

SOME PRELIMINARY INVESTIGATIONS OF CANNIBALISM IN MOSQUITO POPULATIONS COLLECTED FROM URBAN AREAS OF DISTRICT SHEIKHPURA, PAKISTAN

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Abstract In the population of mosquitoes, there is substantial research documentation on cannibalism between larval stages therefore, in our research work cannibalism in mosquito populations was studied in the laboratory experiments comparing old (third and fourth-instars) and young (first-instar) larvae of the mosquito species occurring dominantly in urban areas of District Sheikhpura. Although mosquitoes can grow in temporary aquatic habitats having wildly varied topologies, the significance of the site shape in which cannibals and victims coexist has not received enough attention from researchers. Then, experimental trials in the containers were performed, and the cannibalism rate was estimated at 24 and 48hrs. The effect of spatial form on cannibalism in mosquito larvae of the *A. albopictus* species was assessed, adding a new essential element to those influencing ecological interactions in these habitats. Our data reveals that cannibalism was observed mostly between the fully mature and fully immature larvae of the mosquito population and a decline was observed between the mature population. These basic findings of our work would help devise sustainable control strategies for mosquito population (*A. albopictus* and *A. aegypti*) etc. in vector management programs.

Keywords: mosquitoes; cannibalism; mature population; larvae; sustainable control

Introduction

Mosquitoes consist of around 3,500 species, some of them acting as major disease vectors for both animals and humans. During the immature stages the young ones of mosquitoes present in aquatic environments only for a short period where many intra-specific and inter-specific interactions take place and rapid change in conditions create violent competition resulting in cannibalistic behavior among the individuals (Juliano, 2009; Andreadis and Wolfe, 2010; Silberbush *et al.*, 2014; Urbanelli *et al.*, 2014; Tsurim and Silberbush, 2016; Clements, 1999; Santana AFK, 2012). One of the main advantages of cannibalism is that it frequently leads to increased development and survival in environments with high competitor density and limited supply of food. Consuming conspecific tissues may result in growth that is larger than that produced by eating heterospecific tissues. Instead of these advantages, cannibalism has a significant potential cost in terms of harm or fatality, especially when someone tries to eat a conspecific of a comparable size. (Elgar, 1992).

As a result, cannibalistic encounters frequently involve big individuals attacking and eating smaller, more susceptible conspecifics.

The act of cannibalism involves eating or killing a whole conspecific individual or any part of it (Fox, 1975). The species of both invertebrates and vertebrates show this type of intraspecific interaction between predators and prey, and it is observed across taxonomical categories (Crespi, 1992). Numerous studies have examined insect Cannibalism and its ecological impacts (Richardson *et al.*, 2010). Observations showed that cannibalism greatly decreased the size of the population which contributes to population self-regulation, and the possibility of extinction is reduced.

In addition, studies have shown that cannibalism makes populations more resilient to environmental stress since those cannibalistic individuals who survived attacks were found more successful than others (Tayeh *et al.*, 2014). Additionally, studies have shown that cannibalism reduces propagule

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abilities to disperse, consume nutrients, and influence their ability to colonize and persist in new stressful conditions (Osawa, 1993).

In several mosquito species, the density and duration of contact among larvae play a role in affecting cannibalism as well (Koenraadt and Takken, 2003). According to observations during the late instars (third and fourth instar) when food was available without any restrictions as compared to when it was restricted, then cannibalism was shown to increase in the mosquito species *Armigeres subalbatus* (Rajavel, 1992). There is a probability that observing a vulnerable individual may account for cannibalism in some cases instead of any of the factors listed above. According to studies of the mosquito species *Anopheles arabiensis* and *A. gambiae* s.s. It was not the reduction in food supply that increased cannibalism among elderly fourth and first instar larvae, but a lack of space that made it more likely to happen. However a lack of space probably enhanced larval cannibalism in mosquito species by interacting more frequently within smaller spaces (Koenraadt et al., 2004). Furthermore, short and large containers exhibit less cannibalism between intra-instar larvae of *Toxorynchites ambinensis* than tall and thin ones (Annis et al., 1990).

The Sites which are of spatial shape containing both cannibals and victims seem to influence the rate of cannibalism by influencing how many encounters individuals have with each other. Cannibalism rate can also be affected by spatial shape because cannibalistic attacks can succeed or fail according to location, affecting the chance of the victims escaping. Generally, cannibals who encounter victims in open water are much more likely to escape than those who encounter them in enclosed spaces for instance, within a corner of a container or near the surface of water (Porretta et al., 2016). Although the mosquito breeding sites, which lead to wide variations in larval cannibalism in nature depending on dimensions and origins (for example; man-made containers, small water pools, tree holes, and cattle hoof prints) and its cannibalization role among larvae of mosquitoes remains largely unexplored. Using the tiger mosquito *Aedes albopictus* as the model we hope to investigate the issue.

Over the last several decades, the *Aedes albopictus* population has spread over Europe, Australia, Africa, and the Americas like wildfire from its native range in East Asia (Porretta et al., 2012). Several animal and human diseases are transmitted by these species (Bonizzoni et al., 2013), typically they grow in tree cavities and artificial containers of any shape and material, such as rubber tires and flower buckets commonly present in suburban and rural locations (Hawley, 1988). There have been no studies that have examined the cannibalistic behavior of this

species during larval development. In this case, the aim is to i) Analyze if young and old larvae cannibalize each other ii) To look at the impact of spatial forms on cannibalism. Then, experiments were conducted on cannibalism to estimate the rate of cannibalism at two points (24 and then 48 hours) by using first, third, and fourth-instar larvae present in containers.

Materials and methods

Cannibalism experiments

Collection of Mosquitoes

The mosquito populations prevalent in the urban areas of District Sheikhpura and the main campus of the University of Agriculture, Faisalabad were collected by using ovitraps and kept in a growth chamber in the lab. Several plastic trays filled with 800 ml of distilled water which are (5 cm, 30 cm, 19 cm) used to place larvae (Fig 1).



Fig. 1 Larval collection from District Sheikhpura

These trays in fig. 2 were maintained in a lab at the temperature of $27 \pm 2^\circ\text{C}$ with an approximate relative humidity of $75 \pm 10\%$ and a photoperiod of LD (16:8 h). Crushed 0.33 g dry cat food (Friskies1Adult) mixed with 800 ml of water and this mixture was used to feed larvae (Mastrantonio et al., 2018). We used morphological keys to identify the enclosed adults (Schaffner et al., 2001) and fed them with 10% sucrose solution every day in cages that measure (40×40×40cm). Fresh mechanically defibrinated bovine blood is supplied to female mosquitoes for feeding purposes (Mastrantonio et al., 2018). Females laid eggs on paper towels in water-filled cups. Once the whole egg laying is done for the performing of cannibalism experiment these paper towels are dried and stored at 27°C .



Fig. 2: Dry cat food (Friskies1Adult)



Fig. 3: Crushed dry cat food mixed with 800 ml of water to feed larvae



Fig. 4: Female mosquito feeding on the blood of Albino rat

For experiments the raised colonies were kept at the same humidity, temperature, and lighting conditions. According to above discussed rearing conditions during the experiments, fish food was provided (0.85mg/larvae at the onset of the experiment and after 24 hours) and one larva per milliliter, with no food shortage. Cannibalism between larvae of the first and fourth instar (L1 and L3) as well as between larvae of the third and first instar (L4 and L1) which is more than (48hours old) were written down. The effect of container shape on the cannibalism rate was

also examined. To reproduce common breeding sites of *Aedes albopictus* three distinct surface/water column ratios in plastic containers were used e.g. artificial containers, flowerpots as well and flowerpot dishes: a container of 6×6×12 (hereafter referred to as a tall and thin container); a 12.5×12.5×4.5 cm container (hereafter a medium container) and a container of 25×25×8 cm which is referred as a low and wide container. Each container that had been filled with 150 ml of distilled water was used to place L1 (15) larvae and (1) L3 or (1) L4 larvae.



Fig. 5: Collection of cannibalized first instar of mosquito larvae

Exposure of mosquito population for different time period and temperatures

i. 24 Hours at 27 ± 2°C

Container Shape	No of Required Larvae
Tall and thin	1 (L3) Mature+ 20 (L1)Young
Intermediate	1 (L3) + 20 (L1)
Low and wide	1 (L3) + 20 (L1)
Tall and thin	1 (L4) + 20 (L1)
Intermediate	1 (L4) + 20 (L1)
Low and wide	1 (L4) + 20 (L1)
control	L1 20Young once + 0(L3) 0(L4)

ii. 48 Hours at 27 ± 2°C

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Container Shape	No of Required Larvae
Tall and thin	1 (L3) Mature+ 20 (L1)Young
Intermediate	1 (L3) + 20 (L1)
Low and wide	1 (L3) + 20 (L1)
Tall and thin	1 (L4) + 20 (L1)
Intermediate	1 (L4) + 20 (L1)
Low and wide	1 (L4) + 20 (L1)
control	L1 20Young once + 0(L3) 0(L4)

15 L1 larvae were placed in each container without L3/L4 larvae as a control for each treatment. To examine larval decay and disappearance further control experiments by the use of dead larvae. Experimental containers were used to place fifteen larvae of L1 which were killed at -80°C according to the same experimental conditions described above.



Fig. 6: Cannibalism between young old larvae For 24 and 48 Hours at 27 ± 2°C

Container Shape	No of Required Larvae
Tall and thin	1 (L3) Mature+ 20 (L1)Young+1 (L4)
Intermediate	1 (L3) + 20 (L1) +1 (L4)
Low and wide	1 (L3) + 20 (L1) +1 (L4)
Tall and thin	1 (L4) + 20 (L1) +1 (L3)
Intermediate	1 (L4) + 20 (L1) +1 (L3)
Low and wide	1 (L4) + 20 (L1) +1 (L3)
control	20 (L1) only

After 24 and 48 hours we counted missing larvae, during this period larvae remain in an instar until they moult into next. Experimental containers of every shape were used for each experimental condition (pseudoreplicates) and repeated the experiment three times (biological replicates).

Data analyses

After 24 and 48h from the beginning of the experiment, the total number of larvae named L1 in

every container has been computed. Missing larvae L1 could be taken into consideration that they are cannibalized by larvae L3 or L4 (Porretta et al., 2016). By putting cannibalism as a binary response variable, logistic regression is used to study the effects of experimental components and their compounds on cannibalism rate. All components which are applied for the experiment were: (L3 and L4) larval instars; (tall and narrow 1, low and wide 2, and medium 3) container form and 24 and 48 hours is the time of exposure between larvae). For determine the model's fit to the completely examined data, the Hosmer goodness of fit tests were implemented (Hosmer et al., 2013). The rate of combination on cannibalism and the effect of every experimental factor was analyzed via using the statistical model ANOVA. All analyses were carried out by using the 3.02 R version of the software (Team, 2013).

Results

Section 4.1. Analysis of variance parameters of Cannibalism in mosquito species F2 generation: collection of Different larval instars, emergence of L1 larvae, dead and live ratio of fist instar, l3 larvae presence, container shape (encoded as: tall and thin = 1, low and wide = 2 and intermediate = 3) required food and cannibalized larvae at the period of 24 and 48 hours.

- This experiment was conducted for 24 48 hours according to mosquito life cycle as the emergence of first instar L1. During the experiment temperature of trial area was maintained 27 ± 2°C, 75 ± 10% relative humidity (RH) and an L:D 16:8 h photoperiod. Analysis of variance for cannibalized L1 larvae by older instar larvae L3 at the time intervals of 24 and 48 hours was carried out (Table 1).
- As the value of p is less than 0.05 so the results are significant. The number of larvae were remained in control was significantly different from cannibalized larvae. The dead larvae at control level were zero.
- In figure 7 and 8 graphs represent the amount of cannibalized and alive larvae in different shapes of the container and show the significant results of cannibalism in each size of the defined container while the temperature remained same at each level.

More larvae consumed after 48 hours as compared to 24 hours at tall and thin least cannibalism occurs at intermediate containers.

We observed the highest cannibalism rate in the container with the highest number of encountered

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larvae. We found also that spatial shape can affect cannibalism not only by affecting the number of encounters but also the number of encounters “favorable” for cannibalistic events. There were no missing larvae in the control testing after 24 or 48 hours. In the test trials instead, L1 larvae disappearance was observed under all experimental conditions, while no L3 larvae disappearance was observed. Similarly, the length of contact between old and young larvae (24 and 48 hours) affected the cannibalism rate solely in the tall and thin containers. Our findings supported the hypotheses, demonstrating that in all trials including old and

young larvae, the rate of cannibalism differed considerably between the three container types, with the tall and thin container having the greatest rate. Following the tall and thin containers, the low and wide containers had a higher rate of cannibalism than the intermediate containers (Table 1). As a result, ANOVA and graphical representation revealed that container design has a significant effect on cannibalism rates by influencing the frequency of meetings between individuals.

Table 1 Analysis of variance for number of cannibalized larvae at different stages

Sr No	Character	MSS of treatments
01	Number of cannibalized larvae I1 by I3 in different size of containers after 24 hours.	25.0**
02	Number of cannibalized larvae I1 by I3 in different size of containers after 48 hours.	34.7**
03	Number of cannibalized larvae I1 by I4 at different size of containers after 24 hours.	33.3**
04	number of cannibalized larvae I1 by I4 at different size of containers after 48 hours.	18.2**
05	number of cannibalized larvae I1 by I3 and I4 collectively in each container of different sizes after 24 hours	48.9**
06	number of cannibalized larvae I1 by I3 and I4 collectively in each container of different sizes after 48 hours	101.3**
07	number of cannibalized larvae I1 by I3 and I4 collectively in each container of different sizes after 24 hours	215.26**
08	L1 after 24 hours	179.0**
09	L1 after 48 hours	302.3 **

Table 2 Tukey test for number of cannibalized larvae at different stages

Sr No	Character	MSS of treatments
01	Tukey's 1 Degree of Freedom Test (24 hrs)	2.83**
02	Tukey's 1 Degree of Freedom Test (48 hrs)	5.25

Table 3 LSD All-Pair wise Comparisons Test of decomposed larvae for treatment

TREATMENT	Mean	Homogeneous Groups
T1	20.000	A
T2	12.333	B
T3	10.667	C
T4	9.667	C

Table 4 LSD All-Pair wise Comparisons Test of L1 for treatments

TREATMENT	Mean	Homogeneous Groups
T1	20.000	A
T2	15.000	B
T3	13.000	C
T4	11.000	D

All 4 means are significantly different from one another.

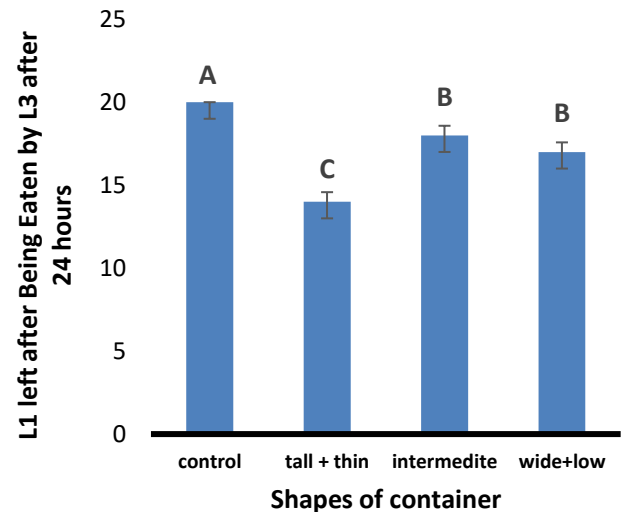


Fig 7: Variation of Cannibalism Rate in I1 larval instar by L3 at different size of containers after 24 hours. As more larvae I1 present at intermediate containers after 24 hours so less cannibalism. More cannibalism was occurred at tall and thin container.

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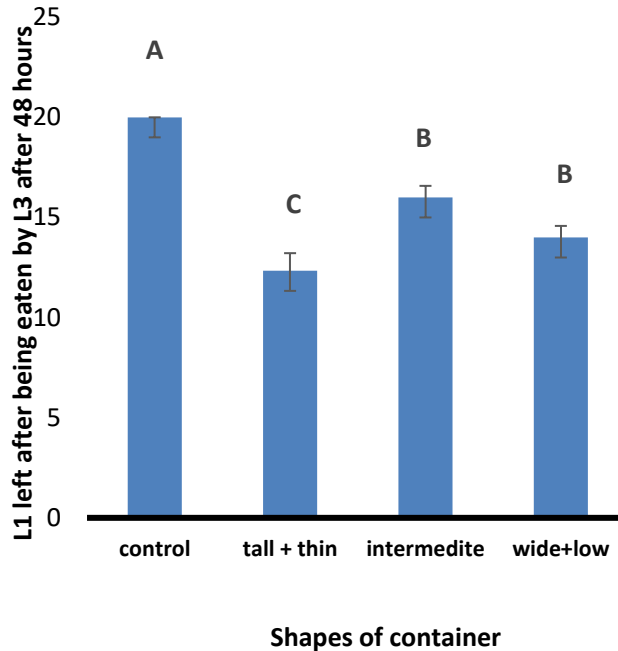


Fig 8 Variation of Cannibalism Rate in I1 larval instar by L3 at different size of containers after 48 hours. As more larvae I1 present at intermediate containers after 48 hours so less cannibalism. More cannibalism occurred at tall and thin container

Section 4.2: Analysis of variance parameters of Cannibalism in mosquito species F2 generation: collection of Different larval instars, emergence of L1 larvae, dead and live ratio of fist instar, 14 larvae presence, container shape (encoded as: tall and thin = 1, low and wide = 2 and intermediate = 3) required food and cannibalized larvae at the period of 24 and 48 hours.

- This experiment was conducted for 24 to 48 hours according to the mosquito life cycle as the emergence of first instar L1. During the experiment temperature of the trial area was maintained $27 \pm 2^{\circ}\text{C}$, $75 \pm 10\%$ relative humidity (RH) and an L:D 16:8 h photoperiod. Analysis of variance for cannibalized L1 larvae by older instar larvae L4 at the time intervals of 24 and 48 hours was carried out (Table 1).
- As the value of p is less than 0.05 so the results are significant. The number of larvae were remained in control was significantly different from cannibalized larvae. The dead larvae at the control level was zero.

In figure 9 and 10 graphs represent the amount of cannibalized and alive larvae in different shapes of the container and show the significant results of

cannibalism in each size of the defined container while the temperature remained the same at each level.

More larvae are consumed after 48 hours as compared to 24 hours at tall and thin least cannibalism occurs at intermediate container. We observed the highest cannibalism rate in the container with the highest number of encountered larvae. We found also that spatial shape can affect cannibalism not only by affecting the number of encounters but also the number of encounters favorable for cannibalistic events. In the control tests no missing larvae were found after 24 and 48 hours. In the test trials instead, L1 larvae disappearance was observed under all experimental conditions, while no L4 larvae disappearance was observed. Likewise, the length of contact between old and young larvae (24 and 48 hours) affected the cannibalism rate only in the tall and thin container. Our results were concordant with this prediction showing that in all trials between old and young larvae, the rate of cannibalism significantly differed among the three container shapes, and was the highest within the tall and thin container. After the tall and thin container, the rate of cannibalism was higher in the low and wide than in the intermediate container (Table 1). Therefore, ANOVA and graphical representation jointly suggested that container shape significantly affects the cannibalism rate by affecting the probability of encounters between individuals.

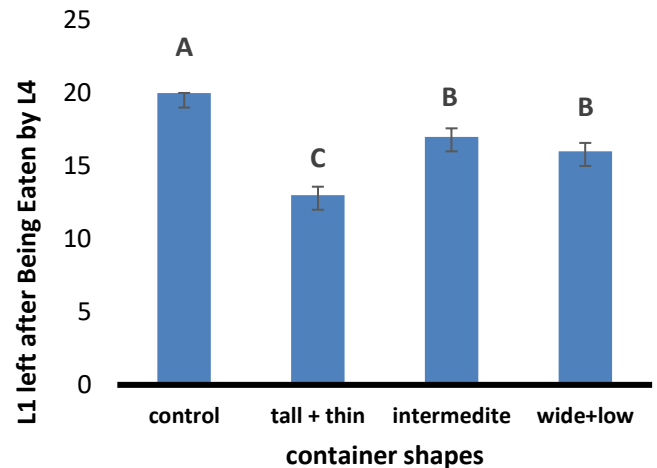


Fig 9 Variation of Cannibalism Rate of I1 larval instar by L4 at different size of containers after 24 hours. As more larvae I1 present at intermediate containers after 24 hours so less cannibalism. More cannibalism occurred at tall and thin container

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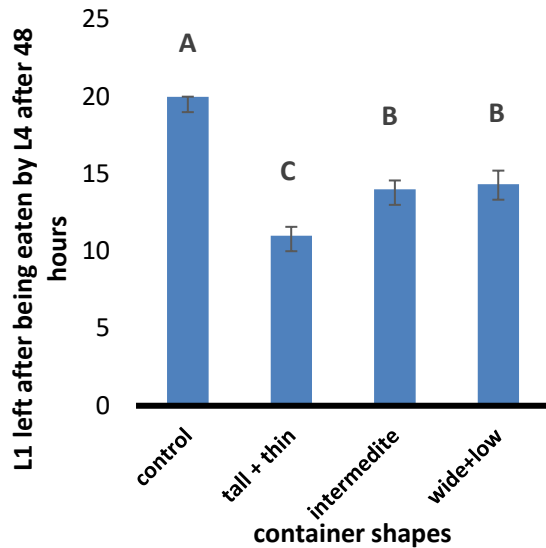


Fig 10 Variation of Cannibalism Rate of I1 larval instar by L4 at different size of containers after 48 hours. As more larvae I1 present at intermediate containers after 48 hours so less cannibalism. More cannibalism occurred at tall and thin container.

Section 4.3: Analysis of variance parameters of Cannibalism in mosquito species F2 generation: collection of Different larval instars, emergence of L1 larvae, dead and live ratio of fist instar, I3 and I4 larvae presence, container shape (encoded as: tall and thin = 1, low and wide = 2 and intermediate = 3) required food and cannibalized larvae at the period of 24 and 48 hours.

- This experiment was conducted for 24 and 48hours according to mosquito life cycle as the emergence of first instar L1. During the experiment temperature of trial area maintained $27 \pm 2^{\circ}\text{C}$, $75 \pm 10\%$ relative humidity (RH) and an L:D 16:8 h photoperiod. Analysis of variance for cannibalized L1 larvae by older instar larvae L3 and L4 collectively at the same space and time intervals of 24 and 48 hours was carried out (Table 1).
- As the value of p is less than 0.05 so the results are significant. The number of larvae were remain in control were significantly different from cannibalized larvae. The dead larvae at control level was zero.

In figure 7 and 8 graphs represents the amount of cannibalized and alive larvae in different shapes of container and show the significant results of

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More number of larvae consumed after 48 hours as compared to 24 hours at tall and thin least cannibalism occurs at intermediate container. We observed the highest cannibalism rate in the container with the highest number of encountered larvae. We found also that spatial shape can affect cannibalism not only by affecting the number of encounters, but also the number of encounters favorable for cannibalistic events. In the control tests no missing larvae were found after 24 and 48 hours. In the test trials instead, L1 larvae disappearance was observed under all experimental conditions, while no L3, L4 larvae disappearance was observed. Likewise, the length of contact between old and young larvae (24 and 48 hours) affected the cannibalism rate only in the tall and thin container. Our results were concordant with this prediction showing that in all trials between old and young larvae, the rate of cannibalism significantly differed among the three container shapes, and was the highest within the tall and thin container. After the tall and thin container, the rate of cannibalism was higher in the low and wide than in the intermediate container (Table 1). Therefore, ANOVA and graphical representation jointly suggested that container shape significantly affects the cannibalism rate by affecting the probability of encounter between individuals.

Under both hypotheses, however, the probability of cannibal and victims encountering each other is a key factor in determining cannibalism rate. According to our results, the container shape plays a role by affecting the number and the propitiousness of encounters between cannibal and victims.

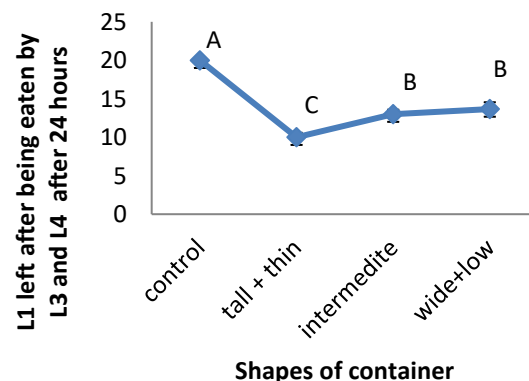


Fig 11 Variation of Cannibalism Rate of I1 larval instar by L3, L4 at different size of containers

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after 24 hours. As more larvae I1 present at intermediate containers after 24 hours so less cannibalism. More cannibalism occurred at tall and thin container.

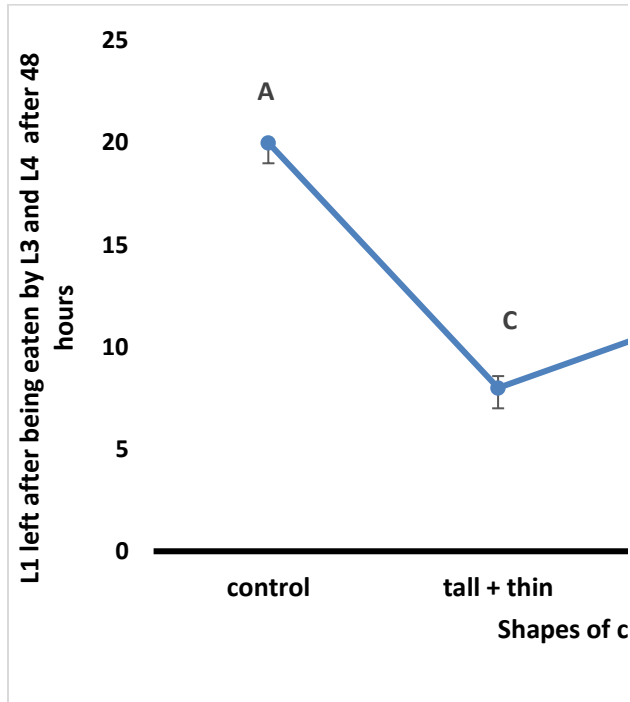


Fig. 12 Variation of Cannibalism Rate of I1 larval instar by L3, L4 at different size of containers after 48 hours. As more larvae I1 present at intermediate containers after 48 hours so less cannibalism. More cannibalism occurred at tall and thin container.

Section 4.4: Analysis of variance parameters of Cannibalism in mosquito species F2 generation: collection of Different larval instars, emergence of L1 larvae, dead and live ratio of first instar, I3 and I4 larvae presence, container shape (encoded as: tall and thin = 1, low and wide = 2 and intermediate = 3) controls using dead larvae (killed at -80°C for 5 min) were also performed to test for possible larval decomposition and disappearance. Cannibalized larvae at the time period of 24 and 48 hours.

- This experiment was conducted for 24 and 48 hours according to mosquito life cycle as the emergence of first instar L1. During the experiment temperature of trial area maintained at $27 \pm 2^{\circ}\text{C}$, $75 \pm 10\%$ relative humidity (RH) and an L:D 16:8 h photoperiod. Analysis of variance with three factorial designs considered Tukey's Test for cannibalized L1 larvae by older instar larvae L3 and L4 collectively at the same space and time intervals of 24 and 48 hours and different control conditions were carried out (Table 1).

- As the value of p is less than 0.05 so the results are significant. The number of larvae were decomposed and disappeared in control were significantly different from cannibalized larvae. The dead larvae at control level was zero.

DISCUSSION

A preliminary study was designed to understand the cannibalism and cannibalistic behaviour in mosquito population collected from urban areas of district Sheikhpura, Pakistan. The experimental work was carried out in Insect Molecular Biology Laboratory, Department of Entomology, University of Agriculture Faisalabad, Pakistan.

Briefly, first different larval stages of mosquito species collected from different breeding sites by using ovitraps. All collected samples put in different plastic trays of (5cm height, 30 cm breadth, 19 cm length) and kept them in growth chamber inside the lab. All Plastic trays filled with 800ml of water. These trays were maintained in a lab at temperature of $27 \pm 2^{\circ}\text{C}$ with approximate relative humidity of $75 \pm 10\%$ and a photoperiod of LD (16:8 h). Crushed 0.33 g dry cat food (Friskies1Adult) mixed with 800 ml of water and this mixture used to feed larvae (Mastrantonio *et al.*, 2018). We used morphological keys to identify the eclosed adults (Schaffner *et al.*, 2001) and fed them with 10% sucrose solution every day in the cages that measure (40×40×40cm). Females which fed with Fresh mechanically defibrinated bovine blood laid eggs on paper towels in water-filled cups (Mastrantonio *et al.*, 2018). For performing of cannibalism experiment we dried these paper towels and stored at 27°C .

In the next set up for experiments the raised colonies kept at the same climatic conditions due to required rearing conditions during our experiments, fish food was provided (0.85mg/larvae at the onset of the experiment and after 24 hours) and one larva per milliliter, with no food shortage. We checked the cannibalism between different larval stages and also the effect of container shapes. Cannibalism between larvae of the first and fourth instar (L1 and L4) as well as between larvae of the third and first instar (L3 and L1) which is more than (48hours old) had been observed. To reproduce common breeding sites of *Aedes albopictus* three distinct surface/water column ratios in plastic containers had been used e.g. artificial containers, flowerpots as well as flowerpot dishes: a container of 6×6×12 (hereafter referred to as a tall and thin container); a 12.5×12.5×4.5 cm container (hereafter an medium container) and a container of 25×25×8 cm which is referred as low and wide container. At this level of experiment each

container filled with 150 ml of distilled water and used to place L1 (15) larvae and (1) L3 or (1) L4 larvae. 15 L1 larval has been placed in each container without L3/L4 larvae as a control for each treatment. After 24 and 48 hours we counted the missing larvae. Experimental containers of each shape have been used for each experimental condition (pseudo replicates) and repeated the whole experiment three times (biologically replicates). In the whole experimental set up evidence says that *Aedes albopictus* L3 and L4 larvae has been cannibalized the L1 larvae. In the control tests, where we do not kept L3 and L4 larvae into the experimental containers, no disappearance of L1 larvae was observed (Annis *et al.*, 1990; Rajavel, 1992). According to Bonizzoni *et al.* (2013), *A. albopictus* is a vector species that is a cause of spreading of many harmful viruses, including the dengue, chikungunya, and zika viruses. The observations regarding the ingestion of immature L1 larvae by the late instars may be important from an epidemiological perspective. Cannibalistic activity, which affects the number of individuals emerging from a breeding site, may have a substantial impact on this species' vector capacity. One way to keep the population size below the carrying capacity and still allow it to expand is to restrict the number of larvae in the breeding sites.

Furthermore, cannibalism, offers a nutritional benefit to cannibal that can lead to the emergence of larger adult females with high fitness, flight performance, host-finding ability and dispersal potential. (Alto *et al.*, 2008). On the other hand, however, cannibalism could reduce the number of emerging adults, and it harmed vector capacity (Brady *et al.*, 2016). The effect of spatial form on the cannibalism rate of late (L3 and L4) and immature (L1) larval instars of the tiger mosquito *A. albopictus* was studied using both experimental and modeling methods.

The simulation findings revealed that the three containers had varying numbers of contacts between cannibals and victims, with the tall and thin containers having the most. If the number of interactions between cannibals and victims influences cannibalism rates, we predicted that the cannibalism rate seen in our experimental trials would be higher in tall and thin containers. According to our findings, which is in support of this hypothesis, the tall, thin container had the highest rate of cannibalism among the three container types in all experiments involving both young and elderly larvae. As a result, both empirical and simulation studies revealed that container design had a considerable impact on cannibalism rates by influencing the likelihood of encounter between individuals. In contrast, container design had a substantial effect on cannibalism rate in both the

(L3-L1) and (L4-L1) trials, with tall and thin containers having a much greater cannibalism rate than the other two containers.

The influence of the experimental conditions and their combination on the cannibalism rate was studied using logistic regression, using cannibalism as a binary response variable. The simulation results revealed that the expected number of interactions between larvae varied among the three containers. The tall and thin container had the most interaction between cannibals and victims, followed by the intermediate and low and wide containers. The area provided for the victim to escape when encountered by a cannibal was the smallest in the low and wide container, followed by the tall and thin and intermediate containers.

The tall and thin container had the most encounters weighted for the probability of successful cannibalism. There were no missing larvae in the control testing after 24 or 48 hours. In the test trials, L1 larvae disappeared under all experimental conditions, however L3 and L4 larvae did not disappear. Cannibalism rate was unaffected by larval instar, since no significant changes were seen when comparing cannibalism rates between (L1) and (L4-L1) trials in either container. Similarly, period of contact between old and young larvae (24 and 48 hours) affected cannibalism rate only in the tall and thin containers. In contrast, container design had a substantial effect on cannibalism rate in both the (L3-L1) and (L4-L1) trials, with tall and thin containers having a much greater cannibalism rate than the other two containers. This study concludes that mosquitos can develop in temporary water habitats with highly diverse topologies; however, the function of the site shape where cannibals and victims coexist has been disregarded.

Cannibalistic behavior between older and young larvae may also influence female oviposition behavior and consequently the spread of mosquito vectors of *Aedes albopictus*. Our findings reveal the relevance of spatial shape in cannibalism rates in mosquito larvae, imply that it could be a crucial component influencing interactions in this habitat. This highlights the importance of additional research into its contribution to aquatic insects. Notably, our findings, which reveal that cannibalism rates fluctuate between experimental containers based on their shape, imply that breeding locations might bear variable weights on vector capacity, and that container shape plays a significant role in generating this variation.

Conclusion

These basic findings of this work would be helpful in devising sustainable control strategies for the mosquito population (*A. albopictus* and *A. aegypti*) *etc.* in vector management programs.

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Not applicable.

Consent for Publication

The study was approved by authors.

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All authors contributed equally.

Conflict of interest

There is no conflict of interest among the authors of the manuscript.



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