Research Article GU J Sci, Part A, 11(1): 147-155 (2024) 10.54287/gujsa.1424961

Gazi University
Journal of Science
PART A: ENGINEERING AND INNOVATION
http://dergipark.org.tr/gujsa

Examining the Blood-Feeding Interactions During Intra- and Interspecific Adult Competition between *Aedes albopictus* and *Aedes aegypti* -a Laboratory Study

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Keywords

Aedes

Interspecific Interactions

Oviposition

Abstract

Aedes albopictus and *Aedes aegypti* are striped mosquitoes with similar behavioral characteristics. They are known vectors of dangerous arboviruses and are expanding their reach globally. These species have established in specific areas of Türkiye, with *Ae. albopictus* expanding significantly than *Ae. aegypti*. While the larval competition of these two mosquito species has been studied, not much is known about their interactions during adulthood. We first investigated the effects of interspecific and intraspecific larval competition on the survival rate into adulthood and the wing length of females and males in containers of different sizes and food level conditions. This research also explored how females of these mosquitoes compete for blood meals in confined environments and its effects on blood feeding rates and female fecundity. Larval competition hinders *Ae. aegypti* emergence, *Ae. albopictus* remains resilient across varying resource levels. Notably, both species displayed increased blood feeding rates when housed together, suggesting potential facilitation or competitive avoidance strategies. Interspecific pressure caused a decrease in the number of eggs laid in mixed species cages for *Ae. aegypti* only. This study highlights the complex competitive dynamics between *Ae. albopictus* and *Ae. aegypti*. While larval competition appears to affect *Ae. aegypti* emergence. Further understanding of adult interactions is crucial for predicting their co-occurrence and effectively managing their populations, especially as *Ae. albopictus* shows greater adaptability and expansion within Türkiye.

Cite

Bursalı, F. (2024). Examining the Blood-Feeding Interactions During Intra- and Interspecific Adult Competition between *Aedes albopictus* and *Aedes aegypti* -a Laboratory Study. *GU J Sci, Part A*, *11*(1), 147-155. doi:10.54287/gujsa.1424961

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Article Process

Submission Date	24.01.2024
Revision Date	05.02.2024
Accepted Date	09.02.2024
Published Date	19.02.2024

1. INTRODUCTION

Aedes albopictus Skuse, 1894 and *Ae. aegypti* Linnaeus, 1762 (Diptera: Culicidae) are medically important vectors and closely related mosquitoes (Diptera: Culicidae) spreading across the world (WHO, 2022). They are vectors of important arboviruses. Infection from dengue, chikungunya, yellow fever and Zika viruses are threats to public health in endemic areas (Rossati et al., 2015). *Ae. aegypti* originated in tropical Africa and *Ae. albopictus* populations originated from Southeast Asia (Vezzani & Carbajo, 2008). The current global distributions of these *Aedes* species have increased significantly. They are highly adaptive, capable of withstanding ecological changes outside their native range (Lounibos, 2002; Rey & Lounibos, 2015). *Ae. albopictus* has been detected in several coastal provinces in Türkiye including Thrace, Black Sea, and Aegean regions (Akiner et al., 2016; Sakacı, 2021) whereas *Ae. aegypti* distribution is still restricted to northeastern part of Türkiye (Touray et al., 2023).

They share similar ecological niches. Both species breed and thrive in small artificial containers and in tires (Paton & Bonsall, 2019). Their ability to breed in small amounts of water and lay eggs that can survive dry periods for months makes them incredibly difficult to control. *Ae. aegypti* prefers to blood feed on humans, whereas *Ae. albopictus* is an opportunistic feeder, taking blood meals from both humans and animals (Bursali

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& Simsek, 2023). Also, some researchers suggest that these species have distinct environmental preferences: *Ae. aegypti* generally thrive in urban landscapes whereas *Ae. albopictus* prefers suburban areas with higher flora (Heinisch et al., 2019).

Invasive mosquito species can invade new ecosystems and interactions including interspecific competition with native species can influence the ecosystem of invaded areas (ECDC, 2020). Such impacts can be seen in the early invasion of *Ae. aegypti* into Asia which triggered a significant displacement of endemic *Ae. albopictus* from large urban centers (Gilotra et al., 1967). Likewise, In the United States, studies established a competitive advantage of *Ae. albopictus* over *Ae. aegypti* (Braks et al., 2004). Interspecific larval competition among native and invasive mosquito species is one of the primary mechanisms that can cause competitive population reduction or displacement (Lounibos, 2007). When larvae of different species share the same ecological niche, the stronger larvae can negatively impact the growth and development of the weaker larvae through physical or chemical means. The stronger larvae may be better at finding and consuming food, leaving less for weaker larvae or occupy the best spaces in the environment, leaving weaker larvae with less suitable habitat and physically exclude weaker larvae from food sources (Giatropoulos et al., 2022). Hence, interspecific competition between *Ae. aegypti* and *Ae. albopictus* larvae have been widely investigated in the field and laboratory (Murrell & Juliano, 2008) and it has been shown that competitive elimination of *Ae. aegypti* by *Ae. albopictus* mostly relied on the seasonal changes in climate, type of food, and population source (Leisnham & Juliano, 2010; Juliano, 2010).

Both species are reared in various laboratories for scientific research (Juliano, 2010). Rearing in the laboratory holds significant importance for a variety of reasons. Mosquitoes encounter numerous biotic during their lifespan that affect their morphological and physiological characteristics (e.g. body size, blood feeding activity), fitness traits (survival, fecundity) and their population growth (Carrington et al., 2013). These biotic or abiotic factors impact adult mosquito phenotypes as well as their vector competence and transmission (Alto & Bettinardi, 2013). So, rearing mosquitoes enables studying their breeding patterns, feeding preferences, insecticide resistance mechanisms, and susceptibility to pathogens in a controlled environment. Thus, information is crucial and provides valuable insights that can inform disease control strategies. Sometimes, rearing both species in the same laboratory setting poses a significant risk of cross-contamination. Researchers can significantly reduce the risk of cross-contamination by physical separation and implementing strict hygiene protocols to prevent the transfer of immature stages and adult mosquitoes between rearing areas. As mentioned above, several studies have researched the interspecific larval competition between these mosquitoes; but few studies have taken a comprehensive approach of how these mosquitoes compete for blood meals and its effects on blood feeding rates and female fecundity. In this laboratory factorial experimental design study, we assessed the impact of container size, food ration, and their interactions on the mosquito emergence and adult body size of *Ae. aegypti* and *Ae. albopictus* mosquitoes. This study also explores how these mosquitoes compete for blood meals in confined environments and its effects on egg-laying.

2. MATERIAL AND METHOD

2.1. Maintenance of Mosquito Colonies

Aedes albopictus larvae were sampled from Güzelçamlı, Aydin, Türkiye, and *Ae. aegypti* eggs was obtained from the Biology Department of Hacettepe University, Türkiye. These insects are maintained in an insectarium at 70±10 relative humidity, 28±2°C, 12 h:12 h photoperiod, in the Vector Control Laboratory, Aydın Adnan Menderes University, Turkey. Adult mosquitoes were maintained in insect cages (40 × 40 × 40 cm) with ad libitum access to 10% sugary water. Every 2-3 days females were provided with a blood meal using defibrinated sheep blood through a membrane and eggs laid on filter papers in paper cups with water were hatched in tap water and emerged larvae were provided on fish food and maintained at 24 °C. For the experiments, sufficient eggs were hatched synchronously.

2.2. Experimental Design: Larval Competitive Treatments

Experiments were established to assess the impact of container size and food ration on mosquito interaction. The size of the containers was 11 cm length × 10 cm width × 5.5 cm depth filled with 250 ml water as small; 18 cm length × 11 cm width × 6 cm depth with 500 ml water for medium; or 22 cm height × 15.5 cm width ×

8 cm depth with 750 ml water for large. The ratio of the one-by-one interaction of the mosquitoes used (*Ae. aegypti* vs *Ae. albopictus*) was 50:0, 25:25, 0:50 and the interactions were. 0.1 g or 0.15 g of ground Tetramin fish food was added. This resulted in 18 treatment combinations with 2 replicates for a total of 36 containers. Food was added to containers after the first instar larvae were introduced and again 5 d later. All containers were maintained at 27±1°C, 70% RH, and a 12 h dark photoperiod in a cage until eclosion. The developmental rate (ratio of individuals developing into adults) and size of newly eclosed adults were recorded and used as measures of competitive outcome. Newly emerged adults were held for another 24 h at 28°C with access to sugary water until full expansion and sclerotization of the cuticle before measurement of wing span/length which is a proxy for body size of a mosquito (Nasci, 1990; Petersen et al., 2016; Yeap et al., 2013). The adults were freeze-killed, wings were detached, and wing lengths (axial incision to apical margin) of 20 (10 males: 10 females) randomly mosquitoes from each group were measured under a microscope with an ocular micrometer (precision ± 0.03 mm) (Nasci, 1990).

2.3. Blood Feeding and Female Fecundity Experiments

Eggs of both species taken from stock cultures hatched synchronously. Newly emerged female mosquitoes of each species were then housed in cages – either alone (with 25♀ and 15 ♂) or with equal numbers of the other species (*25*♀ and *15* ♂ for each species). These cages were incubated in the insectary for a week before a restrained mouse (*Mus musculus*) was introduced to the cages. This blood feeding procedure was approved by Aydin Adnan Menderes University Ethics committee (Approval number: 64583101/2024/09). The mosquitoes fed for an hour and the number of engorged females was counted to determine the percentage number of blood-fed females. Then 10 females were randomly selected and transferred to a 400-ml screened paper cup with water. The females were left to lay eggs for 4 more days. The number of eggs laid in mixed cages was compared to single cages.

2.4. Statistics

Data on the emergence ratio and adult body size based on the wing length of adult mosquitoes were analyzed using the Shapiro–Wilk normality test and homogeneity of variance to address normal distribution. Differences in adult emergence and adult body size were determined using generalized linear models with competition, amount of food, container size, and their interactions as the main factors taken into consideration. Student t-test was used to compare the differences in the number of blood-fed females and the number of eggs laid per female in the adult experiments. $P<0.05$ was used as the significance level.

3. RESULTS

3.1. Larval Competitive Treatments

Analysis of variance in the adult emergence during competition between *Ae. albopictus* and *Ae. aegypti* are presented in Table 1 and Figure 1. Competition was the only main effect that had an impact on *Ae. aegypti* emergence. Fewer adults were collected from medium and large containers which both species as compared to with only *Ae. aegypti* at high food supply. Besides this, no statistical difference was determined on the effects of competition, food supply, and container size or their interactions on the emergence of both *Ae. aegypti* and *Ae. albopictus* species.

Looking at the size of the adults that emerged after larval competition, *Ae. aegypti* males collected from small containers with mixed species at both low and high food supply were slightly larger than those from containers with single species. Simple main effects of competition, food amount, container size, and their interaction had a statistical impact on *Ae. aegypti* male size (Table 2, Figure 2). No difference was observed in the other container sizes. For *Ae. albopictus* male sizes were similar in all treatments ($P<0.05$).

In the case of female size, *Ae. aegypti* adults from containers with interspecific competition at high food supply were larger than containers with intraspecific competition. At low food, interspecific competition caused a reduction in size as compared to intraspecific competition. For *Ae. albopictus*, female size was found to be larger in containers with interspecific competition regardless of food amount or container type (Table 2, Figure 3).

Table 1. *ANOVA output on Ae. aegypti and Ae. albopictus adult emergence during intra and interspecific competition*

	Ae. aegypti			***Ae. albopictus***		
Source	df	F	P.	df	F	P.
competition	1	6.248	0.02	1	1.09	0.307
ration	1	0.009	0.926	1	0.129	0.723
container	2	3.637	0.042	2	2.102	0.144
competition * ration	1	0.963	0.336	1	0.002	0.967
competition * container	2	3.369	0.051	2	0.309	0.737
ration * container	2	0.229	0.797	2	4.037	0.031
competition * ration * container	2	0.948	0.401	2	1.012	0.379
Footnote: df, degree of freedom; F, F-value; P, significance level (<0.05)						

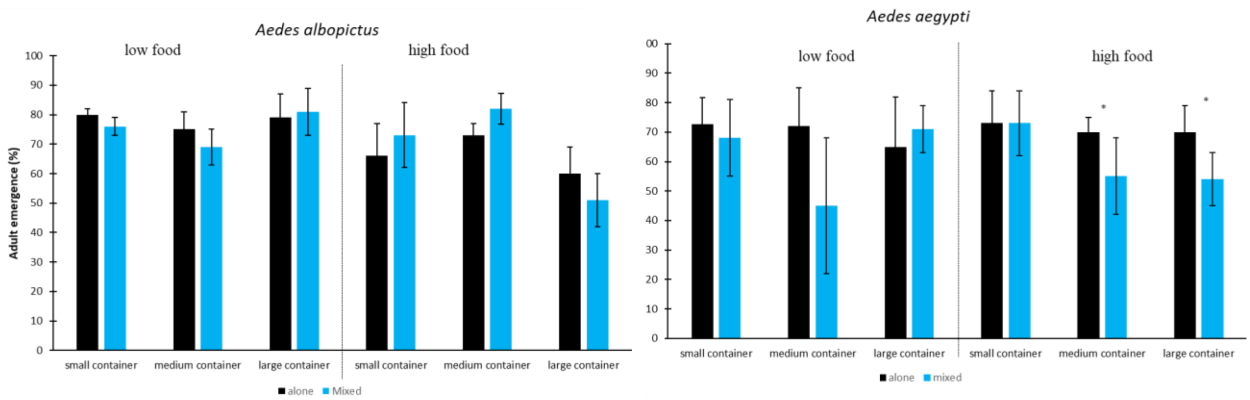

Figure 1*. Adult emergence of Ae. albopictus and Ae. aegypti during intra and interspecific larval competition in different containers and food level. (ANOVA, Tukey's test with significance level P<0.05)*

3.2. Blood Feeding and Female Fecundity Experiments

In these experiments, I investigated the effects of adult competition in confined spaces on blood feeding and female fecundity. Newly emerged female mosquitoes of each species were housed in cages either alone or with equal numbers of the other species. I observed that statistically fewer mosquitoes blood blood-fed in single cages as compared to mixed cases for both species (t=-20.86; df=4; p<0.001) (Figure 4). The number of *Ae. aegypti* that fed in mixed cultures was twice the amount in single cultures. For *Ae albopictus*, 60% of females fed in mixed cultures were as 43% fed in single cages (t=-5.838; df=4; p=0.004). There was a statistical difference in the number of eggs laid by females of *Ae. aegypti* (t=4.437; df=4; p=0.011), no difference was observed for *Ae. albopictus* (t=-1.106; df= 4; p=0.331) (Figure 5).

4. DISCUSSION

This study explored the differential competitive effects during larval competition. Results showed that interspecific competition hinders *Ae. aegypti* emergence, whereas *Ae. albopictus* remains resilient across varying resource levels. This disparity suggests *Ae. albopictus'* greater adaptability, a crucial factor in its observed wider reach in Türkiye.

There are numerous studies on the interspecific competition for space and food resources between *Ae. albopictus* and *Ae. aegypti* larvae, both in the field and laboratory. These studies have shown that competition during the larval stages can impact various growth and development parameters of weaker competitors (Juliano, 2010, Reiskind et al., 2012; Yan et al., 2021). Besides food, factors including seasonal changes in climate, and population origin can also influence this interaction (Leisnham & Juliano, 2010). Among these studies, Noden et al. (2016) comprehensively explored the influence of intra- and inter-specific larval competition on *Ae. aegypti* and *A. albopictus* adult mosquito traits. They measured the impact over the entire life of these mosquitoes and reported that competitive pressures influenced adult emergence and development time for both species' females. Only the median wing length of *Ae. albopictus* females only were affected. They also demonstrated that adults collected from these competitive treatments had no effects on the blood feeding and reproductive success of the mosquitoes. Steinwascher (2020) found that *Ae. aeygpti* intraspecific competition was sex-based, females competed with females, and males with males, and an increase in density caused an increase in competition. The author also showed that the amount and timing of food inputs alter mosquito growth and competition, with effects varying among the sexes. In another study, Yan et al. (2021) provided high or low amounts of larval food to *Ae. aegypti* larval stages and found that low larval nutrition differentially influences female mosquito life history traits, i.e. adult survival, size, and fecundity, and that a positive connection existed between fecundity and size. Similarly, other studies on the interspecific interactions of *Aedes* spp. with other mosquito species have shown that interaction influences the development time, survival, adult body size, vectorial competence, and capacity of less competitive container-dwelling mosquitoes (Lizuain et al., 2022). Giatropoulos et al., (2022) reported that, *Ae. albopictus* larvae outcompeted and developed faster than larvae of *Ae. cretinus* especially when the food amount was low.

Also, I observed an unexpected adult interspecific dynamic. Both *Ae. albopictus* and *Ae. aegypti* show increased blood feeding rates when housed together, hinting at potential resource facilitation or competitive avoidance strategies. This contradicts the expected outcome of interspecific competition and warrants further investigation into the underlying behavioral or ecological factors driving this interaction. Species-specific impact on reproduction showed that while egg-laying of *Ae. aegypti* decreased under interspecific pressure, *Ae. albopictus* remains unaffected. This suggests differential vulnerability at the reproductive stage, which could influence population dynamics and disease transmission. This is the first study to assess the effects of adult competitive dynamics under confined spaces on blood feeding rates and reproduction (female fecundity). Other factors could influence *Aedes* population dynamics and virus transmission. Schmidt et al. (2018) examined the impact of desiccation stress on the survival of female *Ae. aegypti* and *Ae. albopictus* mosquitos and reported that humidity impacts the survival of female *Ae. aegypti* in controlled settings. Further research into the underlying mechanisms of these observed dynamics is crucial for effective control strategies.

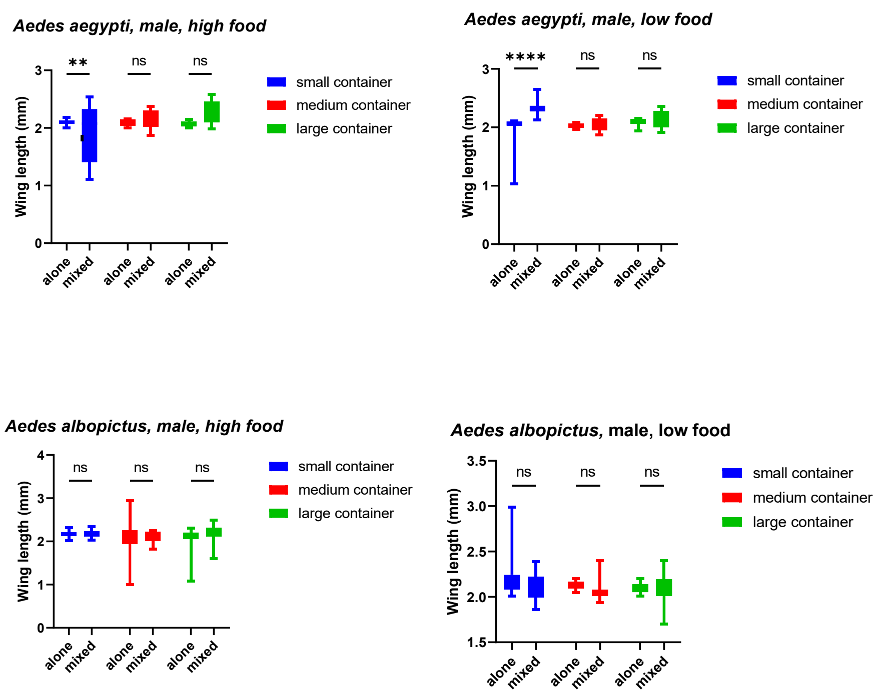

Figure 2**. Male wing length of Ae. aegypti and Ae. albopictus during intra and interspecific larval competition in different containers and food level. ns, non-significant; P ≤0.05*, P ≤0.01 **, P ≤0.001*

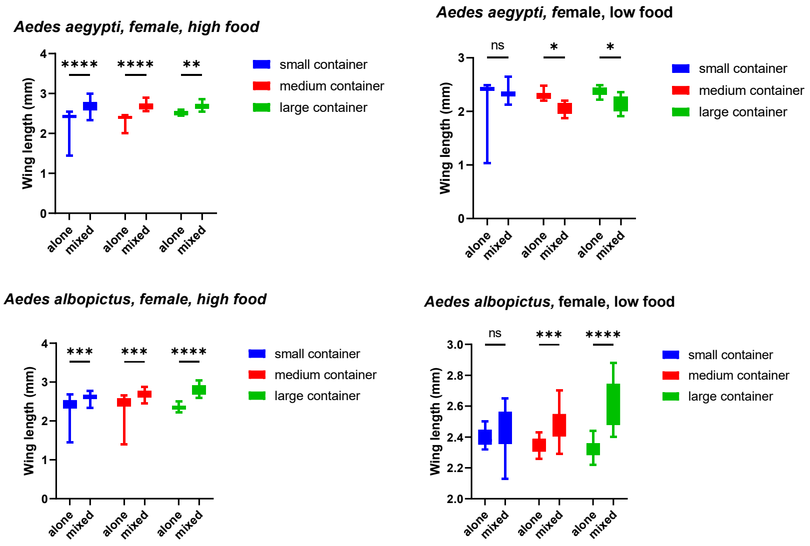

Figure 3. *Female wing length of Ae. aegypti and Ae. albopictus during intra and interspecific larval competition in different containers and food level. ns*, non-significant*; P ≤0.05*, P ≤0.01 **, P ≤0.001****

Table 2. *Analysis of variance output on Ae. aegypti and Ae. albopictus wing length during intra and interspecific competition*

Source	***Ae. aegypti***						***Ae. albopictus***					
	male			**female**			**male**			**female**		
	df	F	*p*	df	F	*p*	df	F	*p*	df	F	*p*
Competition	1	43.1	<0.001	1	41.826	<0.001	1	2.466	0.118	1	4.926	0.028
Food amount	1	6.544	0.011	1	0.321	0.572	1	2.623	0.107	1	4.38	0.038
Container	2	12.113	<0.001	2	24.496	<0.001	2	0.74	0.479	2	4.499	0.012
Competition * food amount	1	0.489	0.485	1	67.843	<0.001	1	0.049	0.825	1	4.51	0.035
Competition * container	2	5.007	0.008	2	26.859	<0.001	2	6.065	0.003	2	4.569	0.012
Food amount * container	2	2.573	0.079	2	21.164	<0.001	2	1.358	0.26	2	4.47	0.013
Competition * food amount * container	2	5.764	0.004	2	16.55	<0.001	2	3.37	0.037	2	4.41	0.014

Footnote: df, degree of freedom; F, F-value; P, significance level (<0.05)

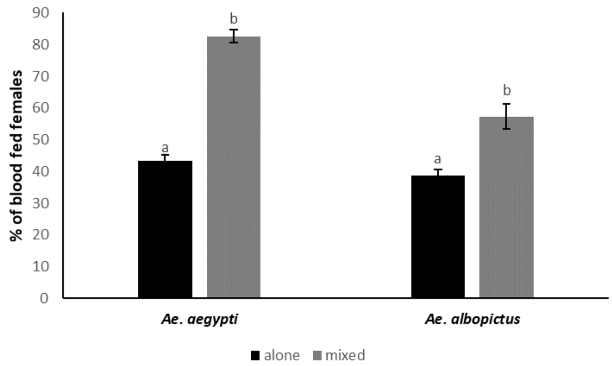

Figure 4. *The impact of adult competition on blood feeding rates of Ae. aegypti and Ae. albopictus. Different letters above bar indicate statistical significance*

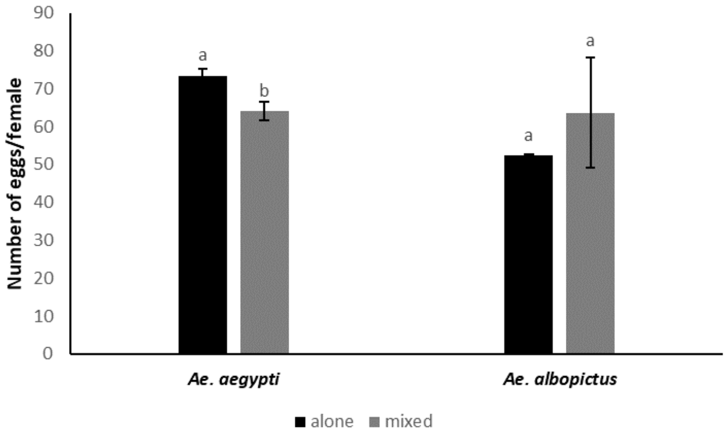

Figure 5. *The impact of adult competition on female fecundity of Ae. aegypti and Ae. albopictus. Different letters above bar indicate statistical significance*

5. CONCLUSION

Understanding the complex competitive dynamics between these mosquitoes allows for more accurate predictions of their co-occurrence and spread patterns. By highlighting *Ae. albopictus'* resilience and *Ae. aegypti's* susceptibility to competitive pressure, this study informs targeted control measures in regions facing both species, reducing the risk of arbovirus transmission in Turkey and globally.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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