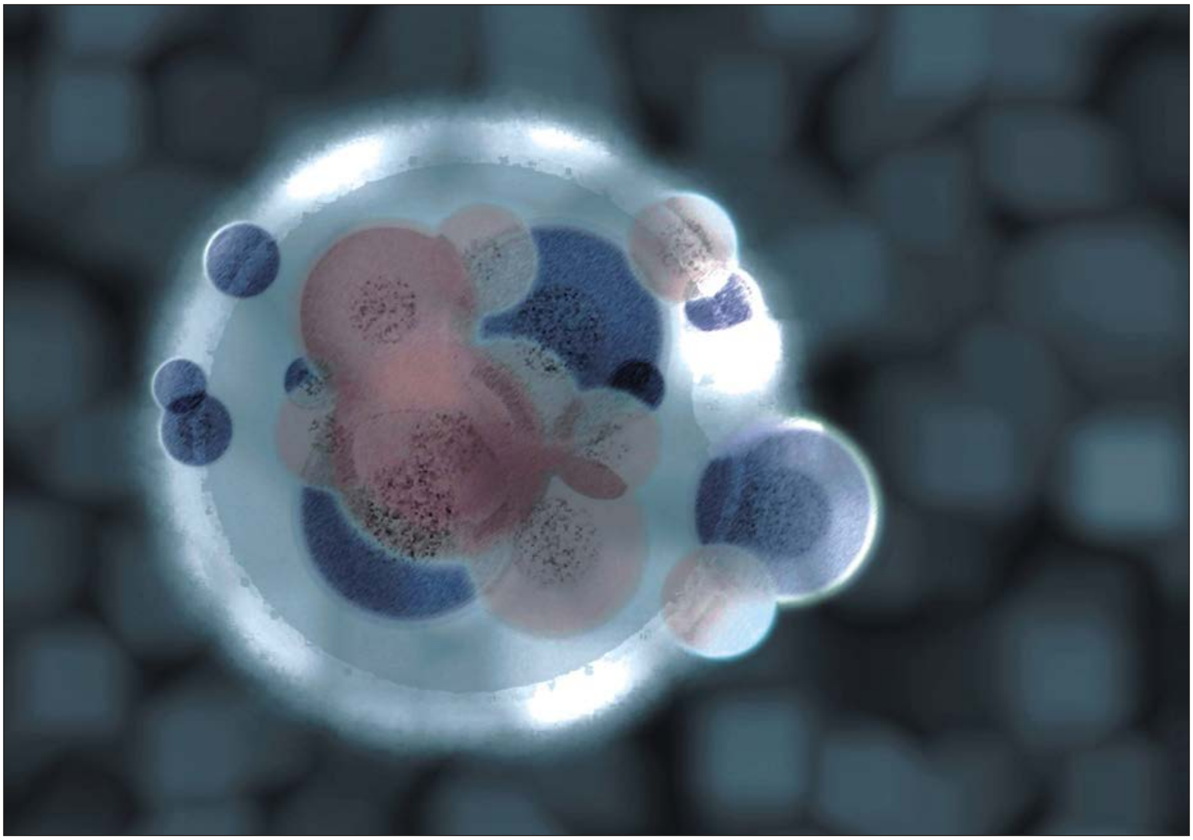

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Studies on Some Physicochemical Factors Affecting the Breeding and Abundance of Mosquitoes (Diptera: Culicidae) in Phytotelmata on *Delonix regia* (Leguminosae: Caesalpinoidea)

D.A. Adebote, D.S. Abolude, S.J. Oniye and O.S. Wayas
Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria

Abstract: Some physical (depth, electrical conductivity, height, surface area, temperature, volume) and chemical (pH, total dissolved solids, concentrations of cadmium, calcium, chlorine, copper, magnesium, sodium, sulphate, zinc) factors affecting the breeding and abundance of preimaginal mosquitoes in phytotelmata on the flamboyant tree, *Delonix regia* were investigated in Zaria, northern Nigeria. Forty-nine (43.36%) of the 113 stands of the tree had phytotelmata while 28 (24.78%) supported mosquito breeding. Of the 541 mosquito preimaginals isolated from phytotelmata were 5 (0.92%) *Aedes aegypti* Linnaeus, 126 (23.29%) *Aedes metallicus* Edwards, 3 (0.55%) *Aedes simpsoni* Theobald, 79 (14.60%) *Culex horridus* Edwards, 272 (50.28%) *Culex nebulosus* Theobald, 52 (9.61%) *Culex pipiens pipiens* Linnaeus and 4 (0.74%) *Culex tigripes* Grandpre. Conspecific, congeneric, heterospecific and heterogeneric breeding behavioural patterns were observed with one, two, three and four mosquito species per phytotelma on 13 (46.43%), 11 (39.29%), 2 (7.14%) and 2 (7.14%) trees, respectively. *Aedes* genus of mosquitoes bred in phytotelmata at significantly lower heights and smaller water volumes than *Culex* genus ($p<0.05$); but depths and surface areas of phytotelmata for both genera did not differ significantly ($p>0.05$). Abundance of each of the seven species of mosquito correlated positively and highly significantly with water volumes in the phytotelmata ($p = 0.0016$; $r = 0.435$). *Culex* horridus population correlates positively and highly significantly with the concentration of cadmium ($p<0.0001$; $r = 0.661$), surface areas ($p<0.05$; $r = 0.283$) and temperature ($p<0.05$; $r = 0.323$) of water in phytotelmata. *Culex nebulosus* abundance correlates positively and significantly with electrical conductivity ($p<0.05$; $r = 0.302$) and total dissolved solids ($p<0.05$; $r = 0.302$) in phytotelmata. Abundance of *C. p. pipiens* in phytotelmata correlates positively and significantly with water depth ($p<0.05$; $r = 0.304$). The study identified some ecological factors regulating mosquito productivity in a relatively unusual larval habitat of disease vectors.

Key words: Physicochemical factors, mosquitoes, phytotelmata, *Delonix regia*

INTRODUCTION

Mosquitoes constitute a very important component in the complex determinants of insect-borne diseases of public health importance, in places where the availability of diverse water bodies support their breeding. The diversities of aquatic habitats for mosquito breeding often make them occur in sufficient population to constitute biting nuisances and or transmitters of disease causing parasites and pathogens. Basically all mosquito species require an aquatic habitat for oviposition, larval and pupal development culminating in adult emergence. Different species of mosquito show individual ecological preferences for breeding habitats (Service, 1993). Amongst the diverse breeding habitats for mosquitoes are the phytotelmata; which are the small volumes of water held in terrestrial plant structures (Kitching, 2000, 2001). Five major classes of phytotelmata habitats are readily recognized, including water-filled tree holes, plant axils, pitchers, tank bromeliads and bamboo internodes (Kitching, 2000, 2001). The mosquito family Culicidae are regarded as ancestrally phytotelm dwellers, with two of its subfamilies, Sabethini and Toxorhynchitinae, consisting of exclusively phytotelm dwellers (Kitching, 2001).

Large literature exists on the mosquito fauna inhabiting phytotelmata worldwide. Most of these publications centre on vectors, suspension-feeding or surface-feeding decomposers and predators (Jenkins and Carpenter, 1946; Haddow, 1948; Corbet, 1964; Service, 1965; Trimble and Smith, 1975; Steffan and Evenhuis, 1981; Bradshaw and Holzapfel, 1983, 1986, 1989; Juliano and Reminger, 1992; Adebote *et al*., 2004). The recent publication of Adebote *et al*. (2004) identifies ten species of mosquito breeding in phytotelmata in Zaria, northern

Corresponding Author: David A. Adebote, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria
Tel: +23469370250, +2348056564878

Nigeria. Over 94% of these mosquito populations were encountered on the flamboyant tree, *Delonix regia* and seven of the ten mosquito species occurred exclusively on the tree. Moreover, 75% of each of the other three species also occurred on *D. regia*. Adebote *et al.* (2004) had suggested an analysis of the physicochemical parameters of water in the phytotelms on *D. regia* as a clue to a better understanding of the factors enhancing the occurrence and association of mosquitoes with the tree. This study reports on some physical and chemical properties of the phytotelmata on *D. regia* tree that are associated with the breeding of mosquitoes.

MATERIALS AND METHODS

The study was carried out within the main campus of the Ahmadu Bello University, situated in Samaru (11°10′N, 07° 39′E), North West of Zaria (11°07′N, 07°44′ E) northern Nigeria. The university, located within the northern Guinea savanna belt of Nigeria, is planted with a dense array of shade trees amongst which the flamboyant, *Delonix regia* is dominant. The climate of the area has variously been described elsewhere with regards to mosquito ecology (Service, 1965; Adebote *et al.*, 2006).

Delonix regia trees growing in various parts of the campus were examined for phytotelms. The stands of the trees that were positive for phytotelms, those with developing stages of mosquitoes in their phytotelms and those without phytotelm were noted. In every phytotelm-positive tree, the following procedures were adopted. The depths and surface areas of water were determined with the aid of a calibrated ruler. The height of each phytotelm from the ground level was determined by lowering a rope from the phytotelm to the ground and measuring the length against a tape rule. A portable HANNA HI 991300 combined pH-electrical conductivity-total dissolved solids temperature meter was used to determine the respective (pH, EC, TDS, temperature) water parameters in the phytotelms.

Habitat evacuation methods, employing a glass and rubber tubing oral aspirator and or a small brass soup ladle dipper (9 cm in diameter and holding 138 mL of water), were used in sampling mosquito larvae and pupae in all positive phytotelms (Service, 1976). The volume of water in evacuated phytotelms was determined with the aid of a plastic measuring cylinder. Water obtained from each phytotelm was poured through a fine sieve in a white enamel bowl to concentrate mosquito larvae and pupae. These preimaginals were then carefully picked with a pair of entomological forceps into appropriately labelled specimen bottles containing 70% ethanol as preservative. Identification of preserved mosquito preimaginals was done under the X50 magnification of a stereomicroscope, aided by the pictorial keys of Hopkins (1952) for larval identification and Edward (1941) for pupal identification. The population of each of the respective identified mosquito species per phytotelm was noted.

Water samples were obtained from every phytotelm in a 250 mL plastic bottle. The water was filtered using filter study in a funnel to rid it of decaying plant detritus often associated with the phytotelms. Digestion of filtered water was carried out based on the methods of APHA (1985) using hot concentrated trioxonitrate(v) acid (HNO_3). The concentrations of Cadmium, Calcium, Copper, Magnesium, Sodium and Zinc in digested water were determined on duplicate samples using a UNICAM 969 (Flame ABS mode) Atomic Absorption Spectrophotometer (AAS). The concentrations of chlorides and sulphates was determined according to standard procedures (APHA, 1985).

Statistical data analysis was done on a personal computer, using System Analysis Statistics (SAS) Programme 6.12, 1998 version. Pearson correlation analysis was used to determine the relationships amongst the physicochemical parameters of the phytotelmata and abundance of mosquito species breeding in them. Student's t-test was employed to check significant differences in the physical attributes of phytotelmata utilized by the two genera of mosquito encountered.

RESULTS

Of the 113 stands of *Delonix regia* trees examined for phytotelmata in this study, 49 (43.36%) had phytotelmata and 28 (24.78%) supported the breeding of seven species and 541 specimens of mosquito larvae belonging to the *Aedes* and *Culex* genera. These included 5 (0.92%) *Aedes aegypti* Linnaeus, 126 (23.29%) *Aedes metallicus* Edwards, 3 (0.55%) *Aedes simpsoni* Theobald, 79 (14.60%) *Culex horridus* Edwards, 272 (50.28%) *Culex nebulosus* Theobald, 52 (9.61%) *Culex pipiens pipiens* Linnaeus and 4 (0.74%) *Culex tigripes* Grandpre (Table 1). Most of the species occurred with low larval populations in the phytotelmata and were predominated by *A. metallicus* amongst the aedine species and *C. nebulosus* amongst the *Culex* species. *Culex nebulosus* was the most abundant species, accounting for over a half of the entire population. About a quarter (24.77%) of the larvae belonged to the *Aedes* genus while about three quarters (75.60%) were *Culex* (Table 1).

Four patterns of breeding were observed with the mosquitoes in phytotelmata, ranging from single species

Table 1: Species of mosquito breeding in *D. regia* phytotelmata in Zaria, Nigeria

Mosquito species	No. of positive phytotelmata (n = 28) (%)	No. of larvae recovered (%)
Aedes aegypti	2 (7.14)	5 (0.92)
Aedes metallicus	12 (42.86)	126 (23.29)
Aedes simpsoni	1 (3.57)	3 (0.55)
Culex horridus	10 (35.71)	79 (14.60)
Culex nebulosns	10 (35.71)	272 (50.28)
Culex pipiens pipiens	6 (21.43)	52 (9.61)
Culex tigripes	4 (14.29)	4 (0.74)
Total		541 (99.99)

Table 2: Breeding patterns of mosquitoes in *D. regia* phytotelmata in Zaria, Nigeria

Mosquito species combination per phytotelma	No. and percentage of phytotelmata (n = 28) (%)
Single species occurrence	
Aedes metallicus	9 (32.14)
Culex horridus	1 (3.57)
Culex nebulosns	3 (10.71)
Total	13 (46.43)
Double species occurrence	
Aedes aegypti and *Aedes simpsoni*	1 (3.57)
Aedes aegypti and *Culex horridus*	1 (3.57)
Aedes metallicus and *Culex horridus*	1 (3.57)
Aedes metallicus and *Culex nebulosus*	1 (3.57)
Culex horridus and *Culex nebulosus*	4 (14.29)
Culex horridus and *Culex p. pipieus*	2 (7.14)
Culex nebulosns and *Culex p. pipiens*	1 (3.57)
Total	11 (39.29)
Triple species occurrence	
Aedes metallicus, *Culex horridus* and *Culex tigripes*	1 (3.57)
Culex nebulosns, *Culex p. pipiens* and *Culex tigripes*	1 (3.57)
Total	2 (7.14)
Quadruple species occurrence	
Culex horridus, *Culex nebulosus*, *Culex p. pipiens* and *Culex tigripes*	2(7.14)
Total	2 (7.14)

in 13 (46.43%) phytotelmata to double, triple and quadruple species that occurred in 11(39.29%), 2 (7.14%) and 2 (7.14%) phytotelmata, respectively (Table 2). *Aedes metallicus* single species per phytotelma predominated this category in 9 (32.14%) phytotelmata. The duo of *C. horridus* and the congener *C. nebulosus* predominated in the double species per phytotelma, in 4 (14.29%) phytotelmata. *Culex tigripes* always featured amongst the triple and quadruple species of mosquito breeding in same phytotelma (Table 2).

Mosquitoes belonging to the *Aedes* genus occurred in phytotelmata located 11-218 cm above the ground; with surface areas of 120-1760 cm^2; 4-16 cm deep, with water volume of 40-2000 cm^3; at temperatures of 21.5-25°C; of electrical conductivity and total dissolved solids values of 61-2518 μS cm^{-1} and 30-1258 ppm, respectively. Those belonging to *Culex* genus occurred on phytotelmata 3-172 cm high; with surface areas of 24-1380 cm^2, 2.3-25 cm deep; with water volume of 40-13000 cm^3; at temperatures of 21.5-28°C; of electrical conductivity and total dissolved solids values of 420-14060 μS cm^{-1} and 209-7045 ppm respectively (Table 3). Mosquitoes of the *Aedes* genus bred in phytotelmata at significantly higher heights than those of the *Culex* genus ($p<0.05$). There was no significant difference in the surface areas of water at which *Aedes* and *Culex* genera of mosquito bred in the phytotelmata ($p>0.05$). Similarly, no significant difference was observed between the water depths at which the two genera of mosquito bred ($p>0.05$). *Culex* species of mosquitoes bred in water of significantly larger volumes than *Aedes* mosquitoes ($p<0.05$). Abundance of *C.p. pipiens* and *C. horridus* larvae highly significantly correlated with the volumes of water in the phytotelmata ($p<0.0001$; $r = 0.555$ and 0.516 respectively). Larval abundance of the pooled species of mosquito also correlates significantly with water volume in the phytotelmata ($p = 0.0016$; $r = 0.435$). Abundance of *C.p. pipiens* larvae correlates significantly with the depth of water ($p<0.05$; $r = 0.304$). Population of *C. horridus* larvae correlates significantly with the surface area ($p<0.05$; $r = 0.283$) and temperature of water ($p<0.05$; $r = 0.279$) in the phytotelmata. Abundance of *C. nebulosus* larvae correlate significantly with electrical conductivity and total dissolved solids ($p<0.05$; $r = 0.303$, 0.302, respectively).

The concentration of calcium in the media ranges from 6.562 mg L^{-1} for *A. metallicus* breeding to 37.63 mg L^{-1} for *C. nebulosus* habitat. Cadmium concentration ranged from 0.002 mg L^{-1} for both *A. metallicus* and *C. nebulosus* to 0.015 mg L^{-1} for *A. metallicus* habitat (Table 4). Abundance of *C. horridus* larvae in the phytotelmata highly significantly correlated with the concentration of cadmium ($p<0.0001$; $r = 0.661$). That of copper ranged from 1.007 mg L^{-1} in both *C. nebulosus* and *C. p. pipiens* habitat to 2.615 mg L^{-1} for *A. metallicus*. Concentration of magnesium ranged from 1.832 mg L^{-1} in habitat supportive of *A. metallicus*, *C. horridus* and *C. tigripes*, to 13.51 mg L^{-1} supportive of *C. horridus* and *C.p. pipiens* breeding. Sodium concentration in the phytotelmata ranged from 0.206 mg L^{-1} where, *A. metallicus* occurred, to 7.129 mg L^{-1} where, *C. nebulosus* bred. Zinc concentration in the phytotelmata ranged from 0.699 mg L^{-1} in *A. metallicus* habitat to 1.586 mg L^{-1} supportive of *C. nebulosus* and *C. p. pipiens* breeding. Chlorine concentration in the phytotelmata ranged from 0.9 mg L^{-1} in *A. metallicus* habitat to 10.8 mg L^{-1} for *C. nebulosus*. Sulphate concentration in the phytotelmata ranged from 9 mg L^{-1} at which *A. metallicus*, *C. horridus* and *C. tigripes* occurred, to 40 mg L^{-1} at which *A. aegypti* and *C. horridus* occurred. The pH of phytotelmata ranged from 5.81 mg L^{-1} at which *A. metallicus* bred to 8.87 mg L^{-1} at which *C. horridus* bred (Table 4).

Table 3: Ranges (Mean±SE) of physical attributes of phytotelmata on *Delonix regia* supportive of mosquito species breeding in Zaria, Nigeria

	Physical parameters of phytotelmata						
Mosquito species	Height above ground (cm)	Surface area (cm^2)	Depth (cm)	Water volume (cm^3)	Water temperature (°C)	Electrical conductivity (µS cm^{-1})	Total dissolved solids (ppm)
Aedes aegypti	11-104 (57.5±32.88)	120-442 (281±113.84)	4-9 (6.55±1.77)	130-630 (380±176.78)	23-25 (24±0.71	1338-2518 (1928±417.19)	667-1258 (962.5±208.95)
Aedes metallicus	12-218 (66.42±17.50)	21-1760 (379.25±137.90)	4-16 (8.08±1.11)	40-2000 (464.67± 153.14)	21.5-25 (23.38±0.32)	61-1522 (360.38±158.68)	30-762 (179.63±79.53
Aedes simpsoni	11 (11±0)	120 (120±0)	4 (4±0)	130 (130±0)	23 (23±0)	307 (307±0)	153 (153±0)
Culex horridus	13-171 (105.9±17.31)	130.5-1380 (496.75±147.63)	2.3-25 (10.18±2.23)	80-13000 (2813.1±1440.88)	21.5-25.5 (23.95±0.44)	813-9350 (3111.63±900.06)	405-4720 (1561±455.09)
Culex nebulosus	3-172 (113.3±16.0)	24-799 (298.55±73.73)	2.3-25 (9.88±2.28)	40-10700 (2189±1089.24)	23-28 (24±0.32)	420-14060 (4756±1536.19)	209-7045 (2381.88±770.68)
Culex p. pipieus	3-171 (78.67±23.74)	140-1380 (694.83±216.18)	2.5-25 (12.83±3.23)	360-13000 (4456.67±2153.41)	21.5-25 (23.75±0.50)	813-14060 (5072.75±2632.57)	405-7045 (2539.5±1319.71)
Culex tigripes	3-113 (82.5±23.01)	120-799 (347.25±132.96)	2.5-25 (13.13 ±4.60)	80-10700 (3112.5±2196.82)	23-25 (24±0.35)	1522-3340 (2313.33±439.14)	762-1670 (1156.67±219.43)

Table 4: Ranges (Mean±SE) of chemical constituents of phytotelmata on *Delonix regia* supportive of mosquito species breeding in Zaria Nigeria

	Chemical constituents (mg L^{-1})								
Mosquito species	Ca	Cd	Cu	Mg	Na	Zn	Cl	SO_4	pH
Aedes aegypti	8.502 (8.502±0)	0.007 (0.007±0)	1.243 (1.243±0)	4.432 (4.432±0)	1.768 (1.768±0)	ND	6.4 (6.4±0)	40 (40±0)	7.54 (7.54± 0)
Aedes metallicus	6.562-12.19 (8.47±0.67)	0.002-0.015 (0.008±0.002)	1.173-2.615 (1.795±0.18)	1.832-6.218 (3.63±0.57)	0.206-6.249 (0.194± 0.95)	0.699-1.047 (0.830± 0.04)	0.9-3.6 (1.43± 0.29)	9-19 (13.13± 2.15)	5.81-7.93 (6.69± 0.21)
Aedes simpsoni	ND^A	ND	ND	ND	ND	ND	ND	ND	ND
Culex horridus	6.678-36.73 (23.26±4.05)	0.004-0.011 (0.009±0.0007)	1.243-2.422 (1.66±0.16)	1.832-13.51 (6.733± 1.46)	0.415-3.039 (0.43±0.72)	0.709-1.479 (1.019±0.10)	1.1-7.5 (4.05± 1.46)	9-40 (22.25±6.62)	7.16-8.87 (7.78±0.20)
Culex nebulosus	8.540-37.63 (27.19±3.89)	0.002-0.011 (0.007±0.001)	1.007-2.481 (1.688±0.22)	5.888- 8.220 (7.298±0.30)	0.209-7.129 (0.75±0.10)	0.709-1.586 (1.093±0.11)	1.2-10.8 (4.6± 2.54)	10-38 (20.67±7.14)	6.94-8.74 (7.75±0.22)
Culex p. pipieus	12.74-35.99 (25.65±4.30)	0.005-0.010 (0.009±0.001)	1.007-2.422 (1.70± 0.29)	5.888-13.51 (8.62±1.46)	0.679-3.039 (0.05±1.08)	0.81-1.586 (1.06± 0.16)	1.2-7.5 (4.35±2.23)	10-30 (20±7.07)	7.16-8.65 (7.77±0.27)
Culex tigripes	6.678-29.97 (20.18±5.70)	0.008-0.01 (0.009± 0.0005)	1.554-2.422 (2.02±0.21)	1.832-7.240 (4.99± 1.33)	1.22-2.772 (0.39±1.34)	0.81-1.047 (0.90±0.06)	1.1-1.2 (1.15± 0.04)	9-10 (9.5±0.35)	7.53-7.93 (7.73±0.09)

ND^A: Not determined for the single phytotelma where, it occurred

DISCUSSION

Delonix regia is an important tree species that provided phytotelmata supportive of mosquito breeding in Zaria, northern Nigeria. Availability of phytotelmata on this tree could be due to its soft white wood that is susceptible to rot and cavity formation due to injury and or die back of parts during growth. The trees were originally planted as ornamentals to beautify and provide shades in gardens in several parts of the campus. Thus, certain aspects of landscape architecture could ultimately result in the provision of breeding habitats for human disease vectors. Both *A. aegypti* and *A. simpsoni* found breeding in phytotelmata in this study are involved as vectors and basic reservoirs in the complex epidemiology of yellow fever in Nigeria and several foci of the disease (Service, 1993; Lutwama and Makwaya, 1994). *Aedes metallicus* is also capable of transmitting the yellow fever virus. Earlier studies in the area by Adebote *et al.* (2006), identified a high risk posture for yellow fever epidemics due to elevated *A. aegypti* breeding indices in villages. This study has further highlighted additional breeding habitats of potential yellow fever vectors. For the prevention and control of the disease in the area, tree hole habitats on *D. regia* should be considered for source reduction alternatives. *Aedes simpsoni* has been associated with phytotelmata habitats on several plant species (Lutwama and Makwaya, 1994); this study has provided additional information on its occurrence on *D. regia*. Other species encountered, including *C.p. pipiens* and the dominant *C. nebulosus* are essentially ornithophilic (Snow and Boreham, 1978; Service, 1993). Four of the seven species of mosquitoes found inphytotelmata (with the exceptions of *A. metallicus*, *A. simpsoni* and *C.p. pipiens*) were also found breeding in peridomestic containers in the study area (Adebote *et al*., 2006). *Culex tigripes* is a predatory species of mosquito with biological control potential. Its presence in triad and quad combinations with other species as congeneric and heterogeneric inhabitants of same phytotelma shows its propensity to feed on these other species and regulate their population.

Relationship between mosquitoes and the phytotelmata they utilize for development is important in habitat and epidemiological studies since vector abundance depends primarily on effective phytotelmata number and conditions inherent in them (Pajot, 1983) *Aedes* and *Culex* genera of mosquito encountered in this study show significantly different height preferences in tree canopies; none occurring at the exposed tree top, but both confined to mid-heights where, deep shade was provided. This segregation could be a behaviour to reduce competition and was attributed to the preferences of ovipositing females (Scholl and DeFoliart, 1977; Sinsko and Grimstand, 1977). Copeland and Craig (1990) showed similar segregation by height of treehole mosquitoes in the Great Lakes region of the United States. The mean pH values of phytotelmata utilized by mosquitoes for breeding in this study hovered around neutrality, (only *A. metallicus* and *C. nebulosus* experienced some tinge of acidity). This contradicts several studies on phytotelm waters that concluded on the acidic nature and acidophilic tendencies of phytotelm communities (Kitching, 2001). The almost neutral pH of this study could be due to plant based (*D. regia*) effect on phytotelm waters since the acidity of earlier studies were observed in phytotelmata on other plant species. This factor could be responsible for the preference shown for the colonization of *D. regia* by mosquitoes amidst other trees in the study area. Survivorship of mosquito larvae in tree holes has been shown to be inversely related to increased acidity (Carpenter, 1982). Phytotelmata on *D. regia* are small water bodies with capacities not exceeding 13 L. The larger volumes (7-13 L) were encountered only on three stands of the plant whose boles experienced major rot resulting in deep and wide cavities for water to accumulate. Such large accumulations of water would provide prolonged breeding habitats for mosquitoes long after others might have dried up. Nonetheless, the greater low water volumes of the phytotelmata are critical to mosquito's survival as abundance of all the species of mosquito therein showed highly significant positive correlation to water volume. Thus water volume is a key factor to the colonization of phytotelmata on *D. regia* by mosquitoes. Similar observations on low water volume were made by Kitching (2000) and Adebote *et al.* (2004). The implication of low water volume in phytotelmata on *D. regia*, that are solely rain fed and occurring in an area of prolonged (six months; November-April) dry season, is habitat dryness that would prevent their availability to mosquitoes for breeding. Volume of water has also been indicated as one of the major factors in the natural habitat of *Aedes simpsoni* complex in plant axils (Lutwama and Mukwaya, 1994). With the exception of cadmium, none of the cations and anions explored appeared to significantly affect mosquitoes in the phytotelmata.

In conclusion, water volume, surface area, temperature, depth, electrical conductivity and total dissolved solids are amongst the physical attributes of phytotelmata on *D. regia* that variously affect mosquito species occurrence and abundance while the concentration of cadmium appeared to be critical to species colonization.

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