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Biotic and Abiotic Factors Influencing *Microsporidia MB* Infection in *Anopheles coluzzii*, Malaria Vector in Burkina Faso

Etienne Bilgo[1,2*#], Issiaka Saré[1,2,3*], Issac Oumar Mahamat[1,2,4], Gladys Sanon[1,2,4], Abel Millogo[1,2,4], Maurice Konkobo[1,2], Abdoulaye Diabaté[1,2#]

[1]Institut de Recherche en Sciences de la Santé (IRSS)-Direction Régionale de l'Ouest (DRO), Centre National de la Recherche Scientifique et Technologique, Bobo Dioulasso, Burkina Faso
[2]Centre Muraz, Institut National de Santé Publique, Bobo Dioulasso, Burkina Faso
[3]School of Life and Earth Sciences, Université Joseph KI ZERBO, Ouagadougou, Burkina Faso
[4]School of Life and Earth Sciences, Université Nazi BONI, Bobo-Dioulasso, Burkina Faso
Email: [*]bilgo02@yahoo.fr, [#]npiediab@gmail.com

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Abstract

Introduction: A recent study in Kenya highlighted a promising advance in malaria control by demonstrating that infecting mosquitoes with the endosymbiont *Microsporidia MB* blocks *Plasmodium* transmission. However, the influence of biotic and abiotic factors such as diet, relative humidity (RH) and temperature on this infection remains poorly studied. This study, aimed to gain a better understanding of this relationship. **Methods:** To highlight the influence of diet quantity, we defined a range of 3 quantities: 0.00375 g, 0.015 g and 0.09 g. Each quantity was tested on two groups of larvae: a group of 150 larvae infected with *Microsporidia MB* (MB^+), and a group of 150 larvae not infected with *Microsporidia MB* (MB^-) (control group), each divided into three replicates of 50 larvae. Each replicate was fed each morning with the assigned quantity until the pupal stage. In addition to this factor, we investigated the influence of temperature and RH. We defined three temperature-RH combinations: 21˚C-80% RH, 39˚C-50% RH, and 27˚C-75% RH. Each combination was tested on two groups of larvae: a group of 150 MB^+ larvae and a group of 150 MB^- larvae, each divided into three replicates of 50 larvae. Each replicate was subjected to the assigned combination until pupation. Pupae that had reached the adult stage were tested by PCR to determine their *Microsporidia MB* infection status for each factor studied. **Results:** The results showed that only the lowest quantity (0.00375 g) significantly reduced the prevalence of *Microsporidia MB* compared with the medium quantity (chi-2 test, χ^2 =

[*]Equal Contribution as co-first authors.
[#]Corresponding authors.

4.9088, df = 1, p = 0.02672) and the high quantity (chi-2 test, χ^2 = 4.7958, df = 1, p = 0.02853). As for temperature and RH, the combination 39˚C-50% RH led to a significant reduction in the prevalence of *Microsporidia MB* compared with the combination 27˚C-75% RH (chi-2 test, χ^2 = 6.3736, ddl = 1, p = 0.01158) and that 21˚C-80% RH (chi-2 test, χ^2 = 9.983, ddl = 1, p = 0.00158). **Conclusion:** This work contributes to a better understanding of some key factors linked to *Microsporidia MB* infection in mosquitoes. However, further research on several generations is necessary to draw more comprehensive conclusions.

Keywords

Diet, Relative Humidity, Temperature, *Anopheles coluzzii*, *Microsporidia MB*, Malaria

1. Background

Burkina Faso is a country with high malaria prevalence, particularly during the rainy season when the number of cases significantly increases. Malaria transmission is primarily carried out by Anopheles mosquitoes, leading to high morbidity and mortality rates, with children and pregnant women being the most affected. To combat malaria, Burkina Faso has implemented several measures, including the widespread distribution and use of insecticide-treated nets (ITNs), indoor residual spraying (IRS), and health education and community engagement programs. These efforts have contributed to reducing the malaria burden; however, challenges such as drug resistance and limited healthcare resources continue to pose significant obstacles.

Malaria, a parasitic disease caused by the hematophagous protozoan genus *Plasmodium*, is transmitted to humans through the bite of female *Anopheles* mosquitoes [1]. It remains a significant global public health challenge. Indeed, according to the World Health Organization (WHO) [2], there were an estimated 247 million cases and 619,000 deaths worldwide due to malaria. Around 95% of these cases and 96% of deaths occur in sub-Saharan Africa, with children under five years of age accounting for 80% of fatalities [2]. In Burkina Faso, malaria is the leading cause of consultations, hospitalizations, and mortality in healthcare facilities [3]. In response to this public health crisis, the Burkinabe authorities have prioritized malaria control through enhanced case management and effective vector control strategies. These include the large-scale distribution of long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS), both of which are recommended by the WHO (WHO, 2022). These initiatives have significantly contributed to a decline in malaria incidence from 600 cases per 1000 inhabitants in 2000 to fewer than 400 cases in 2015 [4]. However, the incidence has since risen to 569 cases per 1000 inhabitants in 2021, equating to 12,231,086 cases and 4355 deaths [3]. This resurgence can be partly attributed to the development of

resistance among malaria vectors due to the excessive use of insecticides [5]. Thus, there is an urgent need for innovative vector control methods to advance malaria prevention efforts.

One promising approach involves biological control using a symbiotic fungi. A recent study in Kenya identified an endosymbiotic fungus, *Microsporidia MB*, isolated from *An. arabiensis*, which inhibits *Plasmodium* development and is transmissible from parent mosquitoes to their offspring [6]. Encouraged by these findings, researchers have investigated *Microsporidia MB* in other malaria vectors in West Africa, revealing that *An. gambiae* and *An. coluzzii*, two additional significant malaria vectors, are also naturally infected [7].

Despite these advancements, to our knowledge, no studies have examined how biotic and abiotic factors such as feeding, relative humidity (RH), and temperature affect the interactions between mosquitoes and *Microsporidia MB*. Previous research has demonstrated that similar factors impact key parameters related to the infection dynamics of other endosymbionts, such as *Wolbachia*, affecting aspects like endosymbiont stability and various life history traits of mosquitoes, including larval development time, pupation rate, emergence rate, and sex ratio [8]-[12]. Fluctuations in these conditions may influence infection from larvae to adult mosquitoes. Given the potential of *Microsporidia* as a malaria control tool, understanding the influence of these factors on its interaction with mosquitoes is crucial.

Here's a brief outline of the currently known vector control methods for malaria prevention:

1) Insecticide-Treated Nets (ITNs):

Use of bed nets treated with insecticides to provide protection, especially during nighttime, significantly reducing mosquito bites.

2) Indoor Residual Spraying (IRS):

Spraying long-lasting insecticides on the walls and ceilings of homes to kill mosquitoes that come into contact with treated surfaces.

3) Larval Source Management (LSM):

Controlling mosquito larvae by modifying habitats and using larvicides to target breeding sites.

4) Use of Repellents:

Applying chemical repellents on skin or clothing to provide personal protection from mosquito bites.

These methods are often used in combination to reduce human-vector contact and control mosquito populations, effectively preventing malaria transmission.

2. Methods

2.1. Sampling Site

The strain of *An. coluzzii* mosquitoes were collected at Soumousso (4˚02'45"W; 11˚00'46"N) using a mouth aspirators (see **Figure 1**). Soumousso is a village situated approximately 55 km southeast of Bobo-Dioulasso. Soumousso has a Sudanian climate and is characterized by Guinean savannah vegetation, with an

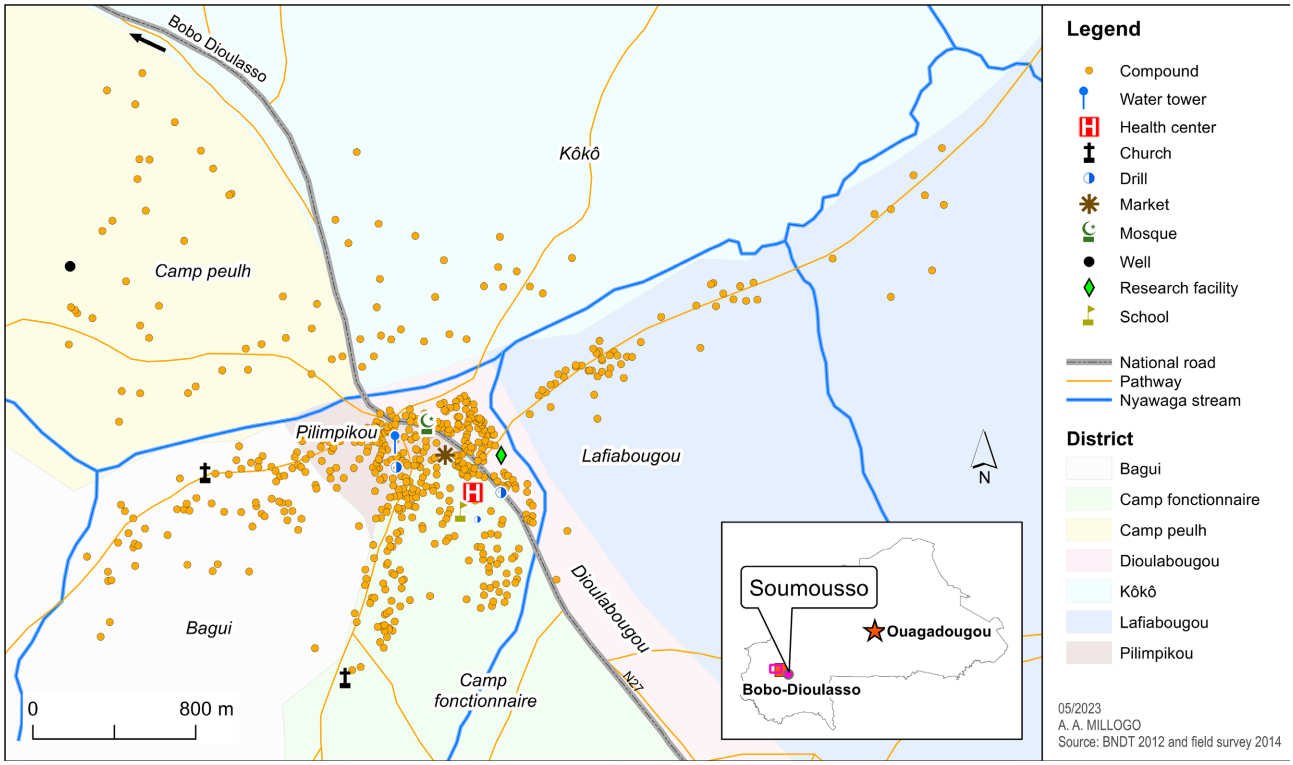

Figure 1. Map of Soumousso showing the locations of study sites.

average rainy season occurring from May to October and annual rainfall ranging between 1000 and 1200 mm. The village is bordered by a semi-permanent stream [13] [14].

Breeding habitats for mosquitoes include swamps, rainwater puddles, and semi-permanent quarries, which are conducive to the development of *Anopheles* mosquitoes. The following five species of malaria vectors are present in the area: *An. coluzzii*, *An. gambiae* s.s., *An. funestus*, *An. nili*, and *An. Arabiensis* [15] [16].

2.2. Confirmation of *Microsporidia MB* Infection Status in *An. coluzzii*

Prior to laboratory analyses, we confirmed the presence of *Microsporidia MB* in mosquitoes using Polymerase Chain Reaction (PCR). This allowed us to distinguish between MB^+ (infected) and MB^- (uninfected) mosquitoes.

After insemination, female mosquitoes were individually oviposited in plastic cups containing approximately 35 mL of tap water. Each female that had laid eggs was stored at −20˚C in 1.5 mL Eppendorf tube.

DNA extraction was performed according to the protocol established by Myriam and Cécile (2003) [17].

For the PCR, specific primers (MB_F: 5' ATA GTA TAC TCG CAA GAG TG 3' and MB_R: 5' CTG TTA TAG CCT CTT TC 3') [6], were used to amplify the 18S rRNA gene region. The reaction mixture for each sample consisted of 15 µL, including 7.5 µL of ultrapure water, 3 µL of master mix, 0.75 µL of each primer,

and 3 µL of purified DNA. The PCR cycling conditions included an initial denaturation at 95˚C for 15 minutes, followed by 35 cycles of denaturation at 95˚C for 1 minute, annealing at 60˚C for 1 minute and 30 seconds, extension at 72˚C for 1 minute, and a final extension at 72˚C for 5 minutes.

The resulting PCR products were analyzed using 2% agarose gel electrophoresis with ethidium bromide staining, migrated for 1 hour, and visualized under UV light at 254 nm. The expected band size was 250 bp (see **Appendix 1**). After visualization, eggs from MB^+ parents were separated from MB^- eggs, and the eggs were hatched separately to obtain stage 2 (L2) larvae.

2.3. Mosquito Rearing

In the insectarium, adult mosquitoes were housed in cages measuring 30 cm × 30 cm × 30 cm, with a density of 200 mosquitoes per cage. They were maintained at a temperature of 27˚C and a relative humidity of 75%, with a photoperiod synchronized with sunset. Mosquitoes were provided with a 5% glucose solution for sustenance, and females were fed rabbit blood for their blood meals. After feeding, females continued to be kept under standard insectarium conditions.

Eggs were collected by placing water-soaked blotting paper in Petri dishes, where females deposited their eggs. The eggs were dried for 24 hours, washed with 0.2% bleach, and filtered before hatching in a plastic tray containing 1 liter of dechlorinated tap water. The water was allowed to stand for at least 24 hours to let chlorine dissipate. After hatching, larvae were maintained under similar conditions as the eggs, with a density of approximately 200 larvae per tray and fed daily with cat food at a rate of 0.06 g/L. Every two days, the water in the larval tanks was replaced to minimize bacterial growth until pupation. Larvae and egg trays were organized on shelves. Pupae were collected with a Pasteur pipette and placed in beakers containing about 70 mL of tap water before being transferred to rearing cages for emergence, thus completing the cycle.

2.4. Evaluation of the Impact of Larval Diet on Life History Traits of *Microsporidia MB*-Infected *Anopheles coluzzii*

To assess the effects of larval diet on the life-history traits of *Microsporidia MB*-infected *An. coluzzii*, we used Tetramin brand fish food at three different concentrations: 0.18 g/L, 0.03 g/L (the average quantity used in our laboratory), and 0.0075 g/L (representing low and high quantities based on Sadia *et al.* [18]. The food quantities were measured using an Explorer Pro (OHAUS®) precision balance.

Each food quantity was tested on two groups of larvae: 150 MB^+ larvae divided into three replicates of 50 larvae each, and 150 MB^- larvae serving as a control group, also divided similarly. Each replicate was fed daily with the assigned food quantity until reaching the pupal stage. After pupation, live pupae were transferred to rearing cages for emergence. The following parameters were measured: larval development time, pupation rate, emergence rate, and sex ratio.

2.5. Evaluation of the Impact of Temperature and Relative Humidity on Life-History Traits

To evaluate the effects of temperature and relative humidity on the life-history traits of *Microsporidia MB*-infected *An. coluzzii*, we established three temperature levels (21˚C, 27˚C, and 39˚C) and three relative humidity conditions (50% RH, 75% RH, and 80% RH). These parameters were selected to represent both extreme and average environmental conditions recorded in the Hauts-Bassins region [19].

Three temperature-humidity combinations were tested: 21˚C - 80% RH, 39˚C - 50% RH, and 27˚C - 75% RH. Each combination was applied to groups of 150 MB^+ larvae and 150 MB^- larvae, each divided into three replicates of 50 larvae. Each replicate was placed in a Sanyo Versatile climatic chamber configured to maintain the designated conditions until pupation. Upon completion of pupation, pupae were transferred to rearing cages for emergence. The same four parameters as previously mentioned were measured.

2.6. Impact of Diet, Temperature, and Relative Humidity on Prevalence of *Microsporidia MB* Infection in *Anopheles coluzzii*

To determine the prevalence of *Microsporidia MB* in adult mosquitoes from the previous phases, we confirmed infection status using conventional PCR. For each factor studied, DNA was extracted following 2% CTAB method. The amplification products were analyzed via 2% agarose gel electrophoresis, to determine the prevalence of *Microsporidia MB* in adult mosquitoes.

2.7. Data Analysis

Data were analyzed using R software (version 4.1.2). We used the Kruskal-Wallis test to compare the larval development times of MB^+ mosquitoes versus their MB^- congeners for each factor studied. Graphics were generated using ggplot 2. The Chi-2 test was used to compare pupation rates, emergence rates, sex ratios and prevalence of *Microsporidia MB* in adults at emergence, for each factor studied. All tests were performed at a significance level of 5% ($p < 0.05$).

The various parameters measured were calculated as follows:

- Duration of larval development (DLD) was calculated by subtracting larvae stage 2 date from the date on which larvae reached pupation:
  DLD = date of stage 2 larvae − date of pupation
- The pupation rate (PR) was calculated by using the following formula:

$$\mathrm{PR}(\%) = \frac{\text{Total number othe pupae}}{\text{Initial number of larvae}} \times 100;$$

- The emergence rate (ER) was calculated by using the following formula:

$$\mathrm{TE}(\%) = \frac{\text{Total number of adult mosquitoes emerged}}{\text{Initial number of pupae}} \times 100;$$

- The sex ratio was calculated by using the following formula:

$$\text{Sex-ratio}(\%) = \frac{\text{Total number of male mosquitoes}}{\text{Total number of adult mosquitoes}} \times 100;$$

The prevalence of *Microsporidia MB* in mosquitoes was determined using the following formula:

$$\text{Prevalence}(\%) = \frac{\text{Number of postive mosquitoe specimens to } MB}{\text{Total number of mosquitoes testde by PCR}} \times 100.$$

2.8. Ethical Considerations

The detailed protocol was submitted to the Comité d'Ethique Institutionnel (CEI) of the Institut de Recherche en Sciences de la Santé (IRSS/DRO) in Bobo-Dioulasso, Burkina Faso, for authorization to conduct the study.

3. Results

3.1. Influence of Diet on Larval Development Time in MB-Positive Versus *MB*-Negative Mosquitoes

The impact of diet on larval development time was assessed by counting the number of pupae daily during pupation. For the low food quantity, the average development time was 9.11 days for *MB*-positive (MB^+) larvae and 8.79 days for *MB*-negative (MB^-) larvae. For the medium quantity, development times were 4.56 days for MB^+ larvae and 4.74 days for MB^- larvae. For the high quantity, MB^+ larvae averaged 2.98 days, while MB^- larvae averaged 3.65 days (Figure 2). Statistical analysis indicated significant differences in development times across food quantities (Kruskal-Wallis test, $\chi^2 = 21.897$; df = 2; $p < 0.0001$). However, no significant difference was found between MB^+ and MB^- larvae (Kruskal-Wallis test, $\chi^2 = 17.807$; df = 1; $p = 0.9850$).

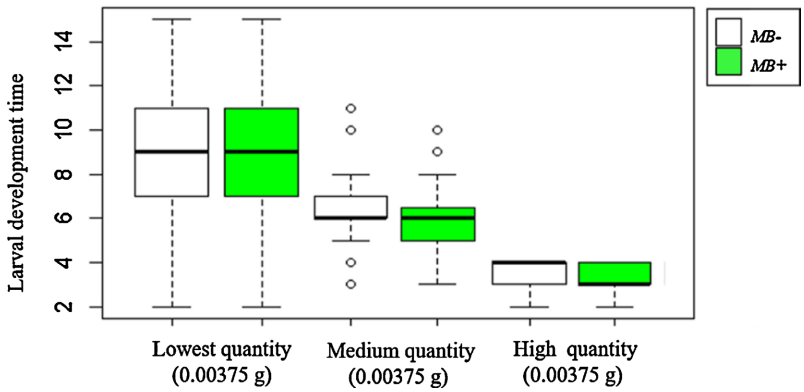

Figure 2. Larval development time of *MB*-positive versus *MB*-negative mosquitoes per food quantity.

3.2. Influence of Diet on Pupation Rate of *MB*-Positive Versus *MB*-Negative Mosquitoes

A total of 841 pupae were collected, with 420 from MB^- larvae and 421 from MB^+ larvae across the three food quantities tested. At the low quantity, 128 MB^+ pupae

(85.33%) and 125 MB^- pupae (83.33%) were recorded. For the medium quantity, 148 MB^+ pupae (98.67%) and 147 MB^- pupae (98%) were collected. At the high quantity, 145 MB^+ pupae (96.67%) and 148 MB^- pupae (98.67%) were obtained (Figure 3). Statistical tests revealed no significant difference in the numbers of MB^+ and MB^- pupae across food quantities (Kruskal-Wallis test, $\chi^2 = 21.897$; df = 1; $p > 0.05$).

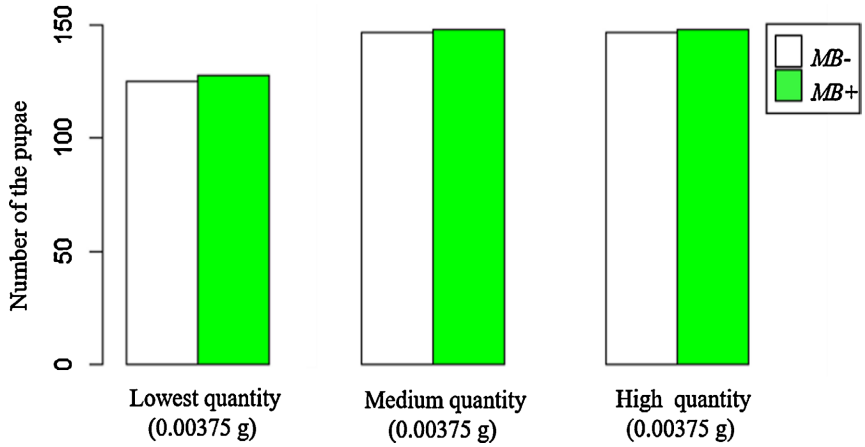

Figure 3. Influence of food quantity on pupation rate of *MB*-positive versus *MB*-negative mosquitoes.

3.3. Influence of Diet on Emergence Rate of *MB*-Positive Versus *MB*-Negative Mosquitoes

In total, 714 adult mosquitoes were collected, with 336 from MB^+ larvae and 378 from MB^- larvae. At the low quantity, 71 MB^+ adults (55.47%) and 100 MB^- adults (80%) were recorded. For the medium quantity, 133 MB^+ adults (89.86%) and 135 MB^- adults (91.84%) emerged. At the high quantity, 132 MB^+ adults (91.03%) and 143 MB^- adults (96.62%) were collected (Figure 4). Statistical analyses showed a significantly lower number of MB^+ adults compared to MB^- adults at the low quantity (chi-squared test, $\chi^2 = 16.271$; df = 1; $p < 0.0001$). For the medium and

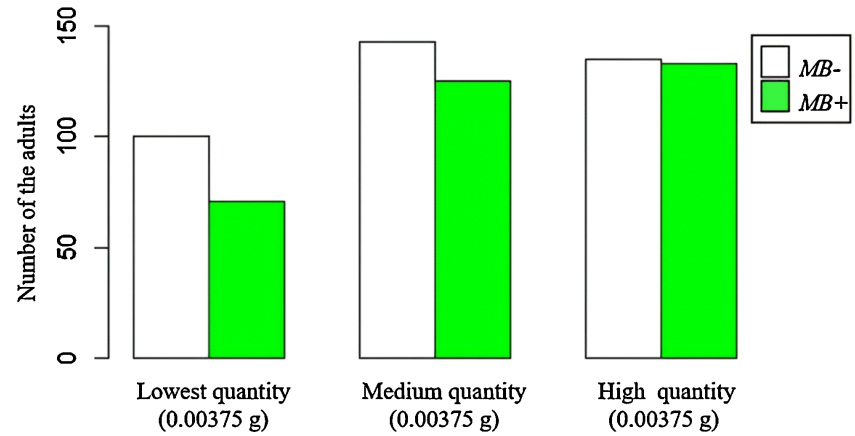

Figure 4. Influence of food quantity on emergence rate of *MB*-positive versus *MB*-negative mosquitoes.

high quantities, emergence rates were similar between the two groups (chi-squared test, $p > 0.05$).

3.4. Influence of Diet on the Sex Ratio of *MB*-Positive Versus *MB*-Negative Mosquitoes

Overall, males outnumbered females (Figure 5). At the low food quantity, MB^+ mosquitoes had a higher percentage of females (54.84%), while MB^- mosquitoes showed a male predominance (58.67%). At the medium quantity, MB^+ mosquitoes had a male ratio of 58.21%, while MB^- mosquitoes displayed an equal sex ratio (50% for each sex). At the high quantity, males were predominant in both groups. Nevertheless, statistical analysis indicated no significant difference in sex ratios between MB^+ and MB^- mosquitoes (chi-squared test, $\chi^2 = 4.3406$; df = 5; $p = 0.5015$).

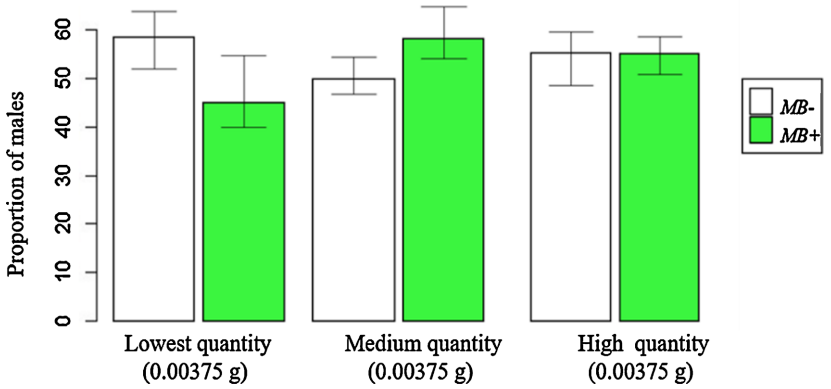

Figure 5. Distribution of sex ratio of *MB*-positive versus *MB*-negative mosquitoes by food quantity

3.5. Influence of Diet on the Prevalence of *Microsporidia MB* in Adults at Emergence

The prevalence of *Microsporidia MB* varied with food quantities. At the low quantity, 23 of 71 mosquitoes tested positive for *Microsporidia MB*, resulting in a prevalence of 32.39%. At the medium quantity, 66 out of 133 mosquitoes were positive (49.62%). For the high quantity, 56 of 125 mosquitoes tested positive, yielding a prevalence of 44.8% (Figure 6). Statistical analysis indicated that larvae fed the low-quantity diet had a significantly lower prevalence of *Microsporidia MB* compared to those fed the medium (chi-squared test, $\chi^2 = 4.9088$; df = 1; $p = 0.02672$) and high (chi-squared test, $\chi^2 = 4.7958$; df = 1; $p = 0.02853$) quantities. No significant difference was observed between medium and high quantities (chi-squared test, $\chi^2 = 4.1053$; df = 1; $p = 1$).

3.6. Influence of Temperature and Relative Humidity on Larval Development Time for *MB*-Positive Versus *MB*-Negative Mosquitoes

At 21˚C and 80% RH, the average larval development time was 5.94 days for MB^+

larvae and 6.46 days for MB^-. At 27˚C and 75% RH, it was 4.74 days for MB^+ larvae and 4.56 days for MB^-. At 39˚C and 50% RH, the average development time was 3.64 days for MB^+ larvae and 3.79 days for MB^- larvae (Figure 7). Statistical testing revealed that different temperatures and humidity levels led to varying development times (Kruskal-Wallis test, $\chi^2 = 11.7978$; df = 2; $p < 0.0001$). No significant difference in development time was found between MB^+ and MB^- larvae (chi-squared test, $\chi^2 = 21.1864$; df = 1; $p = 0.8530$).

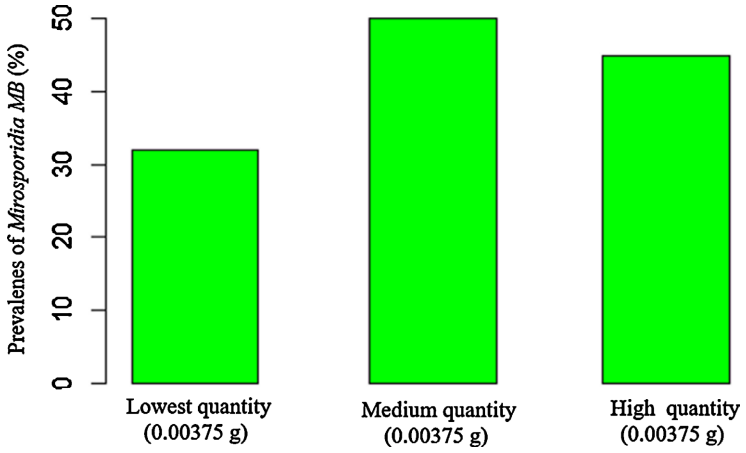

Figure 6. Prevalence of *Microsporidia MB* in adults by feed quantity

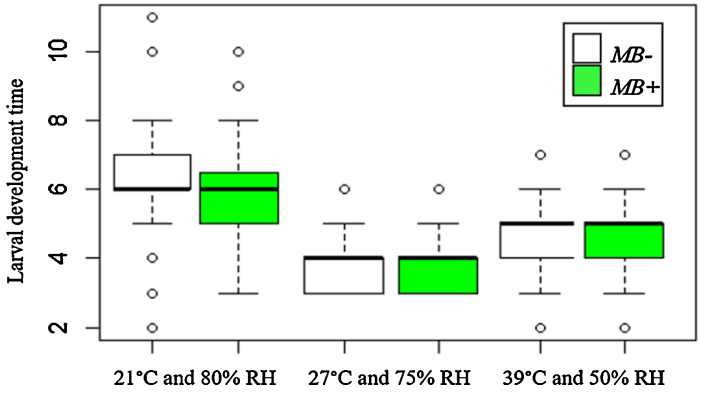

Figure 7. Influence of temperature and relative humidity on larval development time of *MB*-positive versus *MB*-negative mosquitoes.

3.7. Influence of Temperature and Relative Humidity on Pupation Rate of *MB*-Positive Versus MB-Negative Mosquitoes

A total of 827 pupae were collected, with 422 from MB^- larvae and 405 from MB^+ larvae across the three temperature-humidity combinations. At 21˚C and 80% RH, 143 MB^+ and 142 MB^- pupae were recorded, resulting in pupation rates of 95.33% and 94.67%, respectively. At 27˚C and 75% RH, 148 MB^+ pupae (98.67%) and 147 MB^- pupae (98%) were collected. At 39˚C and 50% RH, 114 MB^+ pupae (76%) and 133 MB^- pupae (88.67%) were recorded (Figure 8). Statistical analyses revealed a significantly lower number of MB^+ pupae compared to MB^- pupae at 39˚C and 50% RH (chi-squared test, $\chi^2 = 7.4249$; df = 1; $p = 0.006433$). No

significant differences were detected between the two groups at other temperature-humidity combinations (chi-squared test, $\chi^2 = 23.8673$; df = 1; $p > 0.05$).

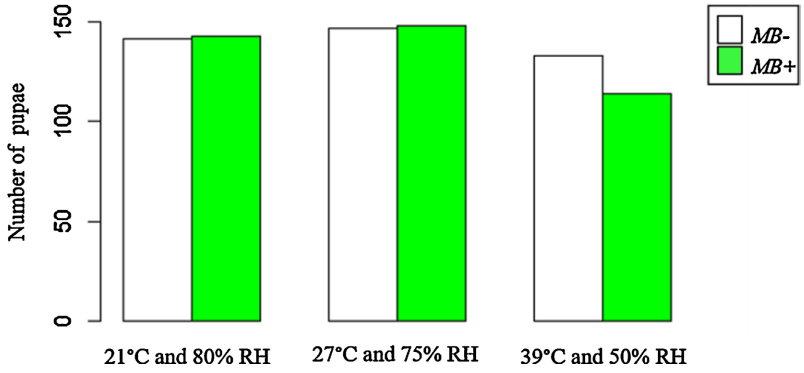

Figure 8. Influence of temperature and relative humidity on pupation rate.

3.8. Influence of Temperature and Relative Humidity on the Emergence Rate of *MB*-Positive Versus *MB*-Negative Mosquitoes

A total of 579 adult mosquitoes were collected, comprising 313 from MB^- larvae and 266 from MB^+ larvae. At 21˚C and 80% RH, 112 MB^+ and 141 MB^- adults emerged, with emergence rates of 72.32% and 99.29%, respectively. At 27˚C and 75% RH, 133 MB^+ adults (89.86%) and 135 MB^- adults (91.84%) were recorded. At 39˚C and 50% RH, only 19 MB^+ adults (16.67%) and 40 MB^- adults (30.07%) emerged (Figure 9). Statistical analysis showed that at 21˚C and 80% RH, the number of MB^+ adults was significantly lower than that of MB^- adults (chi-squared test, $\chi^2 = 27.396$; df = 1; $p < 0.0001$). This was also the case at 39˚C and 50% RH (chi-squared test, $\chi^2 = 5.3551$; df = 1; $p = 0.0207$). At 27˚C and 75% RH, however, adult numbers were similar between the two groups (chi-squared test, $\chi^2 = 11.1485$; df = 1; $p = 0.7$).

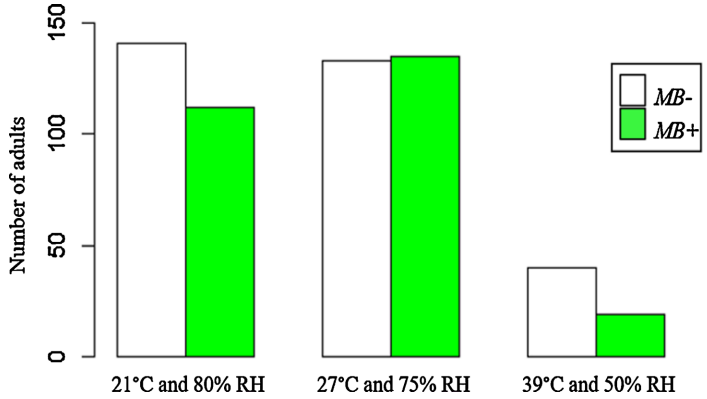

Figure 9. Influence of temperature and relative humidity on emergence rate.

3.9. Influence of Temperature and Relative Humidity on the Sex Ratio of *MB*-Positive Versus *MB*-Negative Mosquitoes

Overall, males predominated over females (Figure 10). At 21˚C and 80% RH, MB^+

mosquitoes exhibited a male ratio of 63.21%, while MB^- mosquitoes had a nearly equal sex ratio. At 27˚C and 75% RH, the male ratio for MB^+ mosquitoes was 58.21%, with MB^- mosquitoes showing equal percentages for both sexes. At 39˚C and 50% RH, the trend reversed, with a higher female percentage (61.54%) observed in MB^+ mosquitoes, while MB^- mosquitoes had a lower female percentage (38.89%). Statistical analysis indicated no significant difference in the sex ratio between MB^+ Fand MB^- mosquitoes (chi-squared test, $\chi^2 = 6.6916$; df = 5; p = 0.2446).

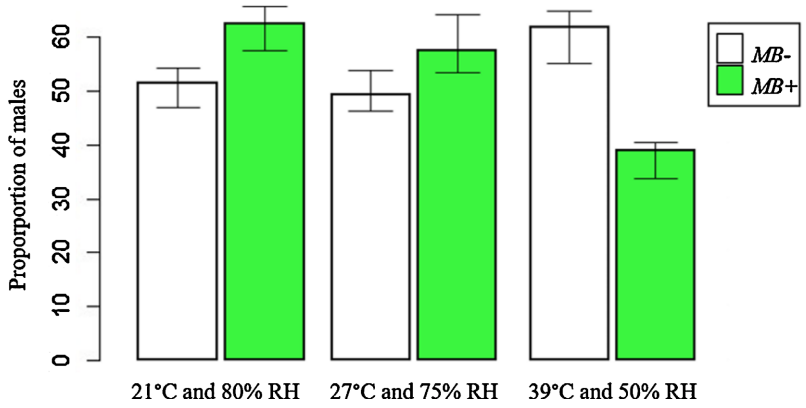

Figure 10. Distribution of sex ratio of *MB*-positive versus *MB*-negative mosquitoes according to temperature and relative humidity.

3.10. Influence of Temperature and Relative Humidity on the Prevalence of *Microsporidia MB* in Adults at Emergence

Prevalence of *Microsporidia MB* varied with temperature and humidity. At 39˚C and 50% RH, 3 of 19 tested mosquitoes were positive, yielding a prevalence of 15.79%. At 27˚C and 75% RH, 66 of 133 mosquitoes tested positive (49.62%). For larvae reared at 21˚C and 80% RH, 65 of 112 mosquitoes tested positive, resulting in a prevalence of 58.04% (Figure 11). The prevalence at 39˚C and 50% RH was significantly different from that at 27˚C and 75% RH (chi-squared test, $\chi^2 = 6.3736$; df = 1; p = 0.01158) and from that at 21˚C and 80% RH (chi-squared test, $\chi^2 = 9.983$; df = 1; p = 0.00158). No significant difference was found between

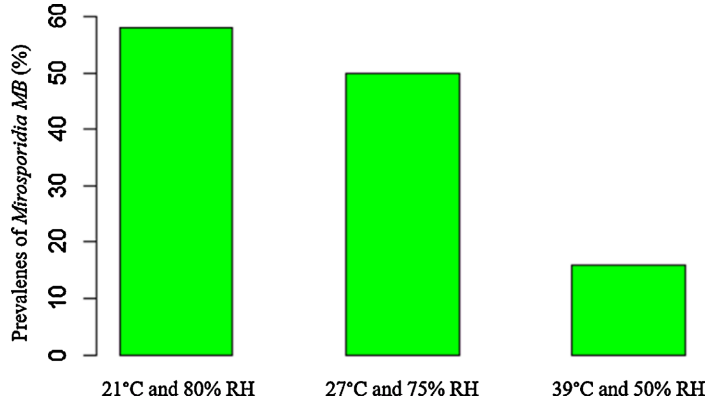

Figure 11. Prevalence of *Microsporidia MB* in adults by temperature and relative humidity.

mosquitoes reared at 27˚C and 75% RH compared to those at 21˚C and 80% RH (chi-squared test, $\chi^2 = 1.4075$; df = 1; p = 0.2355).

4. Discussion

Based on the objectives of this study, our findings indicate that varying food quantities significantly impact the duration of larval development, aligning with previous research on *Anopheles gambiae* s.l. [18]. Notably, no significant differences were observed between *MB*-positive (MB^+) and *MB*-negative (MB^-) larvae regarding food quantity effects. This implies that larval development time is independent of *Microsporidia MB* infection status in *An. coluzzii*. These results contrast with findings from *An. arabiensis* and *An. gambiae* s.s. in Kenya, where MB^+ larvae exhibited a shorter development time under standard laboratory conditions (28˚C, 70% RH) [6] [20]. The discrepancies may stem from genetic diversity among different mosquito species or *Microsporidia MB* strains.

Regarding the impact of food quantity on pupation rates, statistical analysis revealed no significant differences between MB^+ and MB^- mosquitoes, suggesting that pupation rates are also independent of *Microsporidia MB* infection status in *An. coluzzii*. These findings partially support observations in *An. gambiae* s.s. and *An. arabiensis* under similar laboratory conditions [6] [20].

In terms of emergence rates, a significantly lower emergence rate was noted for MB^+ adults at the low food quantity compared to their MB^- counterparts. This may be attributed to limited food availability, which could restrict nutrient access for both *Microsporidia MB* and the larvae. Additionally, *Microsporidia MB* infection may adversely affect mosquito metabolism or other physiological processes, rendering infected larvae more vulnerable under low food conditions. Previous studies have shown that insects infected with symbionts often experience reduced survival in resource-limited environments [21].

Regarding the sex ratio, no significant differences were detected between MB^+ and MB^- mosquitoes across food quantities, indicating that sex ratio is independent of *Microsporidia MB* infection status. Similar results have been documented in *An. gambiae* s.s. and *An. arabiensis* [6] [20].

When examining the influence of diet on the prevalence of *Microsporidia MB* in emerging adults, our results highlighted that food availability affects prevalence rates. Specifically, food scarcity was associated with lower prevalence, while abundance correlated with higher rates. We propose two hypotheses to explain this phenomenon: first, the presence of competing microorganisms may inhibit *Microsporidia MB* prevalence by restricting access to nutritional resources. Second, insufficient food resources may hinder the replenishment of essential nutrients required for *Microsporidia MB* development. Recent studies have demonstrated that the prevalence of certain microbiota in *Aedes* mosquitoes is influenced by larval food intake [22] [23].

Assessing the effects of temperature and RH on larval development time, our study found that increased temperature significantly reduced development time

in *An. coluzzii*, consistent with general observations in *Anopheles* species [24]. However, no significant differences were observed between MB^+ and MB^- mosquitoes, reinforcing the notion that larval development time is unaffected by *Microsporidia MB* infection status. This contrasts with findings by Herren *et al.* [6] and Nattoh *et al.* [20], who reported reduced development times for MB^+ larvae. These differences may again be attributed to genetic variability among mosquito species and *Microsporidia MB* strains.

Concerning the influence of temperature and RH on pupation rates, our results indicated that higher temperatures reduced the number of MB^+ larvae reaching the pupal stage compared to MB^- counterparts. This suggests that elevated temperatures may negatively impact the survival of *Microsporidia MB*-infected larvae. Kikuchi *et al.* [25] reported similar findings, noting reduced survival in bugs infected with the intestinal symbiont *Nezara viridula* when exposed to high temperatures.

In terms of emergence rates, extreme temperatures significantly lowered the emergence rate of MB^+ mosquitoes compared to MB^- mosquitoes, indicating that high temperatures may adversely affect the survival of MB^+ larvae. Adelman *et al.* [26] noted that cooler temperatures disrupt RNA interference pathways, which can influence mosquito immune responses and interactions with microbiota.

No significant differences were observed in the sex ratio between MB^+ and MB^- mosquitoes, suggesting that sex ratio is independent of *Microsporidia MB* infection status. This aligns with earlier findings in *An. arabiensis* and *An. gambiae* s.s. [6] [20].

Finally, regarding the prevalence of *Microsporidia MB* in emerging adults, our results indicated that both temperature and RH significantly influenced prevalence rates. Increased temperatures and decreased RH were associated with lower prevalence of *Microsporidia MB*. Previous studies have linked high prevalences of *Microsporidia MB* in *An. arabiensis* to high rainfall conditions, which coincide with lower temperatures and increased RH [6] [20]. This correlation supports our findings, as does research by Ross *et al.* [27], which identified a negative relationship between rising temperatures and the prevalence of the endosymbiont bacterium *Wolbachia* in its *Aedes* host.

Two hypotheses may explain our results: high temperatures could negatively impact the survival of *Microsporidia MB* in mosquito larvae, resulting in fewer adults emerging with the infection. Alternatively, elevated temperatures may affect food availability, potentially leading to starvation of *Microsporidia MB* due to limited nutrient sharing between the larvae and the symbiont. According to Mourot (2020) [28], high temperatures can influence food availability for mosquito larvae by accelerating organic matter decomposition, promoting larval proliferation, and impacting water quality. These observations further substantiate our hypotheses.

5. Conclusion

This study aimed to elucidate the factors influencing *Microsporidia MB* infection

in *Anopheles coluzzii* to inform the development of alternative or complementary malaria vector control strategies. We examined the effects of diet, temperature, and relative humidity during the larval stage on the life-history traits of *Microsporidia MB*- infected mosquitoes, as well as the prevalence of the infection in adult mosquitoes. Our findings demonstrate that variations in these abiotic factors significantly affect the pupation and emergence rates of infected mosquitoes, alongside the prevalence of *Microsporidia MB* in adults. Notably, the low food quantity (0.0075 g/L) had the most detrimental effect on these parameters. Similarly, the combination of high temperature (39˚C) and low relative humidity (50% RH) proved to be particularly adverse. This research underscores the critical biotic and abiotic influences on *Microsporidia MB* infection within one of the principal malaria vector species in tropical Africa. However, further studies are essential to explore multiple generations and additional mosquito traits such as adult survival, fecundity, fertility, and overall fitness of *Microsporidia MB*-infected mosquitoes. Supplementary research should focus on elucidating the mechanisms underlying the interactions between *Microsporidia MB*, *An. coluzzii*, and the environmental factors examined in this study. Expanding this investigation to include *An. gambiae* s.s. and *An. arabiensis*, both significant malaria vectors naturally infected with *Microsporidia MB*, could enhance our understanding and application of these findings. Additionally, exploring the effects of other environmental variables such as salinity, pH, heavy metals, turbidity, conductivity, and ion content on *Microsporidia MB* infection will provide a more comprehensive view of the ecological dynamics at play. Examining the impact of locally used antifungals and identifying natural nectar sources that may influence *Microsporidia MB* outcomes will also be valuable.

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Authors' Contributions

IS, IM, EB and AD conceived and designed the study. IS and IM performed the experiments. IS and IM drafted the manuscript. MK, GS and AM involved in molecular assays. All authors read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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