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Short Communication

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# Synergistic Effect of Fertilizer and Plant Material Combinations on the Development of *Aedes aegypti* (Diptera: Culicidae) and *Anopheles gambiae* (Diptera: Culicidae) Mosquitoes

**Frédéric Darriet**[1,2]

[1]Institut de Recherche pour le Développement (IRD), UMR MIVEGEC Maladies Infectieuses et Vecteurs, Ecologie, Génétique, Evolution et Contrôle, 911 Avenue Agropolis, BP 64501, 34394 Montpellier, France, and [2]Corresponding author, e-mail: frederic.darriet@ird.fr

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## Abstract

Chemical fertilizers are used everywhere, which often pollute the breeding sites of mosquitoes. In this laboratory study, the consequences on *Aedes aegypti* (L.) (Diptera: Culicidae) and *Anopheles gambiae* (Giles) (Diptera: Culicidae) of water-containing plant matter (PM) alone, or in association with an NPK type of fertilizer (PM+NPK), were evaluated. To obtain a 20% imaginal emergence of *An. gambiae* ($IEt_{20}$), the bioassays carried out with PM have evidenced that its larvae need four times as much food as for *Ae. aegypti* larvae. The PM+NPK combinations significantly improve the survival rates of both mosquitoes multiplying the percentages of imaginal emergence by 1.7–3 (synergistic effect). The log-probit analysis of the adult emergence also reveals that the environments containing fertilizers accelerates by two to four times the development of the mosquito larvae.

**Key words:** *Aedes aegypti*, *Anopheles gambiae*, plant material, fertilizer, synergistic effect

The distribution of mosquito populations depends on the presence of breeding sites with suitable physicochemical and biological characteristics (Bentley and Day 1989; Barrera and Amador 2006; Darriet and Corbel 2008a,b). *Aedes aegypti* (L.) (Diptera: Culicidae) develops in small- to middle-sized domestic and peridomestic collections of clear water (Cordellier et al. 1977). Despite a recent proliferation inside cities (Barbazan et al. 1998, Dossou-Yovo et al. 1998), *Anopheles gambiae* (Giles) (Diptera: Culicidae) remains a lot more rural with breeding sites ranging from rice paddies to smaller puddles. Dengue fever, chikungunya, and zika are arboviral diseases transmitted to humans by *Ae. aegypti* (Hayes 2009, Reiter 2010, Akiner et al. 2016). *An. gambiae* transmits the *Plasmodium* responsible for malaria. Two billions of people still live in areas significantly plagued by this disease (WHO 2011).

Both mosquitoes lay their eggs in water collections of varying nature. Still some factors related to the quality of the water will make some breeding sites more attractive than others. Deciduous and coniferous leaves, hay, and grass brewing have long been known for their attraction properties on mosquitoes (Chadee et al. 1993, Clement 2000, Darriet, 2014). The special attraction of *Ae. aegypti* gravid females to this type of breeding sites is partly determined by the presence of fertilizer in the water (Darriet and Corbel 2008a). As a matter of fact, fertilizers favor the growth of algae and bacteria that account for part of the mosquito larvae diet (Young et al. 2014). The fertilizers home gardeners and owners use the most contain nitrogen (N), phosphorus (P), and potassium (K). The water from plant watering, thus, enriched with NPK fertilizers seeps through the soil into the dishes placed under the pots. Those small yet numerous collections of domestic waters are well-known to the mosquito control services for hosting large populations of *Aedes albopictus* or *Ae. aegypti* (Delatte et al. 2008, Costa et al. 2012). Similarly, in farming areas, many mosquito species are also regularly exposed to massive concentrations of fertilizers and pesticides (Diabate et al. 2002, Sandford et al. 2005, Akogbeto et al. 2006). In rice paddies, the densities of *Anopheles* larvae were reported reaching peaks a few days after nitrogenous fertilizer was poured out at the time of transplanting (Victor and Reuben 2000, Mwangangi et al. 2006). In this laboratory study, we studied the growth rate of *Ae. aegypti* and *An. gambiae* according to whether the breeding sites contained plant matter and/or NPK fertilizer. The effects of the plant matter and fertilizer combinations were also determined in comparison with the activity of each component on its own (additive or synergistic interactions).

## Materials and Methods

### Mosquito Strains

The insecticide-susceptible strains (SS) of *Ae. aegypti* (Bora) and *An. gambiae* (Kisumu = KIS) have been reared for more than 20 years in the Institute of Research for Development (IRD) insectarium in Montpellier (France).

### Plant Matter and NPK Fertilizer

The plant material (PM) used in this study consisted of commercial rodent food (grassland hay) (Zolux, Domazan, France). This particular brand of hay had already been used in two previous research works focused on the biology of mosquitoes (Darriet et al. 2010, Darriet et al. 2012). The liquid NPK fertilizer 5-7-5 (Algoflash, Asnières sur Seine cedex, France) contained 5% of nitrogen (N) consisting in 3% of $NO_3$– and 2% of $NH_4^+$; 7% of phosphorus (P) in the form of $P_2O_5$ and 5% of potassium (K) in the form of $K_2O$.

### PM, NPK, and PM+NPK Compositions and Bioassays Procedures

The quantities of plant material and NPK fertilizer selected for the constitution of larval environment are those that separately induced a low range (0–20%) of imaginal emergence on *Ae. aegypti* and *An. gambiae*. For the plant material, the quantities tested were 1000 mg/liter for *Ae. aegypti* (PM1) and 4000 mg/liter (PM4) for *An. gambiae*. For the fertilizer, four concentrations (NPK1: 4-6-4 mg/liter, NPK2: 17-23-17 mg/liter, NPK3: 33-47-33 mg/liter, and NPK4: 66-91-66 mg/liter) were tested on both mosquitoes. The PM+NPK combinations were done using the same concentrations as the PM or NPK alone. PM, NPK, and the different PM+NPK combinations were prepared in 0.0042 $m^3$ plastic trays (length: 0.30 m; width: 0.20 m; depth: 0.07 m), each containing 1 liter of reverse osmotic water. Twenty-four hours after the preparation of the larval environments, 100 first instars larvae of *Ae. aegypti* or *An. gambiae* were counted and placed in a tray. Each artificial milieu was evaluated on a total of three replicates. Throughout the duration of the experiment, the trays were maintained at a temperature of 27 ± 2°C.

### Statistical Analyses

Every day, female and male adults were counted in each environment to establish the percentages of imaginal emergence with a 95% CI (Vollset 1993). The experiment was stopped when all larvae or pupae died or emerged in adults. Then the percentages of females and males from the different environments were compared for the purpose of statistical analysis (Wilcoxon test using Statistica 2011). The larval environments allowing more than 20% of adult emergences were analyzed using the log-probit software (Raymond et al. 1997) to determine the duration of preimaginal developments leading to 20 and 50% of imaginal emergence ($IEt_{20, 50}$). The results obtained for each combination were compared with the results theoretically expected in the absence of any interaction (additive effect). The expected mosquito emergence (Corbel et al. 2002) for each PM+NPK combination was calculated by 1 − (frequency of global mortality on PM by frequency of global mortality on NPK), the global mortality adding up the mortalities recorded among the larvae, the nymphs, and the adults that had drowned. Synergy (positive interaction) is evidenced when the observed mosquito emergence is significantly higher (chi-square test using Statistica 2011) than the expected mosquito emergence.

## Results

### *Aedes aegypti* Bioassays

In the PM1 environment, adult emergence reached 19% after 55 d of larval development (Table 1). The log-probit analysis of the imaginal emergence along time assesses the time needed for 20% of adult *Ae. aegypti* to emerge ($IEt_{20}$) at 58.8 d. For NPK1 to NPK4 concentrations, 2.7–3.3% adults emerged along the 61–66 d period.

In all the PM1+NPK combinations, the imaginal emergence occurred over a time span of 50–60 d yet with very different rates of emergence from one combination to another. The PM1+NPK1, PM1+NPK2, and PM1+NPK4 combinations considerably improved the living conditions of the mosquitoes compared with the two components separately (synergistic interaction, $P < 0.005$). PM1+NPK3

**Table 1.** Determination of imaginal emergence time 20% and 50% ($IEt_{20}$ and $IEt_{50}$) of *Aedes aegypti* and calculation of synergistic or additive effects of the PM1+NPK combinations

| Plant material and/or fertilizer quantities (mg/liter) | Maximum duration of preimaginal development (d) | $IEt_{20}$ in d (95% CI) | $IEt_{50}$ in d (95% CI) | Slope (± SE) | Adult emergence % observed (95% CI) [expected] | $P$* |
|---|---|---|---|---|---|---|
| PM1 (1000) | 55 | 58.8 (53.1–67.1) | ND | 2.0 (± 0.2) | 19.0 (14.6–23.4) | — |
| NPK1 (4-6-4) | | | | | 0 | |
| NPK2 (17-23-17) | 66 | ND | ND | ND | 3.0 (1.04–5.0) | — |
| NPK3 (33-47-33) | | | | | 3.3 (1.3–5.3) | |
| NPK4 (66-91-66) | 61 | | | | 2.7 (1.0- 4.8) | |
| PM1+NPK1 | | 20.5 (18.2–23.0) | ND | 4.5 (± 0.02) | **44.7 (39.1–50.3)**[c] [21] | <0.005 |
| PM1+NPK2 | 60 | 15.5 (13.3–17.9) | 45.1 (40.7–50.0) | 1.8 (± 0.2) | **53.7 (48.1–59.3)** [21] | |
| PM1+NPK3 | | 32.9 (29.5–36.7) | ND | 1.3 (± 0.1) | 28.7 (23.4–33.8) [22] | 0.06 |
| PM1+NPK4 | 50 | 20.4 (18.0–23.2) | | 1.3 (± 0.2) | **33.3 (28.0–38.6)** [22] | **<0.005** |

Bold font indicates that the adult emergence differed statistically from the expected value.
*$P < 0.05$ = synergistic effect of the PM and NPK combinations. $P > 0.05$ = additive effect.
ND, not detectable.

induced only an additive effect to the actions ($P = 0.06$). The $IEt_{20}$ of the four combinations tested reveal that 20% of the adult *Ae. aegypti* emerge between 15 and 33 d, two to four times faster growth of the larvae compared with PM1 (58.8 d). PM1+NPK2 combination was the most productive combination in allowing three times as many adults to emerge compared with PM1. Besides, its 45 d $IEt_{50}$ is 14 d shorter than the $IEt_{20}$ of plant matter alone.

### *Anopheles gambiae* bioassays

To get 19% of adult *An. gambiae*, its larvae had to be provided with four times as much plant matter (PM4) as what *Ae. aegypti* required to reach the same percentage of survival (PM1) (Table 2). The 4 g/liter rate allows the larvae of *An. gambiae* to complete their preimaginal cycle within 17 d only. The four NPK solutions tested having denied survival to all *An. gambiae* larvae. Consequently, the percentages of expected emergences in PM4+NPK combinations all equaled the percentage of adults recorded in PM4 (19.3%).

The percentages of adults emergences observed in PM4+NPK1 and PM4+NPK2 environments (59.7% and 49.3%, respectively) were way above ($P < 0.005$) the value expected (synergistic interaction). The log-probit analysis of the imaginal emergence has shown that for both combinations the $IEt_{20}$ were two times less with plant matter alone (PM4). $IEt_{50}$ themselves showed that 50% of the adults emerged after 11 and 12 d (as opposed to 17 d for PM4). As for PM4+NPK3 and PM4+NPK4, the comparison between the expected values and the ones actually observed only showed an additional effect of the interaction ($P = 0.14$).

### Female and male emergences

No significant difference was recorded between the percentages of imaginal emergence of females and males ($P > 0.05$) whatever the nature of the breeding sites (Fig. 1). Because the sex ratios are identical for *Ae. aegypti* and *An. gambiae*, it shows that males and females emerge in equal quantities, whatever the composition of the breeding sites.

## Discussion

Mosquito larvae breed in water collections whose physicochemical and biological characteristics differ a lot from one spot to another (Bentley and Day 1989; Darriet and Corbel 2008a,b; Darriet et al. 2010; Darriet et al. 2012). Although the nitrogen, phosphorus, and potassium alone cannot provide for the nutritional needs of the mosquito larvae, adding a chemical fertilizer into water already carrying plant material considerably increases the survival rate of these mosquitoes, multiplying by 1.7–3% of imaginal emergence (synergistic effect). The log-probit analysis of the adult apparitions also reveals a faster development of the larvae in PM+NPK combinations. The faster growth of the larvae in this type of environments partly comes from the production in the water of an organo-mineral complex abundant in nutrients (organic nitrogen and carbon, ammoniacal nitrogen [$NH_4^+$], nitric nitrogen [$NO_3^-$], phosphorus, and potassium) that favors the development of bacteria, algae, and fungi (Darriet et al. 2010; Hao et al. 2010). This supplementary food biomass of the breeding sites is crucial for the mosquito larvae because they cannot digest cellulose (Clements 2000).

*Ae. aegypti* larvae not only grow in urban water collections of medium to larger size (barrels, cisterns, jars…) but also live in smaller breeding sites (vases, gutters, cans, tires, and plant pot saucers) where water volumes can easily dry up. Whether in urban or residential areas, there are many water collections polluted by fertilizers, yet plant pot saucers are undoubtedly the most polluted of all breeding sites for *Ae. aegypti*. In Brazil and Argentina, the plant pot saucers represented 4–14% of the identified breeding sites for *Ae. aegypti* larvae and pupae (Maciel-de-Freitas and Lourenço-de-Oliveira 2011, Costa et al. 2012). In the field, many small breeding sites are characterized by waters containing lots of plant material and fertilizers which favors the growth of *Ae. aegypti* and *Aedes albopictus* larvae (Delatte et al. 2008, Darriet et al. 2010). The faster growth of the larvae should thus allow the imaginal emergence before the water has completely dried up.

*An. gambiae* larvae also can live just as well in rice paddies water and ponds as in smaller water collections such as puddles, footprints, or animal tracks. Similarly, where the waters contain plant matter and fertilizers, the survival rate of the mosquito is greater (synergistic effect) while the time of larval development is shorter.

One hundred and eighty million tons of fertilizers (FAO 2012) and 2.4 million tons of pesticides (Grube 2011) are being used every year worldwide. Attracted by the fertilizers the mosquitoes preferentially lay their eggs in water collections that contain some (Darriet and Corbel 2008a, Young 2014, Kibuthu et al. 2016). The selection

**Table 2.** Determination of imaginal emergence time 20 and 50% (*$IEt_{20}$* and *$IEt_{50}$*) of *An. gambiae* and calculation of the synergistic or additive effects of the PM4+NPK combinations

| Plant material and/or fertilizer quantities (mg/liter) | Maximum duration of preimaginal development (d) | $IEt_{20}$ in d (95% CI) | $IEt_{50}$ in d (95% CI) | Slope (± SE) | Adult emergence (%) observed (95% CI) [expected] | $P$* |
|---|---|---|---|---|---|---|
| PM4 (4000) | 17 | 16.2 (13.1–28.2) | ND | 1.0 (± 0.3) | 19.3 (14.8–23.8) | — |
| NPK1 (4-6-4)<br>NPK2 (17-23-17)<br>NPK3 (33-47-33)<br>NPK4 (66-91-66) | — | ND | ND | ND | 0 | — |
| PM4+NPK1 | 14 | 7.3 (6.6–7.8) | 11.8 (11.4–12.3) | 4.0 (± 0.03) | **59.7 (54.2–65.2)** [19.3] | <0.005 |
| PM4+NPK2 | 11 | 9.0 (8.8–9.2) | 10.8 (10.6–11.2) | 10.6 (± 0.8) | **49.3 (43.6–55.3)** [19] | |
| PM4+NPK3 | 14 | ND | ND | ND | 15.0 (11.0–19.0) [19.3] | 0.14 |
| PM4+NPK4 | 11 | | | | 15.0 (11.0–19.0) [19.3] | |

Bold font indicates that the adult emergence differed statistically from the expected value.
*$P < 0.05$ = synergistic effect of the PM and NPK combinations. $P > 0.05$ = additive effect.
ND, not detectable.

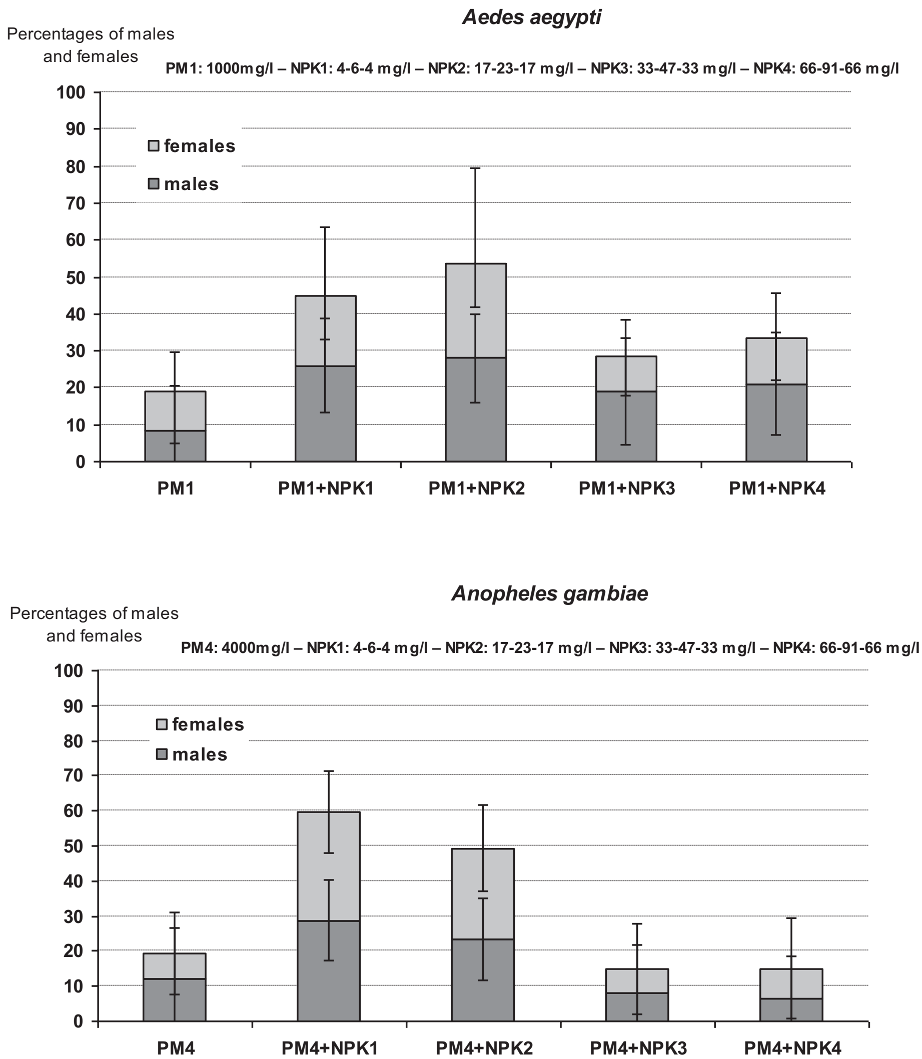


**Figure 1.** Percentages (95% CI) of males and females mosquitoes whose larvae lived in environments containing plant material alone or in combination with NPK fertilizer.

pressure induced by the agricultural inputs is huge, and the resistance mechanisms present in the mosquitoes are selected all the more rapidly and efficiently as the mineral fertilizations and the insecticide treatments are frequent within a time span. One of the vector control strategies to promote for the environments containing plant matter and fertilizers to produce fewer mosquitoes is to add a chemical or biological larvicide to the fertilizer, which kills the mosquito larvae right out from hatching. With less than 5% imaginal emergence, the fertilizer and spinosad combination treatment remained effective for 30 d on *Ae. aegypti*. Both fertilizer and pyriproxyfen or diflubenzuron combinations were effective for 45 d (Darriet 2016).

Nowadays the diseases transmitted by mosquitoes kill many people worldwide. As for the interface agriculture/public health, there is still a huge research area that has only partly been explored. The synergy of such a partnership between the scientists, the rice-growers, and the vector control services would initiate pluridisciplinary research programs whose goal would be to protect the crops while reducing the mosquito populations as much as possible.

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