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Zea mays (L.) Pollen as Nutriment to *Aedes aegypti* (Diptera: Culicidae) Larvae under Laboratory Conditions

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ABSTRACT

This study investigated if maize (*Zea mays* Linnaeus) pollen can serve as a nutrient source to *Aedes aegypti* Linnaeus larvae, the primary dengue mosquito vector in the Philippines. First instar larvae of *Ae. aegypti* were subjected to different treatments of maize pollen reared in mineral water or in rain water to determine their effects on the larval development into adult emergence. Results showed that the overall development time, survivorship, and mean duration of subadult stages differed ($P<0.05$; univariate ANOVA) but not on wing length among the treatments of *Ae. aegypti.* Larvae fed with maize pollen and reared in rain water took 7.36 d to emerge into adults and had 65% survivorship, whereas those reared in mineral water took 7.88 d to become adults and had 62% survivorship. Larvae in positive control took 6.05 d to become adults and had 79% survivorship. Mean duration from first to third instar larval stages differed ($P<0.05$) among treatments. Post hoc analysis using Scheffe's pairwise comparison test showed that larvae fed with maize pollen and reared in rain water did not significantly differ ($P>0.05$) from the positive control (fish food) on their development time, survivorship, and mean duration of subadult stages. These suggest that maize pollen provides nutrients for *Ae. aegypti* larvae similar to the positive control. Carbohydrates and proteins were detected in Molisch, Iodine, and Biuret tests. Results are relevant for future work in establishing the link between dengue mosquitoes and maize plantations in the Philippines.

Key words: Aedes aegypti, dengue, maize pollen, larval development

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INTRODUCTION

Dengue has spread to more than 100 countries worldwide and has become the leading cause of child mortality in many Asian and South American countries (Guha-Sapir and Schimmer, 2005). In September 2014, countries in the Western Pacific Region that were hardest hit by dengue illnesses included: Malaysia (72,604 cases; 136 deaths), Philippines (59,943 cases; 242 deaths), Fiji (26,595 cases; 16 deaths), and Vietnam (15,401 cases; 10 deaths)(WHO-WPRO, 2014). This event has focused attention on *Aedes aegypti* Linnaeus, the primary dengue mosquito vector. This mosquito is anthropophilic and usually prefers artificial breeding sites that are near human dwellings (Schoenig, 1971; Edillo *et al*., 2012). Increase in the use of nonbiodegradable containers and their storage in peridomestic locations have provided more breeding sites for *Ae. aegypti* (Nelson *et al.,* 2001). This situation is especially applicable in urban areas in third world countries where hygiene and sanitation are often neglected. In rural areas, however, breeding sites may be different. Leaf axils of banana (*Musa* sp.), taro (*Colocasia esculenta*), and bromeliads (Bromeliaceae) can serve as potential breeding sites for *Ae. aegypti* (Edillo *et al.,* 2012).

In Ethiopia, maize (*Zea maize* Linnaeus*)* pollen serves as a nutrient source for *Anopheles arabiensis* Patton, one of the primary malaria mosquito vectors (Ye-Ebiyo et al., 2000; 2003[a]; 2003[b]). The proximity of mosquito-breeding sites to sources of maize pollen (<10 m) enhanced development of larval *An. arabiensis.* Furthermore, the intensity of maize cultivation was positively and significantly associated with malaria incidence among 21 villages in Bure district, Ethiopia (Kebede *et al.*, 2005).

In the Philippines, dengue illness is one of the eight leading infectious diseases (WHO, 2010). Cebu city ranked first among cities and municipalities in Cebu province consistently from year-to-year between 1997 and 2008 (Edillo and Madarieta, 2012). Although some parts of Cebu are agricultural, the link between dengue cases and agricultural (maize) plantation has not been established. With the current situation in Cebu, we determined if maize pollen can be a nutrient source for the larval development of *Ae. aegypti* in terms of overall survivorship, the time of subadult development until adult emergence, and wing length of the emerging adults. Results should provide a very important basis in conducting further researches to determine the relationship between proximity of maize field plantations and the abundance of dengue mosquito vectors.

MATERIALS AND METHODS

Nutrient Composition of Maize Pollen

Maize pollen was collected from maize farms such as Adlaon (N 10°24'27", E 123°54'6"), Carmen (N 10°35'34", E 124°1'2"), Compostela (N 10°27'17", E 124°0'39"), Danao (N 10°31'2", E 124°1'20"), Guba (N 10°24'12", E 123°53'58"), Tagaytay (N 10°19'00", E 123°55'00"), and Talamban (N 10°22'15", E 123°55'28") in Cebu, Philippines. The pollen was analyzed for the presence of carbohydrates, lipids, and proteins. Molisch and Iodine tests were performed to detect the presence of carbohydrates and polysaccharides, respectively. Sudan IV Dye test was used to detect the presence of lipids, and Biuret test, the presence of proteins. For each test, 2 g of maize pollen were mixed in 5 mL of distilled water to create an aqueous solution of the sample.

Effects of Maize Pollen Diet on Aedes aegypti

Horizontal survivorship, the time of subadult development until adult emergence, and wing length were measured to determine the effects of maize pollen diet on *Ae. aegypti*. To assess whether the larvae fed on maize pollen, the abdomen of fourth instar larvae (L4) were examined under a compound microscope 3 h after they were fed. The observed larvae were documented using a digital camera.

Collection of Mosquito Eggs

Collected larvae and pupae of *Ae. aegypti* from their breeding sites were segregated, placed in collection bottles, and were covered with fine-mesh cloth. Once larvae metamorphosed into pupae, they were transferred into separate collection bottles. Likewise, adult male and female mosquitoes that emerged from pupae were transferred into coupling jars. Each coupling jar contained a filter paper at the bottom and 50 mL of rain water (RW). RW was obtained from a faucet of stocked RW supply in the laboratory, and was placed in pails which were set aside for a week. When eggs were laid by the female adults they were transferred into Petri dishes with filter paper, labeled as "egg stock", and stored at room temperature. The first generation of first instar larvae that hatched from eggs of the coupled adult mosquitoes was used for the randomized complete block

design (RCBD) experiments. Hatchability of eggs and fervidity of adult mosquitoes were outside of the scope of this study.

RCBD Experiments

A set of RCBD experiment was conducted with replicates as the blocking variable to determine the development of *Ae. aegypti* subadults until adult emergence. The experiment had six replicates (i.e., 30 larvae per replicate) for each of the four treatments (n=720 larvae). Larvae were fed with frozen maize pollen for a week at 0-1 °C instead of fresh ones because maize plantations described earlier were quite far from the laboratory apart from weather constraints. Mosquito eggs obtained from the "egg stock" were hatched by transferring them in Petri dishes containing RW. The newly hatched first instar larvae (L1) were randomly assigned to one of the four treatments. These treatments were: (1) 0.02 g pollen in 200 mL RW (Pr); (2) 0.02 g pollen in 200 mL mineral water (Pm); (3) 0.02 g fish food (Fwusow Industry Co., Ltd., Sha Lu Taichung, Taiwan) in 200 mL RW as positive control (+); and (4) 200 mL RW only and no food as negative control (-). To prevent scum formation, the maize pollen and the water in the larval pans were replaced every three days.

Physicochemical Properties

During the experiments, room and water temperatures, total dissolved solids (TDS), and pH of the medium of the above treatments were measured daily at 8:00 AM and 5:00 PM. TDS was measured with the use of TDS meter (HI 98300, Hanna, Germany); whereas that of pH, with portable pH meter (Orion 3-Star Plus Portable pH Meter, Thermo Electron Corporation, Massachusetts, USA).

Horizontal Life Table

The horizontal life table was determined from the larval development of *Ae. aegypti* (Edillo *et al.,* 2004; Reisen *et al.,* 1982) under different treatments described earlier. Newly hatched L1 were transferred into larval pans (17 cm x 10.5 cm x 6 cm). Larvae were checked daily at 8:00 AM and 5:00 PM at which time all larval skins were removed, scored to instar, and counted. The number of days (d) when larvae molted into the next larval stage and when pupae emerged as adults were noted. When all

adults emerged, stage-specific survivorship was estimated as Si = ni/ (ni-1), where ni = total number of immatures entering life instar i, and ni-1 = number of alive immatures in the previous instar. Mean instar duration in h at molting (Ti) was Di = Ti – (ti-1), where ti-1 was the previous mean age at molting. The percentage of total subadult life spent at each instar was Li = 100 x Di/t5, where t5 was the median time of adult emergence. Survivorship from L1 to adult emergence was estimated by A/I, where A = the total number of adults and I = the total number of L1 originally counted into the larval pans.

Wing Measurement

Once larvae developed into pupae, they were transferred into collection bottles with 30 mL of water from the same treatment and covered with fine-mesh cloth. Emerged adult mosquitoes were placed in separate bottles lined with filter paper at the bottom and suffocated with ethyl acetate. One wing of every adult *Ae. aegypti* was measured since both wings of the mosquitoes are the same (Gleiser *et al.,* 2000). The wing length was measured as the distance from the axillary incision to the apical margin excluding the fringe scales using a compound microscope with an ocular eyepiece (Nasci, 1986).

Statistical Analysis

Univariate Analysis of Variance (ANOVA) Model 1 SPSS version 18 statistical software (SPSS, 2010) was used to test the effects of the maize pollen diet on the percent survival, development time, and on the mean duration period of the different subadult stages of *Ae. aegypti*. The same statistical test was used to analyze the effects of the different treatments on the wing length of adult mosquitoes that emerged from subadults subjected to the different treatments. Scheffe's statistical test was used as a post hoc analysis for multiple pairwise comparison should there be any significant difference. The significance level was set at 95%.

RESULTS AND DISCUSSION

Nutrient Composition and Ingestion of Maize Pollen

Samples of maize pollen frozen and stored for a day and a week were

subjected to Molisch, Iodine, and Biuret tests to indicate the presence of carbohydrates, starch, and proteins, respectively. Results of the three tests detected the presence of such nutrients. Sudan IV Dye test yielded negative results suggesting that either lipids were not detected or the amount of lipids was negligible in maize pollen.

Ae. aegypti larvae were found to feed at the bottom of the larval pans where maize pollen settled. They occasionally rose up to the surface of the water to breathe. Yellowish materials in the gut of L4 were observed under the microscope within 3 h after they were fed with maize pollen, and were absent in the positive and negative controls suggesting that the larvae ingested the maize pollen. Despite differences in feeding preferences between *Ae. aegypti* and *An. arabiensis*, our findings coincide with the previous reports of Ye-Ebiyo et al. (2000; 2003[a]; 2003[b]) who determined that maize pollen serves as a nutrient source for *An. arabiensis*, one of the primary malaria mosquito vectors in Africa.

Although the viability of maize pollen is preserved by freezing at 0-1° C for a week (Anderson et al., 2004), other nutrients, enzymes, minerals, and vitamins not quantitatively measured in this study might have degraded gradually. Freeze-drying of maize pollen induced alterations in enzyme activity of esterases but not for acid phosphatases. Alterations of acid phosphatases appeared to be related to pollen viability in most cases (Anderson et al., 2004). Maize pollen contains members of all the classes of organic and inorganic nutrients, including relatively high levels of starch, primarily amylopectin (Goss, 1968; Stanley and Linskins, 1974; Roulston and Buchmann, 2000). It contains 23-27% protein, proline as one of its constituents (Goss, 1968; Roulston and Buchmann, 2000; Lundgren and Wiedenmann, 2004). It also contains adenine and choline (Goss, 1968; Stanley and Linskins, 1974). Although the lipid test conducted in the current study yielded negative results, Sudan IV Dye test might not be sensitive enough to detect the low lipid contents in maize pollen (Goss, 1968; Lundgren and Wiedenmann, 2004). Sterols comprise 0.1% of the organic constituents in maize pollen (Standifer, 1966).

Physicochemical Properties

The mean physicochemical conditions of the four larval treatments (*Ae. aegypti* larvae fed with maize pollen frozen within a week) did not exceed 30° C for room and water temperature (Table 1). On the other hand, pH 7 was steady for all treatments. TDS for all treatments did not exceed

100 mg/L indicating that the experimental water quality was within tolerable level. TDS is a measure of the combined contents of all inorganic and organic substances contained in a liquid; its operational definition is that the solids must be small enough to survive filtration through a 2 µm-sieve. It is often monitored in order to create a water quality environment favorable for organism productivity (Boyd, 1999). Measuring the physicochemical properties was needed in order to ensure that the larvae for all treatments were not negatively affected by them (Strickman and Kittayapong, 2003, Barrera *et al.*, 2006, Umar and Don-Pedro, 2008).

Table 1. Mean (± S.E.) of physichochemical conditions in which *Ae. aegypti* subadults were fed with maize pollen frozen at 0-1° C for one week together with positive and negative controls.

Physicochemical Attributes	Positive Control	Negative Control	Maize Pollen in Mineral Water	Maize Pollen in Rain Water
Room temperature (° C)	29.212 ± .028	29. 045 ± .058	29.010 ± .240	29.250 ± .075
Water temperature (° C)	29.325 ± .060	29.572 ± .045	29.282 ± .052	29.627 ± .011
Total dissolved solids (mg/L)	81.667 ± 3.499	69.667 ± 2.616	53.167 ± 2.151	71.000 ± 4.139
pH level	7.020 ± .020	7.150 ± .015	6.935 ± .018	6.975 ± .024

Survivorship of Aedes aegypti

The positive control had the highest overall survivorship rate of *Ae. aegypti* among the treatments (79%) (Figure 1). The larvae fed with maize pollen and reared in RW (Pr) had a higher overall survivorship (65%) than those fed with maize pollen in mineral water (Pm) (62%). The overall survivorship rates of *Ae. aegypti* differed among treatments ($P<0.05$; Table 2) although Scheffe's pairwise comparison test showed that the positive control and the Pm treatment were close to borderline level of significance ($P = 0.06$; Table 3).

Development time of Aedes aegypti

The development time of *Ae. aegypti* significantly differed among the treatments ($P<0.05$; Table 2). The larvae in the positive control developed fastest among the treatments (6.05 d). The larvae fed with maize pollen frozen for a week and reared in mineral water took 7.88 d to emerge into adults, whereas those fed with similar pollen but reared in RW took 7.36 d. Using Scheffe's pairwise comparison test between treatments, the

development time between the positive control and the larvae fed with maize pollen in Pm medium significantly differed (P<0.05; Table 3) but not between positive control and the larvae fed with maize pollen reared in Pr medium (P>0.05; Table 3). This suggests that the Pr treatment was more comparable to the actual breeding site of *Ae. aegypti* in nature. No adult mosquitoes emerged in the negative control and larval development was only from L1 to L4.

Table 2. Univariate Analysis of Variance (ANOVA) for *Ae. aegypti* larvae fed with maize pollen frozen for one week in terms of their development time, overall survivorship, mean duration of subadult stages, and wing length.

Horizontal Life Tables	*F*	*df*	*P*
Development time	13.973	3	0.000*
Overall survivorship	227.749	3	0.000*
Mean duration of subadult stages	15.905	12	0.000*
Wing length	0.398	2	0.679

*P < 0.05

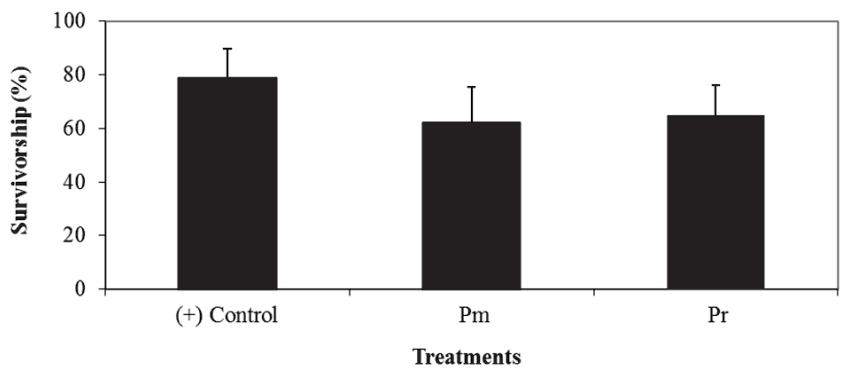

Figure 1. The overall survivorship of *Ae. aegypti* among treatments: positive (+) control, larvae fed with maize pollen and reared in mineral water (Pm), and those fed with maize pollen and reared in rain water (Pr).

Table 3. *P*-values from Scheffe's test for multiple pairwise comparisons between treatments of *Ae. aegypti* larvae fed with maize pollen frozen for one week and reared in either rain water (Pr) or mineral water (Pm). Larvae were fed with fish food for positive control (+).

Horizontal Life Tables	Treatments (*P*-values)		
	+ * Pr	+ * Pm	Pr * Pm
Development time	0.055	0.020*	0.863
Overall survivorship	0.122	0.060	0.921
Mean duration of subadult stages	0.178	0.026*	0.859

*$P < 0.05$

Mean Duration of Aedes aegypti subadults

The mean duration of subadult stages of *Ae. aegypti* among the four treatments differed significantly ($P<0.05$; Table 2; Figure 2). Results from Scheffe's pairwise comparison test showed that the positive control and larvae in the Pm treatment significantly differed ($P<0.05$; Table 3) but not the other pairs ($P>0.05$; Table 3). Mean duration from L1 to L3 instar larval stages differed ($P<0.05$; Figure 2) among the treatments. Results can be attributed to the negative control which took longer time for the larvae to develop until they began to die off by L4 stage (Figure 2).

Wing Measurement

Results showed that wing lengths of the newly emerged adult mosquitoes among the four treatments did not differ ($P>0.05$; Table 2; Figure 3). The mean wing length of adult mosquitoes (N=142) that emerged from the positive control was 83.01 ± 1.39 µm. The mean wing length of those (N=112) treated with maize pollen in mineral water was 81.09 ± 2.54 µm, and those (N=117) treated with the pollen in RW was 81.13 ± 0.82 µm.

In summary, using univariate ANOVA, results showed that the overall development time, overall survivorship, and mean duration of subadult stages differed but not on wing length among the four treatments of *Ae. aegypti* in the RCBD experiments (Table 2). Post hoc analysis using Scheffe's pairwise comparison test revealed that larvae fed with maize pollen reared in RW (Pr) did not significantly differ ($P>0.05$; Table 3) from the positive control (fish food) in terms of their development time, overall

survivorship, mean duration of subadult stages, and wing length. These suggest that maize pollen provides nutrients for *Ae. aegypti* larvae similar to the fish food. This is due to the presence of carbohydrates and proteins as shown in Molisch, Iodine, and Biuret test results. Höcherl et al. (2012) found that the amount of essential amino acids except histidine in maize pollen is greater than that of mixed pollen in the wild. Our findings are consistent with the previous works that maize pollen enhanced development of larval *An. arabiensis* in Africa (Kebede et al., 2005; Ye-Ebiyo et al., 2000; 2003[a]; 2003[b]). Moreover, Pr treatments of the larvae apparently simulated their actual breeding site conditions. However, current results are applicable only to laboratory conditions where larvae are not given diet options.

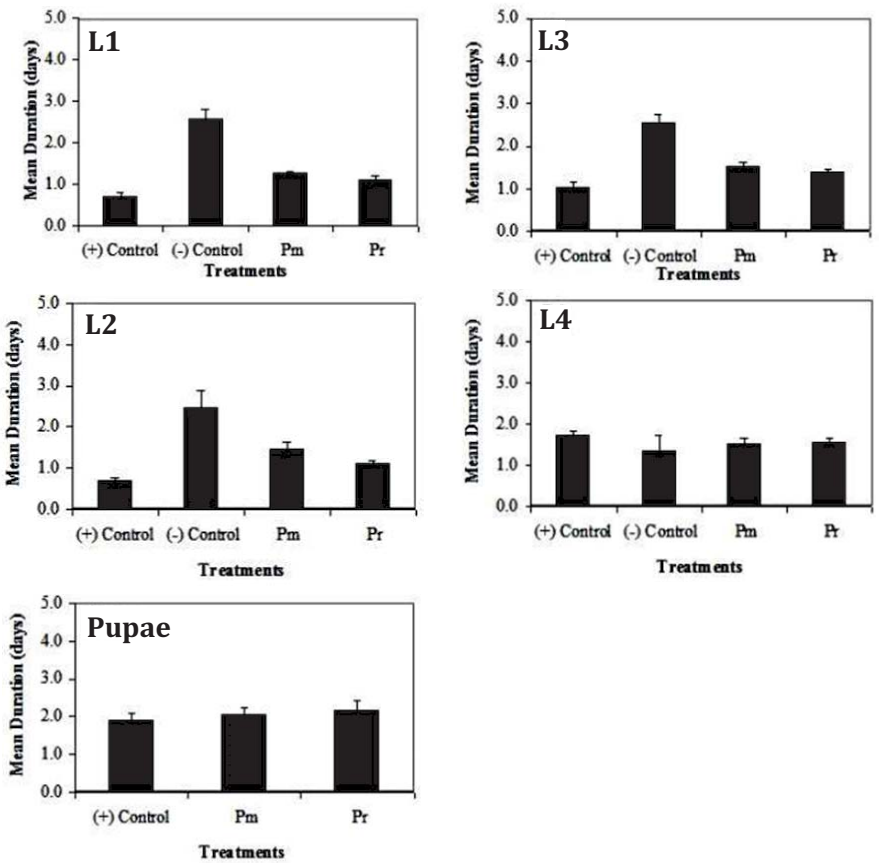

Figure 2. The mean duration (number of d) of *Ae. aegypti* among treatments: positive (+) control, larvae fed with maize pollen and reared in mineral water (Pm), and those fed with maize pollen and reared in rain water (Pr). No pupae in the negative control.

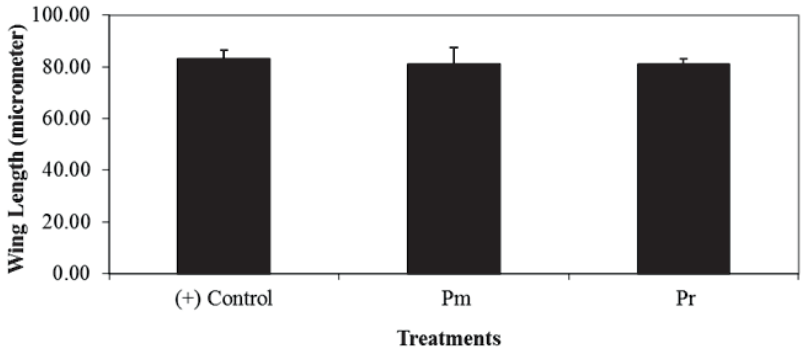

Figure 3. The mean wing length (μm) of adult *Ae. aegypti* that emerged from different treatments, namely, positive (+) control, larvae fed with maize pollen and reared in either mineral water (Pm) and rain water (Pr). No adult *Ae. aegypti* emerged in the negative control.

Larvae of the African *An. gambiae* complex develop readily in turbid water and even when crowded, provided that their breeding sites are located in proximity to flowering maize (Ye-Ebiyo et al., 2003[a]). The intensity of maize cultivation, controlled for differences in elevation between sites using a Poisson regression, was positively and significantly correlated with malaria incidence in Bure, Ethiopia (Kebede et al., 2005). In the Philippines, a correlation study between the proximity of maize plantations and the presence of dengue mosquito vectors may serve relevant for dengue control program if such correlation observed by Kebede et al. (2005) will also apply because maize pollen can be dispersed less than 60 m from its plantation (Raynor et al., 1972).

CONCLUSION AND RECOMMENDATIONS

In conclusion, maize pollen can be a nutrient source to *Ae. aegypti* larvae under laboratory conditions. We recommend an assessment on the natural breeding sites of *Ae. aegypti* larvae whether pollen is available as a nutrient source. We also recommend determining whether the larvae prefer maize pollen over other sources for nutrition (i.e., plant detritus and associated microorganisms) in their natural breeding sites. Further studies in correlating the intensity of maize cultivation with exacerbated human risk of dengue infection in the Philippines or in other tropical countries where maize plants grow may be relevant for dengue control program.

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