

## Research Report

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***Zea mays* pollen for the optimization of colony rearing of *Anopheles arabiensis* Patton (Diptera: Culicidae) mosquitoes under laboratory conditions**Yugi Jared Owiti<sup>1</sup>✉, Ochanda Horace<sup>2</sup> and Mukabana Wolfgang Richard<sup>2</sup>

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**Abstract** *Background & objectives:* Supplements that make food source attractive are crucial as they enhance consumption and optimize on nutrient acquisition from the said foodstuff. In this study we evaluate phagostimulatory effect of *Zea mays* pollen when given alone or together with larval diets to *Anopheles arabiensis* Patton (Diptera: Culicidae) mosquitoes under laboratory conditions. *Methods:* Crushed silver cyprinid fish, *Rastrineobola argentea* and Tetramin<sup>®</sup> baby fish food were given together with or without *Zea mays* pollen or doxycycline to *An. arabiensis* mosquito larvae and their effect as diet sources compared. The effects were determined against larval development time and fecundity, size and longevity of emerged adult female mosquitoes. *Results:* Larvae provided with maize pollen alone emerged to adults that lived for 10 days compared to 12 days for Tetramin<sup>®</sup> baby fish food and 13 day for crushed silver cyprinid fish. The differences in longevity however did not differ significantly ( $p < 0.05$ ). Maize pollen in combination with either crushed silver cyprinid fish or TetraMin<sup>®</sup> baby fish food resulted in rapid pupation. Adults emerging from maize pollen treatments though smaller in size (2.8 mm) compared to those given crushed silver cyprinid fish (3.2 mm) or Tetramin<sup>®</sup> baby fish food (3.4 mm) lived for 10 days. Maize pollen significantly influenced time to pupation ( $p < 0.01$ ), mosquito size ( $p < 0.001$ ) but not adult longevity ( $p < 0.098$ ). *Conclusion:* This study demonstrated that maize pollen enhances consumption of larval diets resulting in larger long lived female mosquitoes. Maize pollen is therefore a phagostimulant and should be harvested and incorporated in the rearing of quality mosquitoes for experimental use.

**Keywords** *Zea mays* pollen; *An. arabiensis*; Fecundity; Longevity; Phagostimulation

## Introduction

Nutrition is a very important factor in the development of all organisms. Both quality and quantity of the diet dictates on the time to maturity and quality of life of the emerging adults of the said organisms. For mosquitoes, maintaining proper nutrition throughout larval development has been demonstrated to have positive effects on mosquito life. Blackmore and Lord (2000) demonstrated that optimizing nutrition for mosquitoes resulted in healthier larvae that gave rise to larger, long lived females that were more fecund. Other studies have also indicated that diets high in protein are superior in producing larger, more fecund mosquitoes. For example, Lang (1978) demonstrated that when *Wyeomyia smithii* (Diptera: Culicidae) were provided with a high protein diet, their fecundity increased from 66 to 80 eggs per female. Similarly when *Culex quinquefasciatus* (Diptera: Culicidae) were fed a high protein diet their egg production rates were increased

from 152 to 220 eggs per female (Akoh et al., 1992). It was also demonstrated that a high protein diet fed to *Toxorhynchites splendens* (Diptera: Culicidae) reduced the development time in their immature (Amalraj et al., 2005) stages.

Many plant products are known to influence insect productivity for example silkworms by increasing food consumption or biomass, thereby enhancing cocoon yield (Maribashetty et al., 2010). The ability of a product to influence a food substance intake or its quality thereby making it attractive to the consumer is known as phagostimulation. Studies on plant essential oils and maize pollen have shown phagostimulatory and growth promoting effects on silkworm (Shahin et al., 2013) and *Anopheles arabiensis* (Ye-Ebiyo et al., 2003) respectively.

Maize pollen is rich in protein but the amount is varied depending on altitude, time or month of year. In general however, the quantity of protein in maize

pollen has been demonstrated to be between 3 to 61% (Roulston et al., 2000; Almeida-Muradian et al., 2005). In this study however we intended to determine the effect of maize pollen on the development of immatures, the size and longevity of emerging *An. arabiensis* adults in an attempt to determine its phagostimulatory effect on conventional mosquito larval rearing diets.

## 1 Results

The experiments were conducted for a period of five days. A total of 600 first instars larvae (L1s) hatching from eggs collected from adult female mosquitoes raised on one of two different diet types: crushed silver cyprinid fish, *Rastrineobola argentea* (Pellegrin, 1904) and TetraMin® baby fish food. Of the 600 L1s, 200 were hatched from eggs laid by mosquitoes raised on crushed silver cyprinid fish and 400 from eggs laid by mosquitoes raised on TetraMin® baby fish food.

### 1.1 Effect of larval diet and maize pollen on pupation and longevity

Pearson's correlation statistics found a significant correlation between the treatment given and pupation time ( $r = 309$ ,  $p < 0.01$ ), mosquito size ( $r = -187$ ,  $p < 0.001$ ) but not with adult longevity ( $r = 079$ ,  $p < 0.098$ ). However, adult longevity was significantly influenced by pupation time ( $r = -436$ ,  $p < .001$ ) and mosquito size ( $r = 217$ ,  $p < 0.001$ ). Mosquitoes emerging from maize pollen treatments lived longer than those from the other treatments irrespective of diet type (Table 2). Mosquito larval stages provided with maize pollen alone survived and emerged to adults' mosquitoes although they survived for only 9 days as opposed to 13 days for those provided with crushed silver cyprinid fish or 12 days for those given TetraMin® baby fish food only. Mosquito larval stages provided with maize pollen treatment in combination with either of the two larval diets: crushed silver cyprinid fish or TetraMin® baby fish food pupated rapidly than those provided with the other treatments in combination with either of the diet types. However, pupation time was delayed when the mosquito larval stages were reared in maize pollen alone (8 days) as opposed to crushed silver cyprinid fish (6 days) or TetraMin® baby fish food (5 days) provided alone. Mosquito larval stages provided with no food did not survive to pupate.

### 1.2 Effect of larval diet and maize pollen on emerged adult mosquito sex and size

Mosquitoes emerging from maize pollen treatment were larger than those from the other treatments irrespective of diet type (Table 3). Maize pollen was able to sustain the larval stages till emergence although the emerging adults were smaller in size (2.8 mm) as compared to those given crushed silver cyprinid fish (3.2 mm) or TetraMin® baby fish food (3.4 mm). More male mosquitoes emerged from all treatments administered in combination with TetraMin® baby fish food as opposed to those administered in combination with crushed silver cyprinid fish.

## 2 Discussions

The quality of food that insects are provided with influences the quality of the resulting adults. In the case of mosquitoes, food availability is particularly important in determining larval developmental success, rapidity of development, and the size of the resulting adults (Agudelo and Spielman, 1984).

In this study it was observed that when the larval diets were provided together with maize (*Zea mays*) pollen, the larvae of *An. arabiensis* mosquitoes, pupated earlier and the adult mosquitoes emerging from these larval stages were larger and lived longer. A similar observation was also made by Ye-Ebiyo et al., (2003) on *An. arabiensis* in habitats near a maize field in Central Ethiopia. In our study maize pollen was observed to better the quality of either of the diet types and this may have been responsible for the rapidity of development and improved adult mosquito size, factors that possibly affected the longevity and fecundity of the mosquito adult (Ameneshewa & Service, 1996).

Interestingly, *An. arabiensis* mosquito larval stages provided with maize (*Zea mays*) pollen alone survived and emerged to adults who however, died sooner than adult *An. arabiensis* raised on either TetraMin® baby fish food or crushed silver cyprinid fish alone. These findings indicate that maize pollen may not only serve as a quality improving supplement but could also be used as food for the mosquito larval stages. The fact that emerged adult mosquitoes die soon after suggest that maize pollen may not contain enough nutrients for the provision of optimal energy to sustain life.

**Table 2** Time in days, the *An. arabiensis* Patton larvae took to develop to pupae and adult mosquitoes emerging from larval stages. Means are followed by SEM

Treatment	N	Crushed silver cyprinid fish				Tetramin <sup>®</sup> baby fish food				Control			
		Adult longevity	n	Time to pupation	n	Adult longevity	n	Time to pupation	n	Adult longevity	n	Time to pupation	n
Rain water alone	50	12.80±0.78	45	5.86±0.11 <sup>a</sup>	50	12.29±0.97	42	5.39±0.07 <sup>a</sup>	46	0	0	0	0
Doxycycline	50	9.00±1.00	2	10.00±2.00 <sup>a</sup>	3	10.00±1.64	18	7.32±0.15 <sup>a</sup>	28	0	0	0	0
Pollen	50	13.63±0.71	46	5.74±0.12 <sup>a</sup>	47	14.22±0.92	45	5.34±0.08 <sup>a</sup>	47	9.48±0.56	33	7.93±0.25	3
													3
Pollen + Doxycycline	50	10.31±1.43	13	8.30±0.34 <sup>a</sup>	20	9.86±0.84	29	6.78±0.19 <sup>a</sup>	36	0	0	0	0

Notes: 1 N is the total number of first instars larvae (L1s) used.

2 n is the number of pupae and adult mosquitoes per treatment,

3 Rows having mean pupation time superscripted with the same letters indicate a significant influence of the time it took the mosquito larval stage to pupate on adult mosquito longevity.

**Table 3** Number of adult *An. arabiensis* Patton mosquitoes emerging from various treatments for the larval stages. Mean mosquito sizes are followed by SEM

Treatment	N	Crushed silver cyprinid fish			Tetramin <sup>®</sup> baby fish food			Control		
		Proportions of adult	wing size	n	Proportions of	wing size	n	Proportions of	wing size	n
		females			adult females			adult females		
Rain water alone	50	22(0.51)	3.22±0.03 <sup>a</sup>	45	19(0.58)	3.36±0.03 <sup>a</sup>	45	0	0	0
Doxycycline	50	1(0.50)	3.08±0.20 <sup>a</sup>	2	6(0.68)	3.13±0.04 <sup>a</sup>	19	0	0	0
Pollen	50	23(0.50)	3.25±0.03 <sup>a</sup>	46	15(0.67)	3.42±0.03 <sup>a</sup>	46	16(0.53)	2.86±0.03	34
Pollen + Doxycycline	50	4(0.60)	2.99±0.06 <sup>a</sup>	20	15(0.50)	3.19±0.03 <sup>a</sup>	30	0	0	0

Notes: 1 N is the total number of L1s used per treatment, the number of female adult mosquitoes emerging per treatment

2 n is the number of mosquitoes emerging per treatment (numbers of male mosquitoes are presented in parenthesis)

3 Rows having mean wing size superscripted with the same letters indicate a significant influence of the mosquito size on adult longevity.

In conclusion it is evident that maize improves the quality of larval mosquito diets and we advise that maize pollen be used in the rearing of mosquitoes in future however, the nutriment components of maize pollen need be evaluated and determined and the contribution of the components be ascertained in a laboratory setting in order to optimize on its potential.

### 3 Materials and Methods

#### 3.1 Study site

This study was carried out at the laboratories and insectaries of the School of Biological Sciences, University of Nairobi. The source, stage and conditions under which the *An. arabiensis* Patton mosquitoes were similar to one described elsewhere (Yugi et al., 2014).

#### 3.2 Mosquito culture

The mosquitoes were reared following standard techniques (Dominic et al., 2005). The adults were offered 10% sucrose solution soaked in cotton pads daily and placed on top of the cages, as a source of energy. Two days after emergence, the females however were offered animal blood collected from an abattoir and mixed with EDTA to prevent coagulation. The blood was provided via Hemotek® membrane feeding apparatus and on the second day after the blood meal, an oviposition dish was placed in the adult-holding cages for the collection of eggs. Mosquito egg collection, dispensing, larvae feeding, changing and the holding conditions for the experimental mosquitoes were done as described in details elsewhere (Yugi et al., 2014).

#### 3.3 Larval diets and doxycycline

One kilogram of silver cyprinid fish *R. argentea* was bought from a local market and ground to powder using a food blender (Silvao®). This together with Tetramin® baby fish ordered from the laboratories at Wageningen, University in Wageningen food were used as larval food. Three sachets containing thirty capsules of doxycycline were bought from the local pharmaceutical shops and used in the experiments. Doxycycline is an antibiotic and was intended for sterilization purpose only. All larval food types and doxycycline were kept at 4 °C.

#### 3.4 Maize pollen

Maize pollen was collected from *Zea mays* plants from a maize farm (-1.260027 °S, 36.732582 °E) at

the College of Agricultural and Veterinary Sciences of the University of Nairobi. The maize plants were of the hybrid variety HB 513 and were three months old at the time of pollen collection. The pollen was dusted from the tassels of the maize plants into a plastic paper bag and immediately transported for further processing at a laboratory in the School of Biological Sciences, University of Nairobi. The processed pollen was put in vials and refrigerated at temperatures of 4 °C.

#### 3.5 Experimental design and bioassays

An informal 'after-only with control' experimental design (Kothari, 2004) was used to investigate the effect of treatments on time to pupation by larvae and longevity and fecundity of emerging adults from the experimental *An. arabiensis* larvae. Each day 40 L1s that had hatched from eggs laid by mosquitoes raised on crushed silver cyprinid fish were placed separately in four sets of larval holding cups measuring 7.5 cm mouth diameter, 5.0 cm bottom diameter and 8 cm height and containing 100 milliliters of solutions of the following treatments in four sets of cups; i) the first a phagostimulant (*Zea mays* pollen) only, ii) the second a phagostimulant (*Zea mays* pollen) and an antibiotic (doxycycline), iii) the third an antibiotic (doxycycline) only and iv) the fourth clean water only (Table 1).

The larvae in these cups were subsequently given crushed silver cyprinid fish as a larval diet. Similarly 80 L1s that had hatched from eggs laid by mosquitoes raised on TetraMin® baby fish food were picked and placed in two separate sets of larval holding cups containing 100 milliliters of solutions of treatments described above. Forty of these were administered with TetraMin® baby fish food while the other 40 were given no food and served as control. Each treatment was provided in a set of ten cups and the procedure replicated five times. The assays were begun at 10.00 hours each day and the set ups left to stand on a table in the insectary.

The larval diets were provided at a rate of 0.03 mg per larvae per day. The food was fed to the larvae thrice a day; at 09.00, 14.00 and 17.00 hours. The phagostimulant (*Zea mays* pollen) and the antibiotic (Doxycycline) were provided to the larvae at a fixed rate of 0.03 mg per larval holding cup. However, this

Table 1 Types of diets in addition to maize pollen or doxycycline administered to *Anopheles arabiensis* Patton larvae.

S/N	Treatments	N	<i>Zea mays</i> pollen	Doxycycline	Replicates
1	Crushed fish	10	+	+	5
2	Crushed fish	10	+	-	5
3	Crushed fish	10	-	+	5
4	Crushed fish	10	-	-	5
5	TetraMin <sup>®</sup> fish baby food	10	+	+	5
6	TetraMin <sup>®</sup> fish baby food	10	+	-	5
7	TetraMin <sup>®</sup> fish baby food	10	-	+	5
8	TetraMin <sup>®</sup> fish baby food	10	-	-	5
9	Rain water alone	10	+	+	5
10	Rain water alone	10	+	-	5
11	Rain water alone	10	-	+	5
12	Rain water alone	10	-	-	5

**Notes:** 1 Columns with positive or negative signs indicate presence (+) or absence (-) of maize pollen or doxycycline. The arrangements are in replicates of four.

2 N indicates the number of L1s exposed in each cup

was done once and was renewed only after changing the water. On pupation, the mouth of each larval holding cup was covered with mosquito netting secured with a rubber band at the base. The time it took the individual larva to develop from L1 to pupa was noted and recorded and the sex of the emerged adult determined by observing the antennae (male mosquitoes have highly feathered (plumose) antennae while antennae in female mosquitoes are sparsely feathered (pilose)).

The emerged adult mosquitoes were fed on 10% sugar solution provided via cotton wool soaked in the solution and placed on the netting material covering the cups. The adult mosquitoes were observed on a daily basis and the time it took each individual to die noted and recorded as adult longevity. The experiment was stopped after the last of the mosquitoes died. The size of each mosquito was determined by detaching one of the wings from each experimental mosquito, mounting it on a microscope stage and its length measured under a magnification of (x 40) from the

distal end of alula to the tip excluding the fringe scales. The obtained measurement was then multiplied with a conversion factor (determined by calibration of the microscope using an ocular micrometer together with a stage graticule) to give the exact size of the mosquito.

### 3.6 Statistical analysis

The effects of larval diets on the eclosion success of pupae, the fecundity of adult female, the efficacy of diet types and the proportion of male and female *An. arabiensis* mosquitoes emerging from feeding arrangements were analyzed as a function of mosquito size (based on wing length) and all possible interaction thereof as predictors. A backward likelihood ratio stepwise logistic regression procedure and Pearson's Correlation statistics were used. All analyses were done using the Statistical Package for Social Scientists (SPSS for windows version 11.5).

### Authors' contribution

YJO did the experiments, collected, analyzed and interpreted the data, wrote and edited the manuscript. HO and WRM

provided supervisory and guidance during experimentation. All authors read and corrected the manuscript.

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#### References

- Agudelo F., and Spielman A., 1984, Paradoxical effects of simulated larviciding on production of adult mosquitoes. *American Journal Tropical Medicine and Hygiene*, 33: 1267-1269
- Akoh J.I., Aigbodion F.I., and Kumbak D., 1992, Studies on the effect of larval diet, adult body weight, size of blood-meal and age on the fecundity of *Culex quinquefasciatus* (Diptera: Culicidae). *Insect Science and Application*, 13: 177-181
- Almeida-Muradian L.B., Pamplona L.C., Coimbra S., and Barth, O.M., 2005, Chemical composition and botanical evaluation of dried bee pollen pellets. *Journal of Food Composition and Analysis*, 18(7): 105-111  
<http://dx.doi.org/10.1016/j.jfca.2003.10.008>
- Amalraj D.D., Sivagnaname N., and Das P.K., 2005, Effect of food on immature development, consumption rate, and relative growth rate of *Toxorhynchites splendens* (Diptera: Culicidae), a predator of container breeding mosquitoes. *Mem Inst Oswaldo Cruz*, 100: 893-902  
<http://dx.doi.org/10.1590/S0074-02762005000800012>
- Ameneshewa B., and Service M.W., 1996, The relationship between female body size and survival rate of the malaria vector *Anopheles arabiensis* in Ethiopia. *Medical and Veterinary Entomology*, 10: 170-172  
<http://dx.doi.org/10.1111/j.1365-2915.1996.tb00724.x>
- Blackmore M.S., and Lord C.C., 2000, The relationship between size and fecundity in *Aedes albopictus*. *Journal of Vector Ecology*, 25: 212-217
- Kothari C.R., 2004, Research design: research methodology, methods and techniques. 2<sup>nd</sup> edition. New Age International Publishers, New Delhi, India,
- Lang J.T., 1978, Relationship of fecundity to the nutritional quality of larval and adult diets of *Wyeomyia smithii*. *Mosquito News*, 38: 396-403
- Maribashetty V.G., Gayathri T.V., Chandrakala M.V., Gururaj C.S., and Shivakumar C., 2010, Utilization of botanicals for improvement in economic traits and control of pests and diseases in silkworm, *Bombyx mori*-An overview. *Indian Journal of Sericulture*, 49(1): 1-11
- Roulston T.H., Cane J.H., and Buchmann S.L., 2000, What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny? *Ecological Monographs*, 70(4): 617- 627
- Shahin R., Gadelhak G., Mahmaud S. M., El-Agamy and Idriss M., 2013, The effects of lettuce seed oil on some economical parameters of the mulberry silkworm *Bombyx mori* L. *Journal of Applied Sciences Research*, 9(4): 2702-2707
- Ye-Ebiyo Y., Pollack R.J., Kiszewski A., and Spielman A., 2003, Enhancement of development of larval *anopheles arabiensis* by proximity to flowering maize (*zea mays*) in turbid water and when crowded. *American Journal of Tropical Medicine and Hygiene*, 68(6): 748-752
- Yugi J.O., Otieno-Ayayo Z.N., Ochanda H., and Mukabana W.R., 2014, The silver cyprinid *Rastrineobola argentea* as the main diet source for rearing *Anopheles arabiensis* mosquitoes. *Journal of Mosquito Research*, 4(17): 1-6