in relation to the malaria mosquitoes either against or in favour of larval and adult stages[5,7]. *Typha latifolia* (common cattail) is a perennial herbaceous plant in the genus *Typha* and among the most common of all aquatic plants. It is an "obligate wetland" species found close to water bodies. This plant is among the wind-pollinated plants that produces large quantities of pollen. *Prosopis juliflora* is a shrub or small tree in the family Fabaceae. It is an evergreen and fast-growing invasive species in arid and semi-arid areas. The flowers of *P. juliflora* are self-incompatible, entomophilous (insect pollinating), depending on insects for seed dispersal. It produces many seeds and is tolerant of a wide range of climatic regimes and soil types which have contributed to making *P. juliflora* one of the worst invasive alien plants. The leafy stalk of *Z. mays* (maize) produces pollen inflorescences and separate ovuliferous inflorescences called ears that yield seeds, which are fruits and its pollen, are produced entirely in the staminate inflorescence that is wind pollinated. The male flower matures earlier than the female flower. The plant specimens were collected from their typical habitat by field press and brought to the Arba Minch University Botany laboratory for further confirmation by expertise.

Pollen collection

Pollen of *T. latifolia* was collected in the shore of the Lake Abaya, *P. juliflora* from the shrub dominated area of Lante (local place in Arba Minch area) and the pollen of *Z. mays* L. (BH660 cultivar) from the farmland of Lemat during the maize flowering season. The pollens were collected by covering the mature flower with a paper bag and shaking. The collected pollen was transferred to vials and sterilized within 24 hours of collection using UV light (10850 Harry Hines Blvd Suite 155, Dallas, TX 75220) overnight for 12 hours and store on silica gel until used for feeding experiments.

Pollen nutrient analysis and measuring grain size

The C and N concentration of pollen diets were determined by Walkley-Black and Kjeldahl methods in Arba Minch University analytical chemistry laboratory. Approximately 5 mg of sample of pollen from each plant was taken and processed based on the standard protocol. Thereafter, the C: N ratio of the pollen was determined by %C/%N × 6.25[17]. The pollen grain sizes were measured according to Roulston et al[17]. Pollen grains were mounted in paraffin oil and measured at 400× with an ocular micrometer. Polar axis measurements were taken for all pollen grains. Volumes were calculated using volume equations for

spheres (4/3πp3). Ten randomly selected grains per slide were measured, and these values were averaged for calculating volumes.

Mosquito larvae collection and rearing

Anopheles larvae were collected from the potential breeding habitats in the Arba Minch area and reared in Arba Minch University Medical Entomology and Vector Control laboratory. The rearing room was maintained at temperature between 25–27°C and relative humidity between 72–80%. The larvae rearing trays were inspected twice a day for the presence of pupae, and if present they were placed in adult cages. The newly emerging adults were provided with 10% sterilized sugar solution. Then, they were allowed feed on live rabbit at 3- to 4-days interval and serving as 'mother' cages. The egg dish was placed in the cage one day after blood feeding and eggs were transferred to larvae tray for hatching. These newly hatched first instar larvae were used for the experimental feeding of pollen diets. The female adult mosquitoes in the mother cage were morphologically identified for *An. gambiae* complex and a sub-sample (150) was confirmed by polymerase chain reaction (PCR) for *An. gambiae* complex. The entire tested *An. gambiae* complex was identified as *An. arabiensis* (hereafter the target mosquito species will be *An. arabiensis*).

Larval development and survival analyses

After 12 hours of hatching, 200 unfed first instar larvae were counted manually and randomly apportioned to larval trays of 10×25 cm and 8 cm high filled with one litter of distilled water. Effective dose of pollen diet and TetraMin fish food was determined by optimizing 0.15, 0.3 and 0.6mg on relative adulthood survival. Then, 0.3mg was established as the effective dose of each pollen diet and Tetramin fish food.

Six replicates each with 25 larvae for each of the plant pollen diet and two as control group were used. The standard TetraMin fish food was used as control. The experiment was repeated three times for each pollen diet and Tetramin fish. A total of 450 larvae were treated for each diet and 150 for each experimental term. For the first and the second instar larvae, the diet was offered only once a day, for the third and fourth, it was twice a day based on procedure of Kivuyo et al[18]. In each replicate, the larval development and survival was recorded twice a day (01:00 and18:00) and dead individuals were counted and removed. Continuous inspection of the number of larvae surviving, pupating, and emerging adults were recorded.

Survival rate of each day (s_j) =

Survival rate total (S) = $s_1 + s_2 \ldots\ldots + sj$

Where, s_1 the no. of larvae at the begning of day 1

s_2 the no. of larvae at the begning of day 2

Measuring Mosquito Size/wing Length

The same batches and same ages of 30 female adult *An. arabiensis* were collected from each pollen diet and TetraMin fish food. They were killed by freezing and, then one wing of each mosquito glued onto a slide. Its length from the distal end of the alula to the tip, excluding the fringe scales, was measured with stage graticule micrometer (ruler etched in side) to examine the effect of larval diet on wing length of adult *An. arabiensis*.

Patient screening for gametocyte stage

Microscopically positive patients with *P. vivax* and *P. falciparum* gametocytes were enrolled. The gametocyte detection was done by standard finger prick and Giemsa stained blood smears from thick blood smear under oil immersion microscopy by experienced laboratory technicians. The gametocyte positive patients were asked to provide approximately 3ml of blood for membrane feeding prior to antimalarial treatment. Blood gametocyte density was estimated microscopically by converting the total number of gametocytes in 1000 leukocytes to 8000 leukocytes/µl. Screening of gametocyte carriers was synchronized into mosquito rearing processes and thus always performed on the same day with the experimental infection. Patients with mixed infection were excluded.

Membrane blood feeding

The same batch of 2 to 3-day-old female *An. arabiensis* were starved overnight and kept for experimental infection through membrane feeding. A total of 1280 female *An. arabiensis* were allowed to feed on *P. vivax* positive blood in four membrane feeding experiments, and another 1280 were exposed to *P. falciparum* positive blood in four membrane feeding experiments. In each feeding experiment, 320 *An. arabiensis* were exposed in 8 paper cups each with 40 mosquitoes.

The circulation water bath was used to allow water circulation through the feeder and its temperature was adjusted on 37°C to keep the blood warm and the parasites alive. A *Plasmodium*-positive blood meal was carefully pipetted from the same blood source into the neck of the feeder and allowed to feed simultaneously for 30 minutes under dark conditions. After carefully looking at the mosquito for abdominal condition, unfed mosquitoes were discarded. The fed *An. arabiensis* were kept in the paper cups and provide with 10% sugar solution under normal insectary condition (27°C ± 2 and 70 ± 10% relative humidity). *Anopheles arabiensis* were kept under sugar for eight days for *P. vivax* and twelve days for *P. falciparum* oocyst detection. Infected mosquito containing cups were kept in a small cage for double protection. The membrane was removed carefully and soaked in 10% bleach to decontaminate and rinse with water.

Dissection to oocyst stage and sporozoite detection

Randomly 40 mosquitoes were taken from each pollen type replicate cages on day 7 post-infection for *P. vivax* and on day 12 post infection for *P. falciparum.* A total of 120 female mosquitoes were dissected for *P. vivax* and *P. falciparum*. For each of the dissection sessions, the infection rate (IR) (infected mosquitoes/total mosquitoes dissected in each feeding (%) was recorded, and intensity of infection (oocyst load/total infected mosquitoes dissected in each feeding) were determined. Non-infected mosquitoes were omitted from the calculation.

The infective stage of the *Plasmodium* parasite was detected by circumsporozoite protein (CSP)-ELISA using the kits specific for *P. vivax*-210, *P. vivax*-247 and *P. falciparum* following standard instruction[19]. A total of 104 and 35 *An. arabiensis* were tested for CSPs *P. vivax* and *P. falciparum*, respectively.

Data analysis

The effect of diets on larval survival was performed, and to conduct survival analysis data were converted to count-time data. Kaplan-Meier curves were used to compare diet-specific survival pattern for each group. Comparison of survival rate in each larval diet to that of TetraMin Fish food was performed using log rank test. ANOVA was used for pupation, emergence rate and wing length to assess the influence of main variables (larval diet). Wald's test was used to determine significant differences between diets based on χ^2 and *P*-values.

The proportion of female adult mosquitoes that survived after infected blood meal determined by counting the number of mosquitoes on their oocyst and sporozoite stage across all larval diets. The median number of oocyst per mosquito mid-gut was compared using a Kruskal-Wallis test, followed by a Dunn's post-hoc test. The prevalence of sporozoite infection, or percent of mosquitoes reaching to infective stage, was compared with a Fisher's exact test.

All statistical analysis performed using GraphPad Prism 5 (Version 5.01). Significance was considered at 5% level of confidence. Single factor experimental design was employed to assess the impact of pollen of some selected plants on the larval survival, pupation, adult emergence, and vector infectivity to malaria parasites. Survival rate of each treatment case was calculated as survival rate of each day (Araujo et al., 2012).

Ethical Consideration

This study was reviewed and approved by the Institutional Review Board of Arba Minch University (No: CMHS/12036564/111). After briefing the objective of the study, consent was obtained from the study participants. Participants in the study who tested positive for *Plasmodium* received free treatment in accordance with the national malaria treatment guidelines. The CSP-ELISA test was performed in accordance with standard protocol[19]. All methods were performed in accordance with the standard protocols and procedures[18,19]. Other plant pollens, aside from maize pollen, were collected with the local

administration approval in open spaces like the lakeshore and by the side of the road. Through permission of farm owners, the maize pollen was collected on farmland. Despite the fact that only the pollen grains were gathered for the experiment, plant specimens were also gathered in order to be identified by senior botanists before collection. The voucher specimens of the plants (*P. juliflora* and *T. latifolia*) have been deposited in a publicly available botanical herbarium at Arba Minch University.

Results

Pollen nutrient content and size

The pollen grain volume was significantly different between grass species with higher pollen volume of *Z. mays* (mean diameter 71.2 ± 1.66 µm) compared to *P. juliflora* (48.1 ± 0.56µm) and *T. latifolia* (25.5 ± 0.32 µm) ($F = 1033$, $DF = 3$, $P < 0.0001$).

The C: N ratio of pollen differed between test plant species with the pollen C: N ratio of *T. latifolia* > *Z. mays* > *P. juliflora*. *T. latifolia* had a higher proportion of carbon (52%), compared with the two other species *Z. mays* (44%), and *P. juliflora* (32%). The higher proportion of nitrogen was belonging to *Z. mays* (31%) followed by *T. latifolia* (28%) and *P. juliflora* (18%).

Larval diet impacts on larval survival

Survivorship of the larvae instars was found to be larval diet dependent. The overall larval survival differed significantly among the pollen diets tested ($F = 23$, $DF = 3$, $P < 0.0001$). The survival rate of larvae fed on the *Z. mays* pollen was 100% until day 8 and slowly declined with the minimum survival rate of 0.4 at day 15. The probability of those larvae fed on *Z. mays* pollen diets was 0.89 followed by *T. latifolia* (0.71) in the follow-up period. The survivorship of larvae due to *P. juliflora* pollen diet was found to be the poorest (highest larval death) with 0.51 probability of survival during the follow-up period. The group of *An. arabiensis* larvae supplied with *T. latifolia* pollen had accelerated development of larval instars, while those supplied with the *P. juliflora* pollen diet experienced extended larval period (Fig. 3).

Effect of larval diets on pupation rate and duration

The effect of pollen diets on the larval average time to complete the larval stage and change to the pupae showed significant differences between the diets ($\chi^2 = 12.8$, $DF = 3$, $P < 0.0001$). *Anopheles arabiensis* pupation was faster on *T. latifolia*, it took 2-to-3-days since the starting day of pupation followed by Tetramin fish and *Z. mays*. Larval instars took longer time to develop into pupae when larvae supplied with *P. juliflora* pollen diet 5-to-11 days (Table 1).

Table 1
The impact of different larval diets on *An. arabiensis* larvae pupation rate, duration of pupation and adult emergence rate

Larval diet	Average no. of pupae/150 larvae (%)	Average no. of pupae/day	Starting day of pupation	Average time to complete pupation since starting day of pupation
T. latifolia	111(74)	5.4	5	2-to-3-days
Z. mays	137 (91.3)	12	8	4-to-5-days
P. juliflora	96 (60.9)	1.95	10	5-to-11-days
Tetramin fish food	128 (85.3)	7.5	6	3-to-4-days

Adult emergence rate

A significant difference has been observed between larval diets from different plants on adult emergence rate (number of adults that successfully emerged from an initial number of larvae) ($x^2 = 17.3$, DF = 3, $P < 0.0001$). The highest adult emergence rate was documented in *Z. mays* diet ($P < 0.05$) followed by TetraMin fish food and *T. latifolia*. The number of adults that emerged in *P. juliflora* was lower compared to the three other diets (Fig. 4). Moreover, high mortality of adult mosquitoes was also documented on this diet. But there was no significant deviation in time needed to reach adulthood in either of the diet ($P > 0.05$), mostly, emerged with in 48h after popuation. More females were emerged from larvae fed on *Z. mays* pollen, compared to other diet sources but not significantly different ($P > 0.05$).

Larval diet impacts on wing length

Wing length was significantly affected by the type of larval diet ($\chi^2 = 69$, DF = 39, $p < 0.0001$). The female *An. arabiensis* emerged from larvae fed on *Z. mays* possessing bigger wings (mean WL = 3.72mm) than females that fed on Tetramin fish (mean WL = 3.255mm) and *T. latifolia* (mean WL = 2.825mm). The mean wing length was 2.9mm (2.00 to 3.72 mm) for *P. juliflora* pollen showed the smallest in wing length (mean Wl = 2.006mm) (Fig. 5).

Larval diet impacts on Adult survival and oocyst density

The proportion of female surviving until the day of dissection for oocyst after feeding on *P. vivax* and *P. falciparum* positive blood was higher among adults emerged from larvae fed on *Z. mays* pollen diet than other diets (Fig. 6).

The proportion of *Plasmodium* oocyst infected *An. arabiensis* following an infected blood meal was significantly different among larvae diets ($P < 0.05$). *Anopheles arabiensis* adults emerged from larvae fed on *Z. mays* pollen showed the highest oocyst infection rate (45% for *P. vivax* and 27.5% for *P. falciparum*) followed by Tetramin fish food (30% for *P. vivax* and 20% for *P. falciparum*) (Table 2). There was no significant difference in *Z. mays* and Tetramin fish ($P > 0.05$). Those *An. arabiensis* fed on *T. latifolia* had infection rates of 22% for *P. vivax* and 8.3% for *P. falciparum*.

The median number of *P. vivax* oocyst was higher in adult *An. arabiensis* emerged from larvae reared on *Z. mays* pollen diet (Fig. 7). The intensity of infection of *An. arabiensis* reared on *Z. mays* was varied significantly from other pollen diets ($P < 0.0001$). The highest median number of oocyst was observed in *An. arabiensis* reared on *Z. mays* (median 43) for *P. vivax* and (median = 0) for *P. falciparum* followed by Tetramin fish (median = 6) for *P. vivax* and (median = 0) for *P. falciparum.* The lowest mean number of oocyst was reported in those *An. arabiensis* reared on *P. juliflora* pollen larval diets (mean = 0.26, 1.04 ± SD, median = 0) for *P. vivax* and no infection for *P. falciparum*.

Larval diet impacts on CSP infection rate

The overall CSP rate for *P. vivax* was 8.6% (95% CI; 1.14–21.02; 9 of 104) (Table 2). *P. vivax* CSP rate of *An. arabiensis* reared on *Z. mays* pollen was 13.2% (95% CI: 4.4–28.1; 5 of 38) followed by 9.1% (95% CI: 1.9–24.3; 3 of 33) for Tetramin fish. No *An. arabiensis* from larvae fed on *P. juliflora* was positive for *P. vivax*. No *An. arabiensis* were found to be positive for *P. falciparum* CSPs (Table 3).

Table 2
The effect of larval diets on feeding efficacy, oocyst density and *P.vivax* sporozite infection rate of *An. arabiensis*

Larval diet	No.of exposed	Feeding efficiency (%)	Adult survival (%)	No. dissected (+; %)	Oocyst/mid-gut ranges	ELISA tested (+)	CSP rate in % (95% CI)
Z. mays	320	87	238 (74.4)	120 (54; 45)	0–80	38 (5)	13.2 (4.4–28.1)
T. latifolia	320	83	148 (46.3)	120 (27; 22)	0–41	22 (1)	4.5 (0.5–22.8)
P. juliflora	320	81	150 (46.9)	120 (6; 5)	0–5	11 (0)	0
Tetramin fish	320	90	224 (70)	120 (36; 30)	0–77	33 (3)	9.1 (1.9–24.3)
Overall	1280		760	480 (123; 25.6)		104 (9)	8.6 (4.0-15.8)

Table 3
The effect of larval diet on feeding efficacy, oocyst density and *P. falciparum* sporozoite infection rate of *An. arabiensis*

Larval diet	No. of exposed	Feeding efficiency (%)	Adult survival (%)	No. dissected (+; %)	Oocyst/mid-gut range	ELISA tested (+)	CSP rate in %
Z. mays	320	82	188 (58.7)	120 (33; 27.5)	0–6	14 (0)	0
T. latifolia	320	79	156 (48.7)	120 (10; 8.3)	0–3	7 (0)	0
P. juliflora	320	77	84 (26.2)	120 (0; 0)	0	3 (0)	0
Tetramin fish	320	89	166 (51.9)	120 (24; 20)	0–5	11(0)	0
Overall	1280					35 (0)	0

Discussion

Here, we looked at how different plant pollen larval diets affected the adult emergence rate, female size, pupation time, larval and adult survival, and infectivity to *P. falciparum* and *P. vivax* parasites in the main malaria vector, *An. arabiensis*. *Zea mays* pollen enhanced larval survival, adult emergence and infectivity to *Plasmodium* parasites. *Anopheles arabiensis* that emerged from larvae fed on *Z. mays* pollen were seemed to be more permissive to *P. vivax* and *P. falciparum* parasite than Tetramin fish food, but there was not statistically different difference. Adults that emerged from larvae that consumed *Z. mays* pollen had the highest mean number of *P. vivax* oocysts and the longest survival times.

The amount and quality of *Anopheles* larval food are important for the development, survivorship and adult emergence rate[7]. In farmland associated habitats, maize pollen grains had positive correlation with larvae abundance[20]. The grasses including *T. latifolia* also determine the abundance of larval stages of *Anopheles* mosquitoes and adult productivity[21]. A study on invasive plant *P. juliflora* documented positive impact on *Anopheles* mosquito population[8]. The positive impact of maize pollen on larval development, vector density, and mosquito longevity has been documented[5]. The maize pollen also used as odor to attract gravid female malaria mosquitoes for oviposition[6]. This study further extended the investigation to the artificial membrane feeding experiment to see the impact of larval diets on the adult survival, oocyst infection density and permissiveness to the *Plasmodium* parasites.

In this study, a substantial difference in larval survival rate was observed between larvae fed on different pollen diets. *Zea mays* pollen diet enhanced the survivorship of malaria mosquitoes both at adult and larval stages. A *Z. may* crop is the most common one in the immediate vicinity of homes and planted throughout the year particularly where irrigation access is possible. *Zea mays* pollen larval diet enhanced

the larval survival, which was documented by Asmare et al[21]. On the other hand, larvae fed on the pollen of *T. latifolia* taken shorter time to pupation than *Z. mays*, but those supplied with *P. juliflora* pollen diet took longer time to develop into pupae. The carbon and nitrogen ratio in the diet may play a significant role in the development and survival of *An. arabiensis* mosquito[22]. The protein content of *Z. mays* pollen is a key driver of enhanced development and survival of *An. arabiensis* larvae[5]. Poor diet causes an extended larval period and hence the larvae require enough food supply for ecdysis to avoid a high mortality rate[23].

In addition, *Z. mays* pollen enhanced adult fitness to *Plasmodium* parasites infection. Adults from larvae fed on *Z. mays* pollen had high mean wing length. Adult *An. arabiensis* emerged from larvae fed on *Z. mays* pollen were more permissive to *P. vivax* and *P. falciparum.* The highest number of *P. vivax* oocyst was observed in adults emerged from larvae fed on *Z. mays* pollen. Several studies have shown that by manipulating the amount and type of food, one could produce small, intermediate and large size mosquitoes[24]. In our study, adult wing length varied depending on the larval diet. The size of a mosquito was measured as the length of its wing. Overall larvae fed on *Z. mays* produced the largest female adult compared to the other two diets. This could be due to the nutritional values. A study has documented that having appropriate nutritional content permits relatively big size adult mosquitoes[25]. It has been already documented that the larger size *An. arabiensis* survive longer and produce more eggs than the smaller one[26].

In the present study, the larval food source substantial changes in infection rate of *An. arabiensis*. Among pollen diets, adults from *Z. mays* fed larvae were more permissive to *P. vivax* and *P. falciparum*. The least was documented among adults emerged from larvae fed on *P. juliflora* pollen. This might be justified by the nutrient reserve of adult during larval stage with high nutritional quality and adult body size, which is also linked with the larval food[7,25]. Moreover, larger mosquitoes take more blood meal which is positively correlated with the number of oocyst development in *Anopheles* species including *An. gambiae*[7,27]. The nutritional quality *Z. mays* might be superior over other diets in producing large size female adults that contributes the probability of harbouring relatively high oocyst number along with enhanced survivorship to infected mosquitoes, which would lead to the highest prevalence of infective mosquitoes. Higher risk of malaria infection in maize cultivating community has been documented in Ethiopia[3]. Therefore, supplementary malaria vector control intervention could be considered in maize growing malarious areas, especially in maize flowering seasons.

Further, the effect of larval nutrition on larval survivorship, adult fitness, oocyst density and infection suggests that intensifying cultivation of crops like *Z. mays* could supplement the larval diet and might create suitable conditions for increased vector populations and malaria transmission. The contemporary vector control is targeting adult mosquitoes. However, mosquito larvae and pupa control might be integrated with adult control tools especially during the favourable transmission season like maize cultivation still remains critical. As to this study, the plant found near the breeding habitats could contribute for high vector population and parasite transmission as the diet from these plants impacts the

vector capacity. Therefore, there is need to consider vector control options like vector-plant interactions in in controlling malaria transmission.

Conclusions

In conclusion, our results provide new insights into the effect of larval diet on mosquito larval survival, pupation, adult wing length and infectivity to *Plasmodium* parasites. Of the diets, *Z. mays* pollen diet was superior to the other diets including the standard Tetramin fish food. Larvae fed on this diet produced adults with larger wing size and longer longevity, and greatly increasing the infectivity to malaria parasites. In contrast, the larval development time was significantly longer and mortality rate was higher when the larvae fed on *P. juliflora* pollen diet. These conditions also resulted in a reduction in adult longevity and adult fitness.

The finding of this study encourages, emphasis could be given to consider interventions in villages growing *Z. mays* close to breeding habitats to monitor malaria transmission. Particularly, locally applicable intervention like larval source management could be promoted to render mosquito larval production with pollen access. It also recommended to further understanding of malaria mosquito larval diet to further intervene and suppress malaria transmission, and to plan and implement evidence based vector management in malaria endemic areas. So, further investigation should be continued on the food components of pollen plants as a source of mosquito larva diet as well as larval diet relation with parasite development in vector.

Declarations

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions

Conceptualisation and study design: S.A., T.W., F.M.; Formal analysis and interpretation of data: S.A., F.M.; Laboratory work: S.A., G.T., N.E.; Supervision: F.M., T.W., BL.; Project administration: F.M., B.L.; Validation: F.M., B.L.; Drafting and revising the manuscript: F.M., B.L. All authors reviewed the manuscript.

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Competing interests

The authors declare no competing interests

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Figures

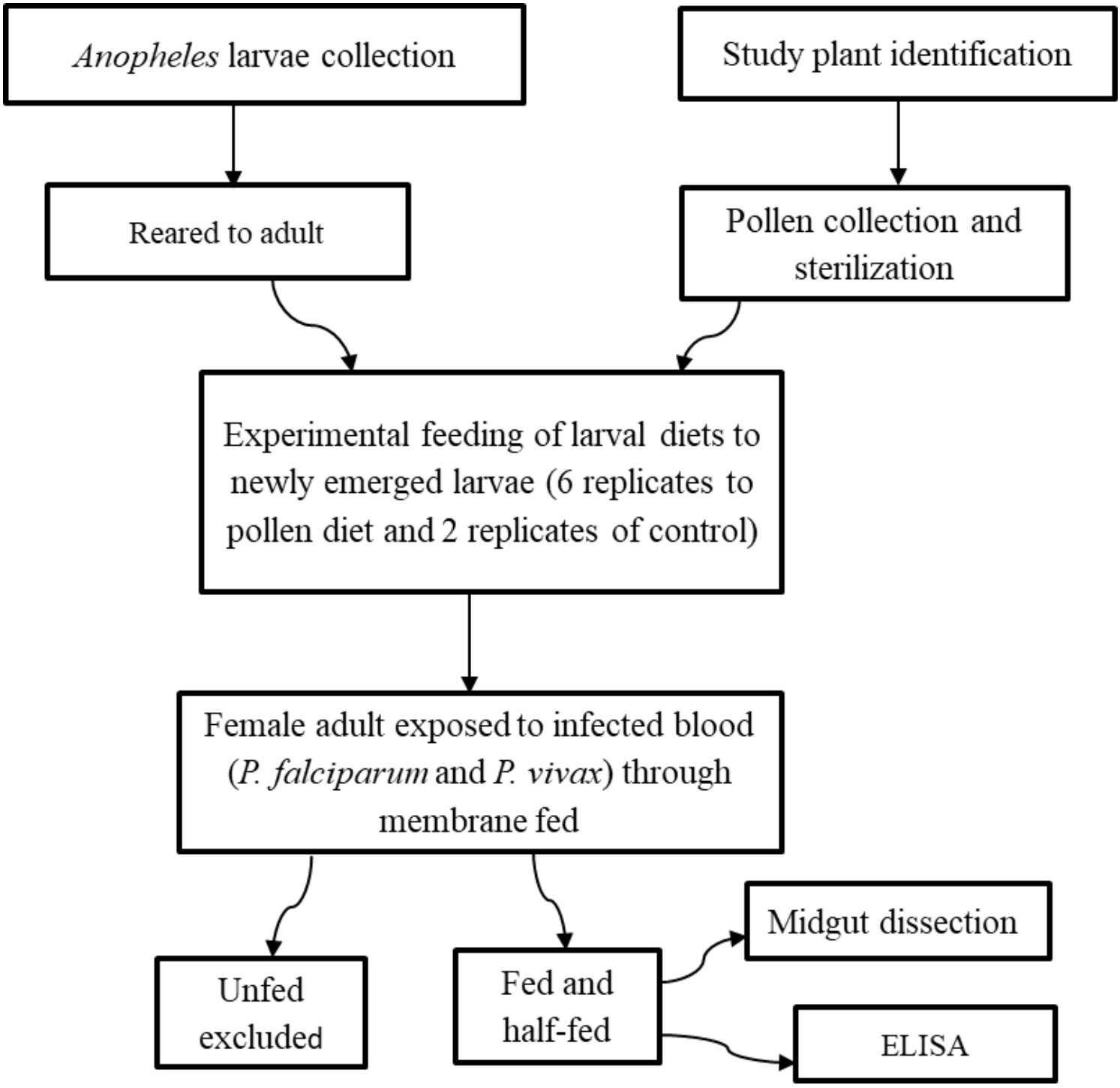


Figure 1

The study flow chart

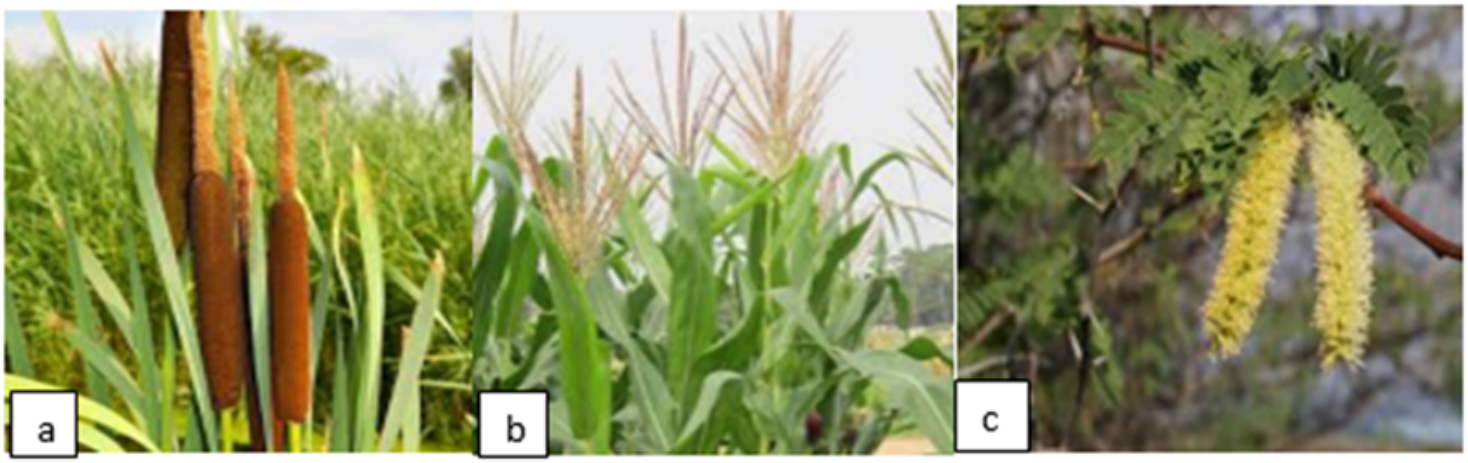


Figure 2

Study plants (a) *T. latifolia*, (b) *Z. mays*, (c) *P. juliflora*

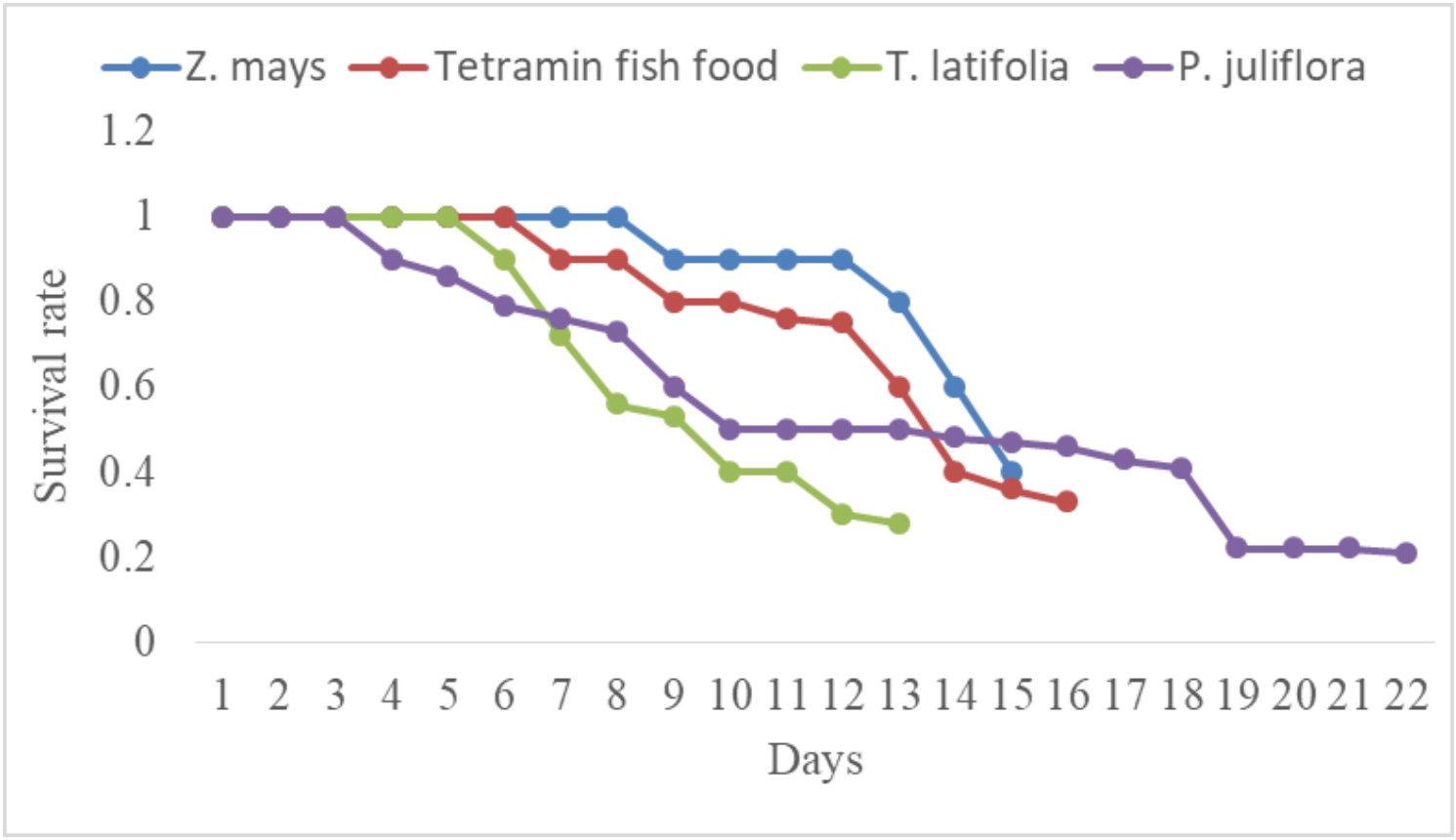


Figure 3

Anopheles arabiensis larvae survivorship and duration of larval development in larval diet of pollens of *T. latifolia*, *Z. mays* and *P. juliflora* and TetraMin fish food.

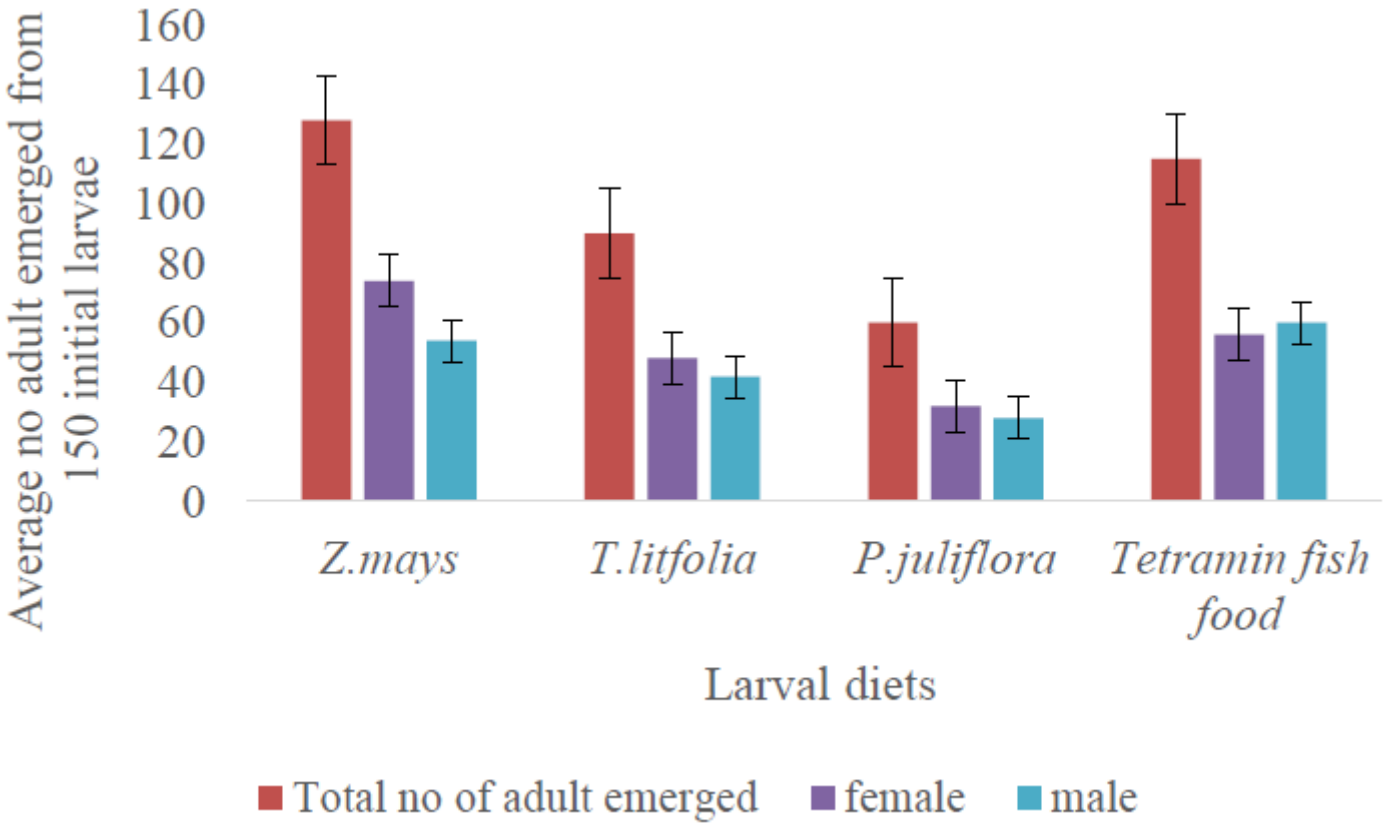


Figure 4

The number adults emerged from initial number (n = 150) of larvae in different larval diets.

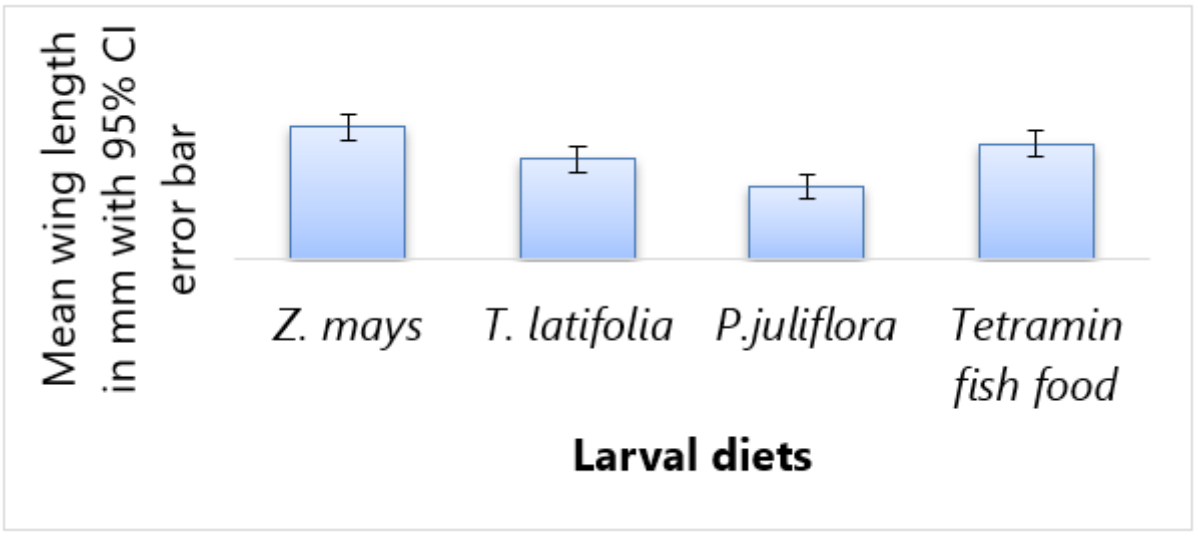


Figure 5

The effect of larval diets on wing length (wl) of female *An. arabiensis*

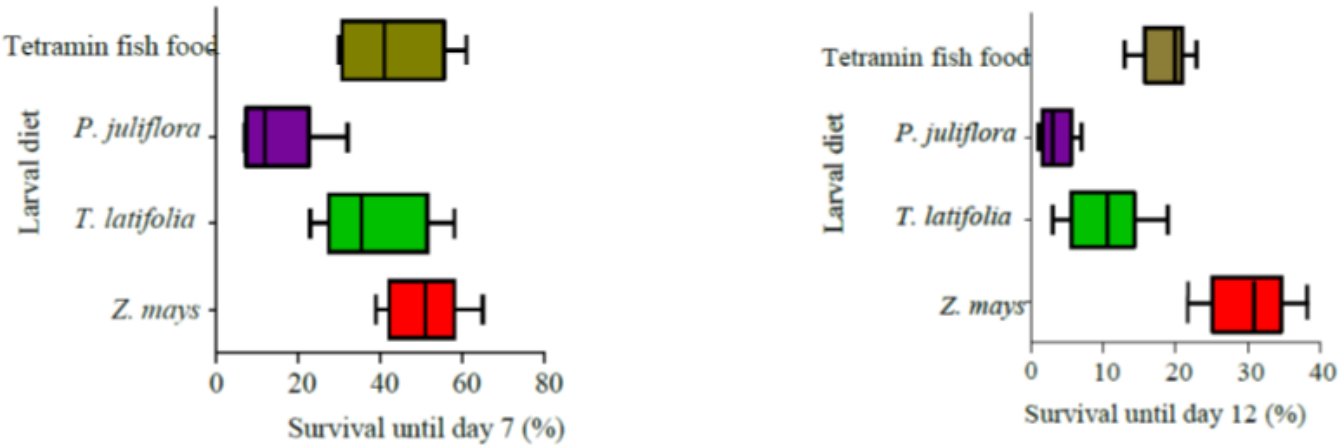


Figure 6

The mean percentage of adults from various larval diets who survived until day 7 after feeding on blood that was positive for the *Plasmodium*parasite until day 7 for *P. vivax* and day 12 for *P. falciparum* midgut dissection for oocyst detection (boxes show the 95% confedance interval).

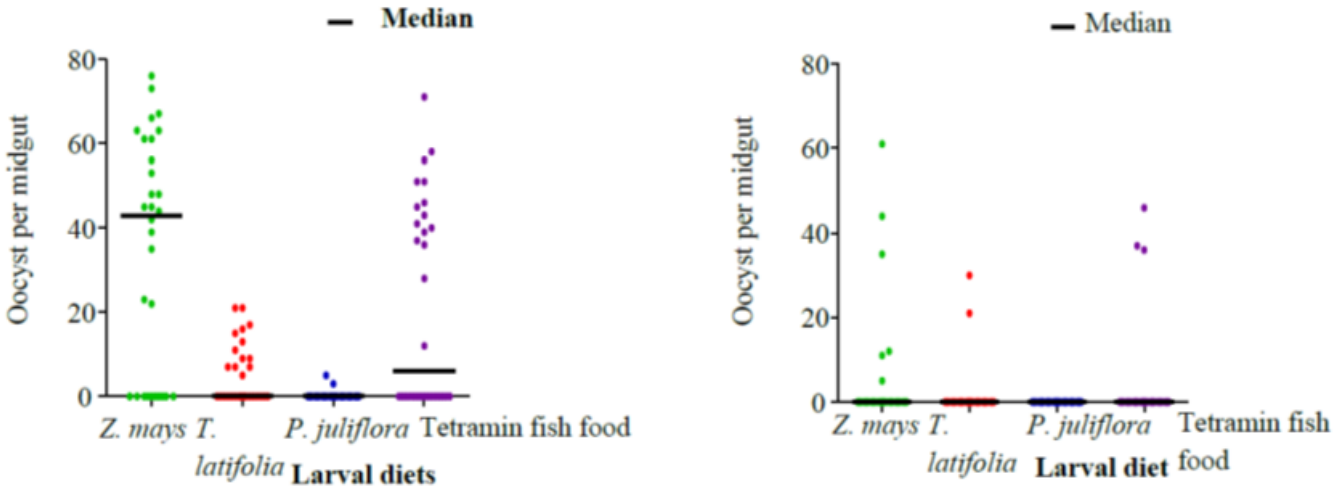


Figure 7

Median number of oocyst of *P. vivax* (left) and *P. falciparum*(right) in the midgut of female *Anopheles*mosquitoes emerged from different larval diets.