An investigation into the influence of leaf species on the growth performance and oviposition preference of Aedes albopictus (Asian tiger mosquitoes) in Sri Lanka.

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Research Article

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Abstract

Aedes albopictus is a container breeder which utilizes a wide range of water-holding containers for oviposition. The attraction of the gravid females to the oviposition site is based on several physical, chemical, and biological cues. Hence the primary objective of the study was to determine the individual and combined effects of *Swietenia macrophylla*, *Pterospermum suberifolium*, *Carallia brachiata*, and *Azadirachta indica* on the oviposition preference, larval mortality, larval duration, pupal duration and fecundity of *Ae. albopictus*. Microcosms were prepared with senescent leaves of the selected plant species and they were used for the tests. Accordingly, the number of eggs laid in *A. indica* leaf infusion was the lowest, while *C. brachiata* and *P. suberifolium* were the highest. Furthermore, the highest larval mortality, larval and pupal duration and lowest fecundity of the emerged adults were observed in *A. indica.* The leaf combination *A. indica* - *C. brachiata* (ND) showed the highest larval mortality and the lowest fecundity of the emerged adults, while the leaf combination *S. macrophylla* - *A. indica* - *P. suberifolium* (MNW) showed the highest larval and pupal durations. However, it can be postulated that *A. indica* leaf species hinders the growth performance and oviposition preference of *Ae. albopictus.*

1. Introduction

Aedes aegypti and *Aedes albopictus* are two major mosquito vectors of dengue viruses in Sri Lanka, where *Ae. aegypti* is the primary vector, and *Ae. albopictus* is the secondary vector. (Preechaporn et al. 2007). *Aedes albopictus* is a container breeder that utilizes a wide range of water-holding containers for oviposition. Often, its breeding sites can be found in outdoor environments compared to indoor environments. The breeding sites can be natural containers like tree holes and artificial containers like tires, tins, buckets, plastic cups, cans, gutters, ornamental ponds, vases, and yoghurt cups (Alto and Juliano, 2001).

Selection of an oviposition site is an essential component of the life history of mosquitoes affecting the longevity of eggs, development and growth performance of larval stages, predator avoidance, intra, and inter-specific interactions, as well as phenotype and the overall fitness of the resulted offspring (Yoshioka et al. 2012). In natural conditions, the attraction of the gravid mosquitoes to the oviposition site is based on several physical, chemical, and biological cues. However, these cues interact with the other factors specific to each mosquito species at site selection (Yanoviak, 2001; Ponnusamy et al. 2008).

Aedes albopictus mosquitoes follow the preference-offspring performance (p-p) hypothesis to select an oviposition site. Hence, adult female mosquitoes assign their eggs to habitats with high food content (organic matter), resulting in low intraspecific competition (Urbinatti, 2001; Ellis, 2008). Thus, *Ae. albopictus* lay more eggs in dark coloured water sources than in clear and colourless water (Derraik and Slanely, 2005; Li et al. 2009; Obenauer et al. 2009). Since the mosquito larvae consume the allochthonous plant matters present in the breeding site, they provide the required nutrient content for larval development, resulting in high growth performance of offspring and increase fitness of the adults (Dieng et al. 2002; Reiskind et al. 2012; Yoshioka et al. 2012).

Heterotrophic microbial fauna, mainly bacteria and fungi, are the basal consumers involved in decomposing leaf detritus in larval mosquito habitats. Also, they serve as the primary food source for mosquito larvae (Juliano, 2009). The higher the leaf detritus in the breeding habitat higher the microbial population (Dieng et al. 2002). However, the intake of too many or too few essential nutrients will lead to low fitness. Thus, there should be an optimum level that increases fitness (Kaufman et al. 2002). Dieng et al. (2003) have shown a direct impact of leaf matter, or an impact mediated through microbial intermediates on mosquito larvae.

Since the highly abundant mosquito species found in the study site (6º58'20.91" N; 79º54'52.83" E) at the premises of the University of Kelaniya was *Ae. Albopictus* (Weerakoon, 2012) and the area was comparatively dense with vegetation such as *Swietenia macrophylla* (Mahogani), *Pterospermum suberifolium* (Welan), *Carallia brachiata* (Dawata), and *Azadirachta indica* (Neem), the overall objective of this study was to investigate the effects of four selected plant leaf matter on the oviposition preference and growth performances of *Ae. albopictus*.

2. Methodology

A preliminary study was conducted to identify the most suitable concentration of the hay infusion to collect the eggs of *Ae. albopictus*. Oviposition traps filled with a series of hay infusions of 5%, 10%, 15% and 20% were prepared. Hay infusion was prepared by adding 125.0 g of dried hay into 15 L of aerated tap water. The mixture was allowed to undergo fermentation in a large plastic container for seven days. Later the infusion was strained through a sieve having a small mesh to remove the hay and was kept in plastic containers at $28 \pm 2\ ^{0}$C (Polson et al. 2002). A filter paper strip of length 22.0 cm and width of 5.0 cm was placed along the plastic cup's interior circumference as the oviposition substrate. Ten ovitraps filled with hay infusion from each concentration were placed randomly in a shady outdoor area at the premises of the University of Kelaniya (6º 58' 20.91" N: 79º 54' 52.83" E). The ovitraps were observed daily for seven days for the presence of mosquito eggs. Once the eggs had been laid, they were submerged by increasing the volume of the hay infusion in the ovitap. Afterward, the eggs were allowed to hatch and develop up to third instar larvae. All the third instar larvae in each oviposition trap were subjected to species identification using larval identification keys (Weerasinghe et al. 2011). The total number of mosquito larvae in each species was counted concerning each concentration. This experiment was repeated three times.

Low hay infusion concentrations (5%, 10%) attracted *Ae. albopictus* mosquitoes, whereas high concentrations attracted (15%, 20%) *Ae. aegypti*. Although both 5% and 10% concentrations attracted 100% *Ae. albopictus*, the productivity was higher in 10% hay infusion. Therefore, ovitraps baited with 10% hay infusion were used to collect the eggs of *Ae. albopictus* to maintain a colony of mosquitoes during the study.

Maintaining the colony

Based on the observations and conclusions of the preliminary study, 40 ovitraps filled with 10% diluted hay infusion were placed randomly on the university premises to collect the eggs of *Ae. albopictus.* A colony was maintained throughout the study by rearing the adults who originated from the eggs collected. Larvae were fed with crushed shrimp powder. A sample of dried shrimp (*Penaeus* spp.; 100 g) was used to prepare larval food. Shrimps were dried in an oven for 6 hours under 70 ^{0}C and crushed using a mortar and pestle until they became a powder. All the tests were performed with the larvae taken from the maintained colony. The conditions were maintained as; temperature 28 ± 2 ^{0}C, relative humidity 75–80% and photoperiod of 12L: 12D.

Effect of decaying rate of the leaf infusions of a single plant species on oviposition preference

Sixteen microcosms were prepared from each leaf species of *S. macrophylla* (M-Mahogani), *P. suberifolium* (W-Welan), *C. brachiata* (D-Dawata), and *A. indica* (N-Neem). Senescent leaves (150.0 g) were taken, washed with water and air-dried for ten days. Subsequently, they were kept in an oven for two days at 50^{0} C. Then, leaves were removed from the oven and crushed using a mortar and a pestle (Yanoviak, 2001). After that, the crushed leaf matter (1.0 g) was added to black-coloured plastic cups (250.0 mL), having a diameter of 7.0 cm and a depth of 6.0 cm. One-third of the cup was filled with aerated water, and the leaf matter was allowed to decay for 14 days, 12 days, 10 days, 8 days, 6 days, 4 days, 2 days, and 0 days. Two microcosms from leaf species with different decomposition periods were placed separately in four mosquito rearing cages (Fig. 2.1). A filter paper strip of length 22.0 cm and width of 5.0 cm was placed along the plastic cup's interior circumference as the oviposition substrate. Fifteen mated blood-fed gravid females of 5 days old were released to each mosquito rearing cage for the oviposition. Mating was ensured by the visual observation when the mating copulas had formed for more than 5 seconds. A guinea pig (*Cavia porcellu*s) was used to supply a blood meal to trigger the gonotrophic cycle in adult female mosquitoes. A small portion at the back of the ear of the guinea pig was shaved to make it easy for the mosquitoes to suck blood. The number of eggs laid in each microcosm was recorded three days after introducing female mosquitoes. This experiment was repeated four times.

Effect of single leaf species on the growth performance

Swietenia macrophylla, *P. suberifolium*, *C. brachiata*, and *A. indica* leaf species were used to determine the individual effect of each leaf species on the growth performance of *Ae. albopictus*. Four microcosms were prepared from each leaf species by adding 1.0 g of crushed leaf matter into containers with 200.0 mL of aerated water. Another four containers filled only with aerated water were used as the controls. Twenty, first instar larvae were introduced to each microcosm. Each microcosm was observed twice daily (0700h, 1600h) until the emergence of adults. Larval mortality, total larval duration, pupal duration, and fecundity of the emerged adults were recorded in each treatment. This experiment was repeated three times.

Fecundity was recorded on the day of emergence of the females by measuring the length of the right-wing (Armbruster and Hutchinson, 2002). Emerged females were killed with ethyl acetate, and the right-wing was removed. It was mounted on a microscopic slide with a drop of distilled water. Wing length was measured from the axial incision to the apical end (Schneider et al. 2004) using the compound microscope (Olympus© CX21 Microscope) with 40X magnification. The wing was aligned along an ocular micrometre (1 ocular unit, 0.025 mm at 40X), and the wing length was measured to the nearest 0.01 mm (Menge et al. 2005).

Effect of combination of leaf species on growth performance

The combinatory effect of four selected leaf species *S. macrophylla*, *P. suberifolium*, *C. brachiata*, and *A. indica* was determined on the growth performance of *Ae. albopictus*. Eleven combinations were used: six combinations of two-leaf species (T1-T6), four combinations of three-leaf species (T7-T10) and one combination of four-leaf species (T11) (Table 2.1). The two-leaf combination was prepared by mixing 50% of plant material from each plant species. Three-leaf combination was prepared by mixing 33% of plant material from each plant species, and the four-leaf combination was prepared by mixing 25% of plant material from each plant species.

Table 2.1: Arrangement of the microcosms prepared from the combination of leaf species of *Swietenia macrophylla*, *Pterospermum suberifolium*, *Carallia brachiata*, and *Azadirachta indica* in separate mosquito rearing cages

Treatment	Leaf combination	No. of replicates	No. of controls
T1	MD	4	1
T2	MW	4	1
T3	MN	4	1
T4	DN	4	1
T5	DW	4	1
T6	NW	4	1
T7	MNW	4	1
T8	MND	4	1
T9	MDW	4	1
T10	NDW	4	1
T11	MNWD	4	1

M – Mahogani (*Swietenia macrophylla*), N – Neem (*Azadirachta indica*), W – Welan (*Pterospermum suberifolium*), D – Dawata (*Carallia brachiata*)

One gram of plant matter from each leaf combination was separately added to black-coloured plastic cups (250.0 mL) having 200.0 mL of aerated water. Twenty, first instar larvae of *Ae. albopictus* were added to each container (Reiskind et al. 2012). The different microcosms were placed in separate mosquito rearing cages with the controls. Only the aerated water was used for the controls, and no leaf matter was added. The experiment was repeated three times. Larval mortality, total larval duration, pupal duration, and fecundity of the emerged adults were recorded in each treatment.

Statistical analysis

Statistical analyses were performed using MINITAB 14 (Minitab Inc. 2004). All the data were first tested for homogeneity of variance. When variances were homogeneous, it proceeded with a one-way analysis of variance (ANOVA) to identify the effect of the decaying rate of a single plant species leaf infusions on oviposition preference and the impact of single leaf species and combination of leaf species on the growth performance of *Ae. albopictus.* Tukey's test was used to determine the differences among sample means for significance. Consequently, Pearson's correlation was applied to determine the functional relationship between the number of eggs laid by *Aedes albopictus* with the number of decaying days of each leaf species.

3. Results

The number of decaying days of *A. indica* ($F_{7,56}$ = 138.05, P = 0.0001), *P. suberifolium* ($F_{7,56}$ = 328.20, P = 0.0001), *S. macrophylla* ($F_{7,56}$ = 139.82, P = 0.0001) and *C. brachiata* ($F_{7,56}$ = 226.47, P = 0.0001) had a significant effect on the oviposition preference of *Ae. albopictus*. Accordingly, the mean number of eggs laid showed a positive correlation with the number of decaying days of each leaf species. (*A. indica*: r = 0.985, P = 0.0001; *P. suberifolium*: r = 0.992, P = 0.0001; *S. macrophylla*: r = 0.977, P = 0.0001; *C. brachiata* leaf: r = 0.985, P = 0.0001). The highest number of eggs were recorded in all the containers with 14-days decayed leaf infusions. However, *P. suberifolium* and *C. brachiata* leaf infusions resulted the highest number of eggs each decaying day (Fig. 3.1).

Besides, the current study showed that different leaf species had a significant effect on larval mortality ($F_{4, 175}$ = 898.51, P = 0.0001), total larval ($F_{4,175}$ = 29.75, P = 0.0001), and pupal duration ($F_{4,175}$ = 562.88, P = 0.0001). The mean total larval mortality and total larval duration were highest in *A. indica* and were lowest in *S. macrophylla* (Fig. 3.2a and 3.2b). Similarly, the highest mean pupal duration was observed with *A. indica*, and the lowest was observed in both *P. suberifolium* and *S. macrophylla* (Fig. 3.2c).

Furthermore, the wing length of the emerged adults was significantly affected by different plant leaf infusions ($F_{4,175}$ = 73.51, P = 0.0001). Accordingly, the highest mean wing length was observed in adults who emerged from *P. suberifolium* leaf infusion and the lowest in *A. indica* leaf infusion (Fig. 3.2d).

Moreover, the combinations of leaf species significantly affected the larval mortality ($F_{11,192}$ = 859.85, P = 0.0001), total larval duration ($F_{11,192}$ = 13.15, P = 0.0001), pupal duration ($F_{11,192}$ = 15.67, P = 0.0001) and fecundity ($F_{11,192}$ = 22.23, P = 0.0001) of *Ae. albopictus*. High larval mortalities were observed in *A. indica* - *C. brachiata* (ND) and *A. indica* - *S. macrophylla* (MN) leaf combinations, while low larval mortalities were observed in *S. macrophylla* - *P. suberifolium* (MW), *S. macrophylla* - *C. brachiata* (MD) and *S. macrophylla* - *A. indica* - *C. brachiata* (MND) leaf species combinations (Fig. 3.3a). In contrast, the combined effect of leaf species was significantly different from that of the impact of a single leaf species. However, larval mortality was still high when *A. indica* was combined only with *S. macrophylla* (MN) or *C. brachiata* (ND). The highest larval and pupal durations were observed in the combination of leaf species *S. macrophylla* - *A. indica* - *P. suberifolium* (MNW), while the lowest larval and pupal duration was observed in the leaf species combination of *A. indica* - *C. brachiata* (ND) (Fig. 3.3b and 3.3c). Hence, it is obvious that the controlling effect of *A. indica* is drastically reduced due to the chemical compounds produced by the decomposition of *C. brachiata* and *S. macrophylla.* On the other hand, this indicates that the chemical compounds produced by the decomposition of *A. indica* leaf species can minimise the effect of both *P. suberifolium* and *S. macrophylla* species.

The highest wing length was observed in the adults who emerged from the leaf species combination of *S. macrophylla* and *P. suberifolium* (MW), while the lowest mean wing length was observed in the adults who emerged from the leaf species combination of *A. indica* and *C. brachiata* (ND) (Fig. 3.3d). Therefore, the combination of MW affects positively, and the combination of ND negatively affects the emerged adults' fecundity. Moreover, relatively high fecundity was observed when all the four types of leaf species were combined. It represents that the controlling ability of *A. indica* on the adult fecundity is reduced by *C. brachiata*, *P. suberifolium* and *S. macrophylla* leaf species when they are decaying together.

4. Discussion

The current study revealed that the decaying duration of different leaf species significantly affected the oviposition preference of gravid female *Ae. albopictus*. The degree of decomposition is high when the number of decaying days increases. Therefore, the present study indicates that the gravid *Ae. albopictus* show a more significant attraction towards the ovipositing sites comprised of highly decayed leaf species. Hence, high levels of decayed products in tree holes attract gravid females as it signals the availability of sufficiently good quality food (Paradise and Kuhn, 1999). Although decaying leaf detritus is the primary source of organic carbon for the container inhabitants, the nutritional quality of the leaf substrate varies widely owing to the associated microbial flora and their decomposition rates (Dieng et al 2002). Therefore, the nutrient concentration of the breeding site is related to the decomposition rate of the leaves, as rapidly decomposing leaves are known to produce higher nutrient concentrations (Juliano, 2009; Dieng et al. 2002).

Moreover, the microbial communities produce non-volatile and volatile chemicals through the decomposition of detritus, and those compounds serve as semiochemicals that mediate the selection of an oviposition site (Ponnusamy et al. 2008). In the current study, a specific strong aroma was released by *C. brachiata* during the decomposition, which may be due to the volatile chemicals produced. Consequently, *Ae. albopictus* laid significantly more eggs in *P. suberifolium* and *C. brachiata* than in *A.*

indica and *S. macrophylla* leaf infusions. Although *A. indica* leaf infusion produced a strong aroma during the decaying process, the number of eggs laid was low compared to the other leaf infusions. This may be because the aroma of *A. indica* leaf infusion does not stimulate the mosquitoes to lay eggs there.

Although pupa is a non-feeding stage, favourable environmental conditions within the habitat are required for the emergence of adults. The variation of the life-history parameters is because the decomposition of leaf matter of *A. indica* produces chemicals that reduce the growth performance, while *S. macrophylla* and *P. suberifolium* produce chemicals that enhance the growth performances of larvae and pupae of *Ae. albopictus*. Furthermore, larval growth and survival are high in leaf species with faster decay rates (Dieng et al. 2002). Consequently, the increased developmental period in the slow decaying leaf matter will lead to smaller population sizes of mosquitoes (Dieng et al. 2002).

As wing length was used as an estimate of the fecundity, the mosquitoes from *P. suberifolium* had the highest fecundity. In contrast, the mosquitoes from *A. indica* leaf infusion had the lowest fecundity. This is because *P. suberifolium* is a rapidly decomposing leaf species that produce nutrient-rich microcosms. The nutrients enhance the growth of larvae and ultimately make adults who have a larger body size (Blackmore and Lord, 2000).

Even though microcosms of *S. macrophylla* showed the lowest larval mortality, larval duration, and pupal duration, the fecundity of the emerged adults has become low due to the increased competition among larvae. Also, relatively higher fecundity and larval mortality were shown by *C. brachiata* leaf species. This is due to a decrease in larval population density, thereby decreasing the competition. Since the wing measurements were taken immediately after the emergence of adults in which they have not taken any food for development, their development is entirely due to the egg nutrients and larval feed. However, environmental factors such as temperature and chemical composition of water may influence embryo development and directly affect the wing length.

Accordingly, *A. indica* plant leaves can weaken the overall growth performance by increasing larval mortality, total larval duration, pupal duration, and decreasing fecundity. Moreover, a study conducted by Reiskind et al. (2009) showed that larval survival and growth performance of *Ae. albopictus* is affected by the plant leaves of *Vitis aestivalis, Quercus virginiana, Psychotria nervosa*, and *Nephrolepis exaltata*. Additionally, the fascinating thing was all the emerged adults in the *A. indica* leaf infusion were male mosquitoes, while the other infusions resulted in the usual male: female ratio. Hence, it gives a new clue to producing sterile males to control the population size of vector mosquitoes.

Conclusion

The mean number of eggs laid shows a positive correlation with the number of decaying days of leaf matter in all the selected leaf species, *S. macrophylla*, *P. suberifolium*, *C. brachiata*, and *A. indica*. Moreover, *A. indica* plant leaves can increase the larval mortality, larval and pupal duration, and decrease the fecundity of the emerged adults *of Ae. albopictus*. Hence, using A. indica plant leaves can be considered to formulate a biological control for *Ae. albopictus.*

Declarations

Authors' contributions

GASMG conceived and designed the experiments, NNMNG conducted all field and laboratory work, performed data analysis, and was the major writer of the manuscript. RAKMG contributed to the data analysis and improvement of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figures

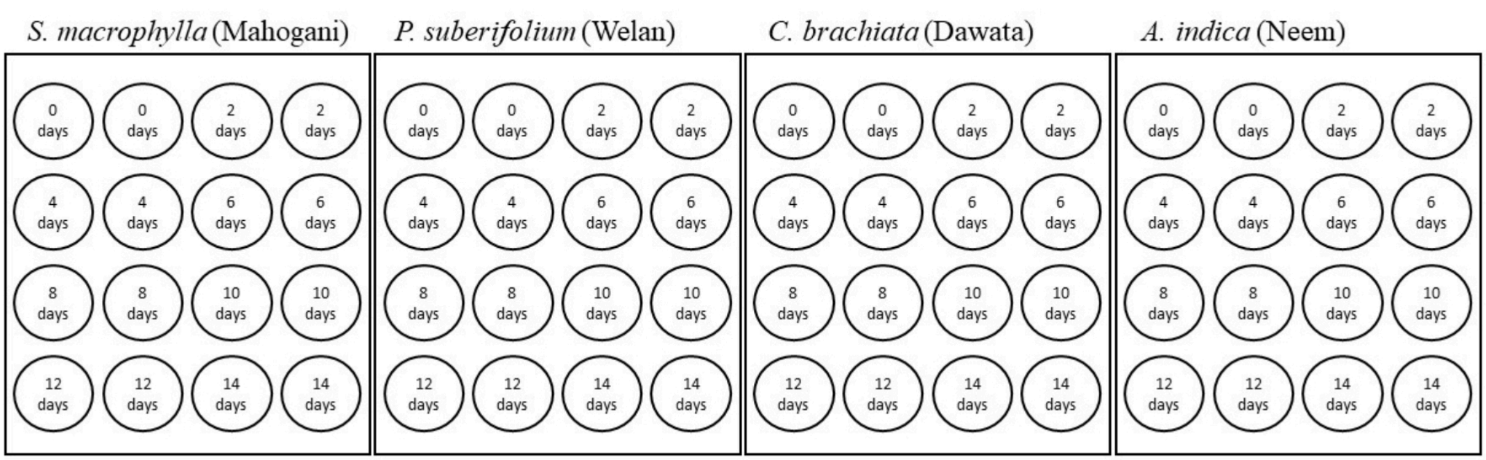

Figure 1

2.1: Arrangement of the microcosms prepared from the leaf species of *Swietenia macrophylla*, *Pterospermum suberifolium*, *Carallia brachiata*, and *Azadirachta indica* in separate mosquito rearing cages

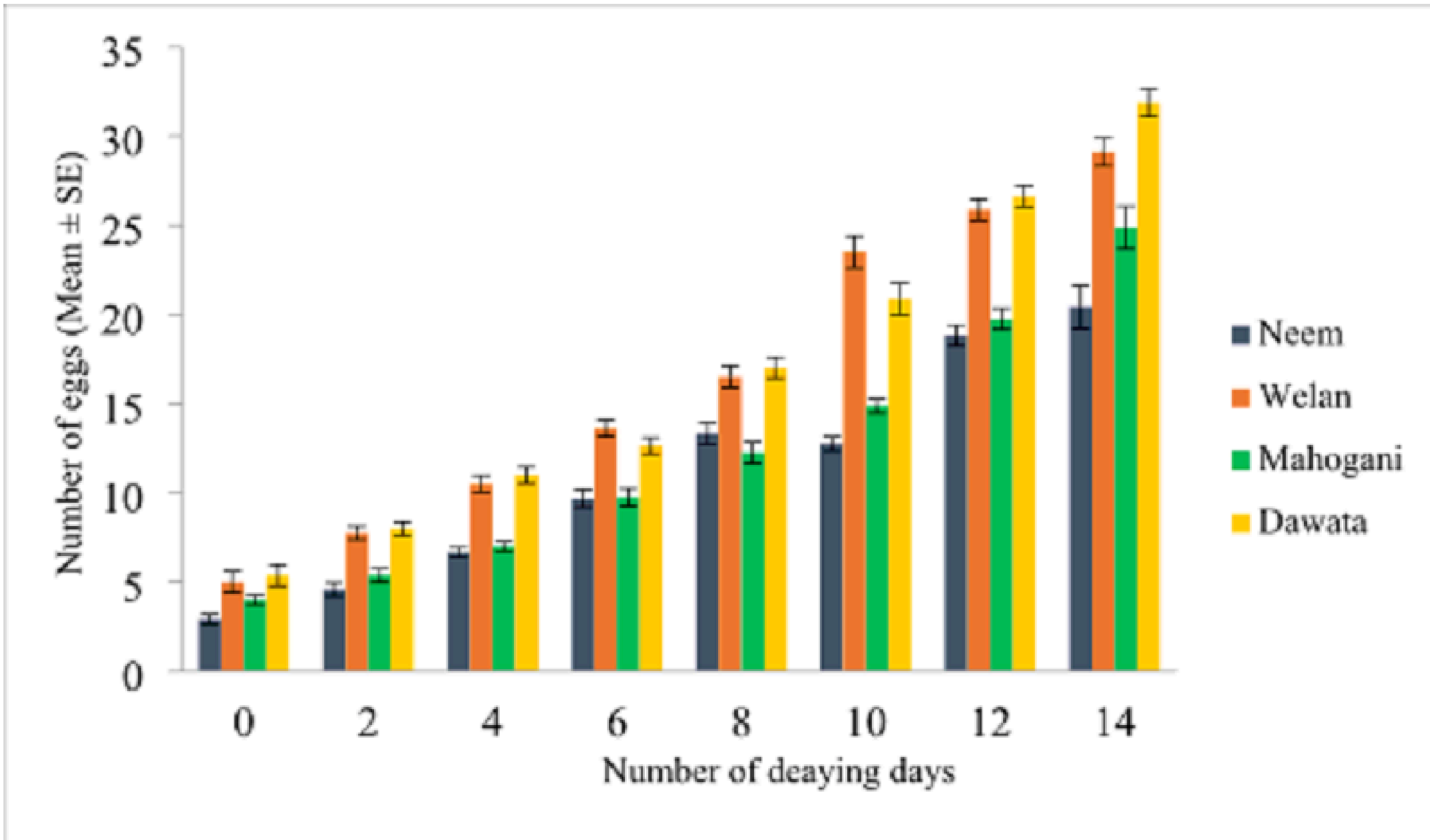

Figure 2

3.1: Mean number of eggs laid by *Aedes albopictus* with the number of decaying days of each leaf species; 0, 2, 4, 6, 8, 10, 12, and 14 days.

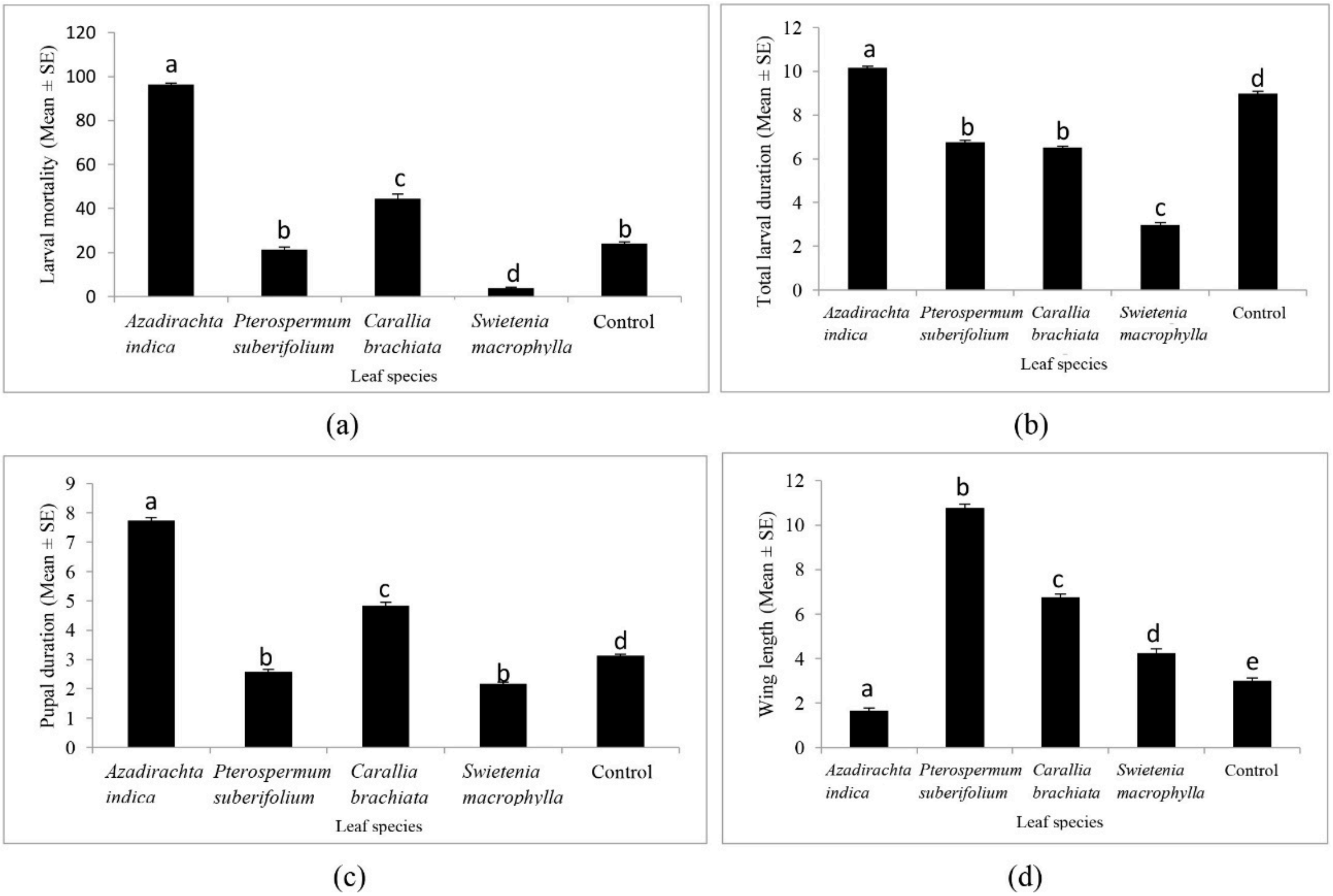

Figure 3

3.2: (a) Larval mortality (b) total larval duration (c) pupal duration (d) adult fecundity of *Aedes albopictus* with different leaf species: *Azadirachta indica*, *Pterospermum suberifolium*, *Carallia brachiata* and *Swietenia macrophylla*.

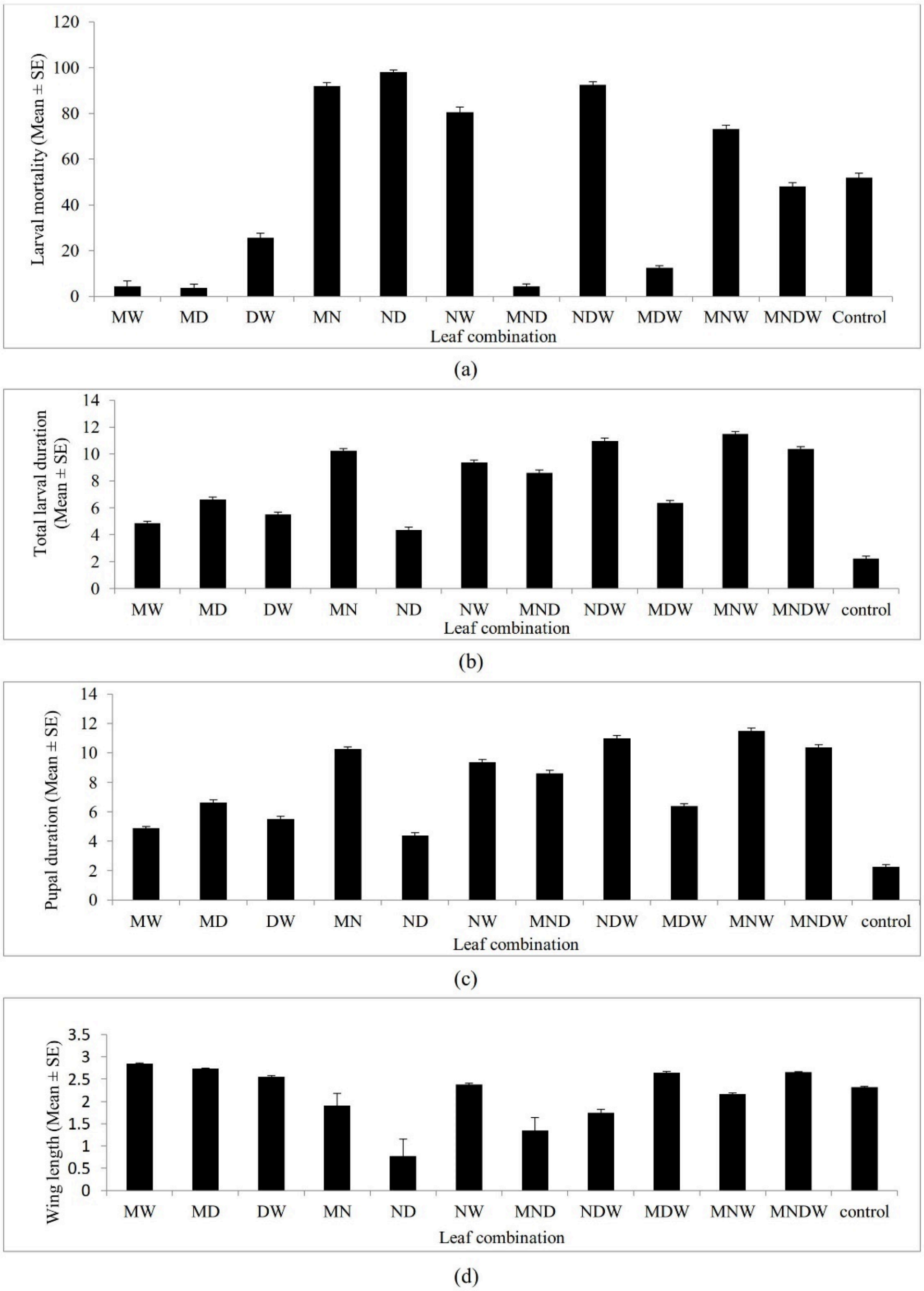

Figure 4

3.3: (a) Larval mortality (b) total larval duration (c) pupal duration (d) adult fecundity of *Aedes albopictus* with different leaf species combinations: *Swietenia macrophylla* - *Carallia brachiata* (MD), *Swietenia macrophylla* - *Pterospermum suberifolium*(MW), *Swietenia macrophylla* - *Azadirachta indica* (MN), *Carallia brachiata* - *Azadirachta indica* (DN), *Carallia brachiata* - *Pterospermum suberifolium* (DW), *Azadirachta indica* - *Pterospermum suberifolium*(NW), *Swietenia macrophylla* - *Azadirachta indica* -

Pterospermum suberifolium (MNW), *Swietenia macrophylla* - *Azadirachta indica*- *Carallia brachiata* (MND), *Swietenia macrophylla* - *Carallia brachiate* - *Pterospermum suberifolium* (MDW), *Azadirachta indica* - *Carallia brachiate* - *Pterospermum suberifolium* (NDW) and *Swietenia macrophylla* - *Azadirachta indica* - *Pterospermum suberifolium* - *Carallia brachiata* (MNWD).