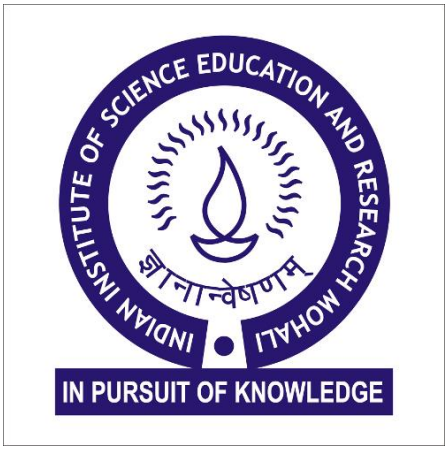
Time period in which mated *Nasonia vitripennis* females can lay maximum number of female progenies

Anargha Sai K K

(MS15092)

A dissertation submitted for the partial fulfilment of

BS-MS dual degree in Science



Indian Institute of Science Education and Research Mohali

April 2020

Certificate of Examination

This is to certify that the dissertation titled "**Time period in which mated *Nasonia vitripennis* females can lay maximum number of female progenies"** Submitted by Ms. Anargha Sai K K (Reg. No. MS15092) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Rhitoban Ray Choudhury

(Supervisor)

Dated:

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Rhitoban Ray Choudhury at the Indian Institute of Science Education and Research Mohali. This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or Institute. Whenever the contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgements of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all the sources listed within have been detailed in the bibliography.

Anargha Sai K K

Date

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Rhitoban Ray Choudhury

(Supervisor)

Date

Acknowledgements

I must thank Dr. Rhitoban Ray Choudhury, Who has been guiding me as my supervisor and mentor during this work. I would like to express my gratitude and admiration for his invaluable support throughout the work to make the study fruitful.

I would like to extend my gratitude to Alok Tiwary for being there as a source of constant support, motivation and guidance. I am thankful to each and every Evogen lab members for helping me to improve myself by providing useful suggestions, and making the lab a friendly environment.

I also thank my parents for their motivation and soulful support throughout this course.

Thank You,

Anargha Sai K K

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Abstract

Wolbachia is an endosymbiotic bacteria that infects terrestrial arthropods and nematodes. It belongs to the class of gram negative alphaproteobacteria. Since it is a maternally inherited bacteria, it tries to increase the number of infected females in the host population by introducing different reproductive alterations in the host such as parthenogenesis, feminization of genetic females, cytoplasmic incompatibility (CI) and male killing. According to recent studies, *Wolbachia* have been divided into 18 supergroup (A-R). Out of these, supergroup A and B are commonly found in arthropods.

Nasonia vitripennis (a parasitoid wasp species, which is infected with both *Wobachia* A and B supergroup) was the model system of this study. A mated *N. vitripennis* female can lay both fertilized and unfertilized eggs. Primary investigation of this work was to figure out a time period (time window) to host the mated females to get maximum number of female progenies. In future work, this time period will be used to collect the different female developmental stages to study the dynamics of different *Wolbachia* supergroup across female development. This study also aims to figure out the fecundity of mated *N. vitripennis* females of all the four infection types (A, B, AB and Cured) by hosting it at different time periods. The wide goal of this study is to understand the existence of multiple infections (supergroup A and B) in same *Nasonia vitripennis* host and why such infection type is more prevalent in natural environment than single infections.

Chapter 1: Introduction

6.1 Basic theory

Wolbachia

Endosymbiosis is a mutually beneficial relationship between two dissimilar organisms, in which an organism is inhabiting inside other organism (host). An example for such an endosymbiont is *Wolbachia*, a gram-negative alphaproteobacteria which is maternally inherited in its host population. This intracellular bacteria infect most of the terrestrial arthropods and nematode species (Werren *et al*., 2008).

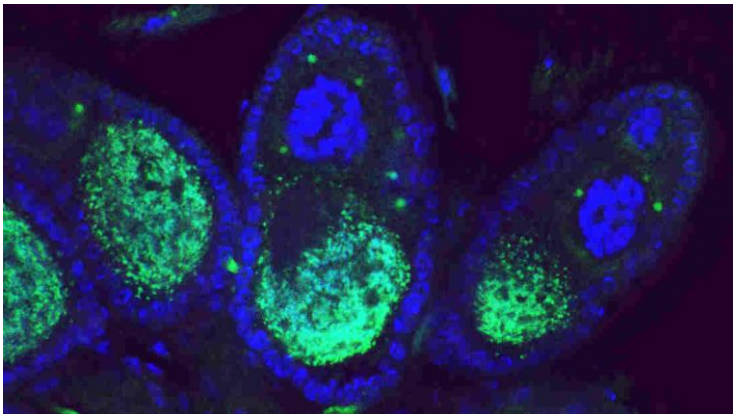


Image: Zhiyong Xi/ Michigan State University

Figure1: *Wolbachia* (green) infects the ovaries of the malarial transmitting mosquito-*Anopheles stephensi*

Wolbachia was first identified in *Culex pipiens* (Hertig *et al*., 1936). According to the recent studies, *Wolbachia* have been divided into 18 supergroups (A-R) (O' Neil *et al*., 1992; Werren *et al*., 1995; Zhou *et al*; 1998; Lo *et al*., 2002., Bordenstein and Rosengans, 2005; Lo *et al*., 2007; Landmann *et al*., 2019). Among these supergroups, A and B are commonly found in land arthropods. C and D are found in filarial nematodes, by following a mutualistic relationship (Werren *et al*., 2008). Transmission of this bacteria among the species can occur by different modes such as horizontal transmission, vertical transmission and through hybridization (Werren

et al., 1997). Since it is a maternally inherited bacteria, it tries to increase the number of infected females in the host population by various reproductive alterations such as parthenogenesis, feminization of genetic males, cytoplasmic incompatibility and male killing (Minnot *et al*., 1996). Vertical transmission of this bacteria is enhanced by these reproductive alterations and therefore they will be maintained in host population (Minnot *et al*., 1996).

From last decade, due to its prevalence in nature, *Wolbachia* biology is being extensively studied (Werren *et al*., 2008). Researchers are now interested in looking *Wolbachia* as a pest and disease vector control (Werren *et al*., 2008).

Cytoplasmic incompatibility (CI)

This is one of the reproductive alterations observed in most of the arthropods (Werren *et al*., 2008). During fertilization, there occurs an incompatibility between a sperm having one *Wolbachia* supergroup infection and an egg which does not contain the same *Wolbachia* infection (Yen and Barr 1971; Hoffman *et al*., 1986; Breeuwerand Werren 1990; O'Neill and Karr 1990). This is known as CI. Since the molecular mechanism is unknown, 'modification and rescue hypothesis' is the most accepted hypothesis for the occurrence of CI (Bossan *et al*., 2011). This hypothesis states that *Wolbachia* infection will introduce a modification in the sperm during a particular male developmental stage and a rescue in egg during female development. During first embryonic mitosis, DNA from modified sperm cannot properly participate, which results in asynchronous development of male and female pronuclei, except if *Wolbachia* action in the egg "rescue" (recovers) the functionality of the sperm DNA (Bossan *et al*., 2011).So, this modification and rescue should be compatible to each other for obtaining viable female progenies. This will be possible only in case of haplodiploid organisms (produced from unfertilized eggs). The figure (2) illustrates CI.

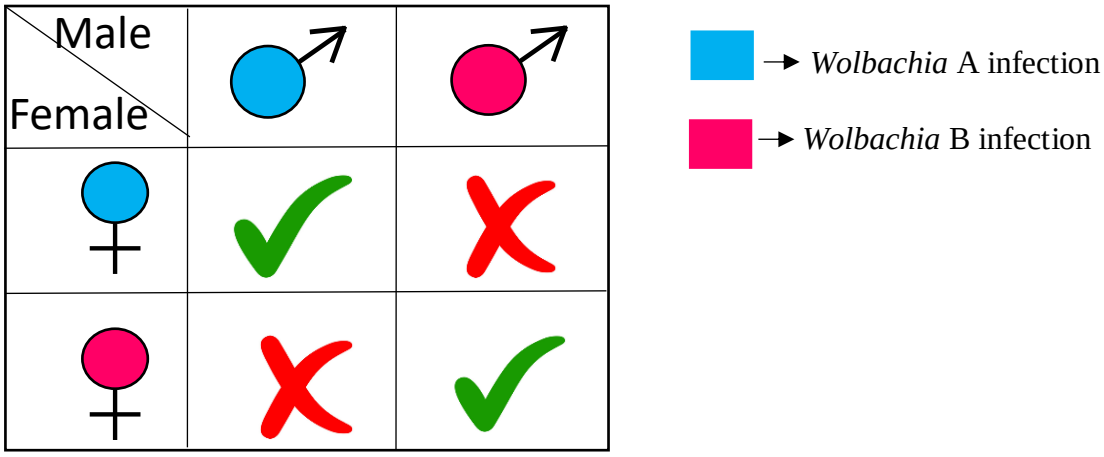


Figure 2: Cytoplasmic Incompatibility induced by *Wolbachia*

***Nasonia vitripennis* (Model Organism)**

Wolbachia infection is also found in *Nasonia vitripennis* (Walker, 1836), the model system of this work. It is a cosmopolitan species. The other three closely related species in the *Nasonia* genus are *N. longicornis*, *N. girault*i and *N. onneida*, which are only found in North America (Darling and Werren, 1990; Raychoudhury *et al*., 2008). Eventhough these *Nasonia* species are closely related, they are genetically isolated from each other due to *Wolbachia*, which induces sperm-egg incompatibilities (Werren *et al*., 1997). Their short generation time (two weeks at 25°C), large family sizes and easiness to handle makes them an excellent model system to work with (Whiting *et al*., 1967).

Image: Michael .E. Clark

Figure 3: *Nasonia* female parasitizing host pupa.

Nasonia follows a haplodiploid sex determination. That suggests, females are diploid which arises from fertilized eggs whereas unfertilized eggs result in haploid, male progenies. The offspring sex ratio is female biased (Werren *et al.,* 1983).

All *Nasonia* species lay their eggs upon pupae of various flies such as flesh flies, blow flies and house flies. The *Sarcophaga dux* pupae is used as the host for *N. vitripennis* in this work. These pupae can be generated regularly in our lab by following the standard protocols required for its life cycle. The figure (4) indicates *S. dux* fly and its pupa generated in our lab.

Image: Alok Tiwary, Evogen lab

Figure 4: *Sarcophaga dux* fly and its pupa

The advantage of *N. vitripennis* over the other species is, it carries only Single *Wolbachia* supergroup A and B infections. So it is easy to study the effect of single and multiple infections in this species. Uninfected (*Wolbachia* cured) strain is taken as the control in this experiment.

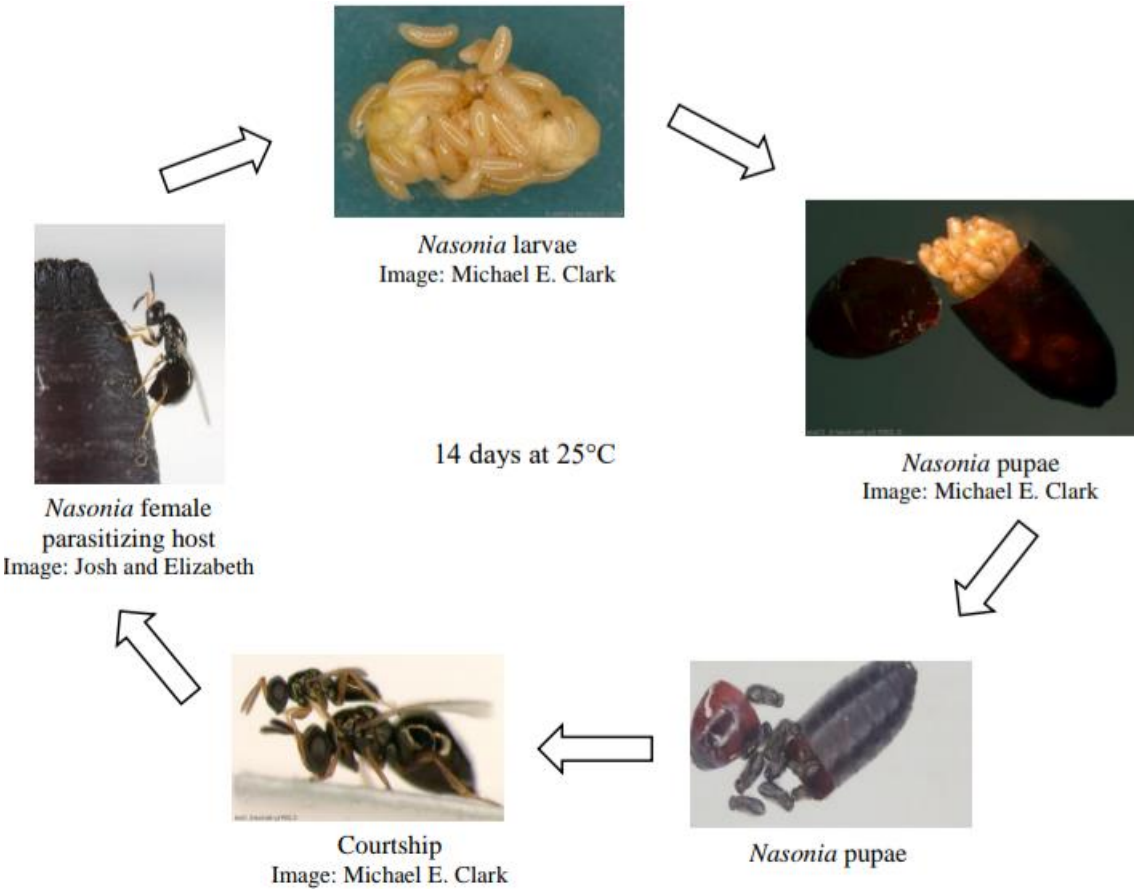


Image: Abhilasha Sharma, Evogen lab

Figure 5: Life cycle of *Nasonia*

Eventhough the *Wolbachia* infection in *N. vitripennis* causes CI, multiple infections (both A and B *Wolbachia* supergroup in single host) are more prevalent in the natural environment whereas single infections (A or B) are very rarely found (Minnot *et al.* 1996).

So, to understand how these multiple infections are of multiple infections in the same host, the questions of investigation in this work are:

1) How multiple infection is maintained in the same *N. vitripennis* host?

2) Is there any compromise between supergroup A and B during development of *N. vitripennis*?

6.2 Aims and Experimental Methods

Aim (1): The primary objective of this project is to identify the time period in which mated *N. vitripennis* females can lay maximum number of female progenies.

Why is it necessary to figure out the time period for obtaining maximum female progenies?

A mated *N. vitripennis* female can lay both fertilised and unfertilised eggs. That indicate a mated female can change offspring sex ratio (Werren *et al*. 1980). In the early larval stages it is impossible to distinguish the sex whereas in pupal stages it is very easy to distinguish males and females.

Male pupa **Female pupa** **Larvae (indistinguishable)**

Image: Anargha Sai K K

Figure 6: Morphological difference between *N. vitripennis* male and female pupa and indistinguishable larval stages.

Female pupae in *N. vitripennis* can be identified by the presence of wing pads and ovipositor in the last abdominal segment of the pupae.

Previous work done in lab: From multiple infected (AB) wild type strain of *N. vitripennis*, single supergroup infected strains (A and B) and uninfected strain were generated in the lab (Done by Alok Tiwary, Evogen lab).

Experimental protocol

Isofemale sublines from a single mated mother were generated.

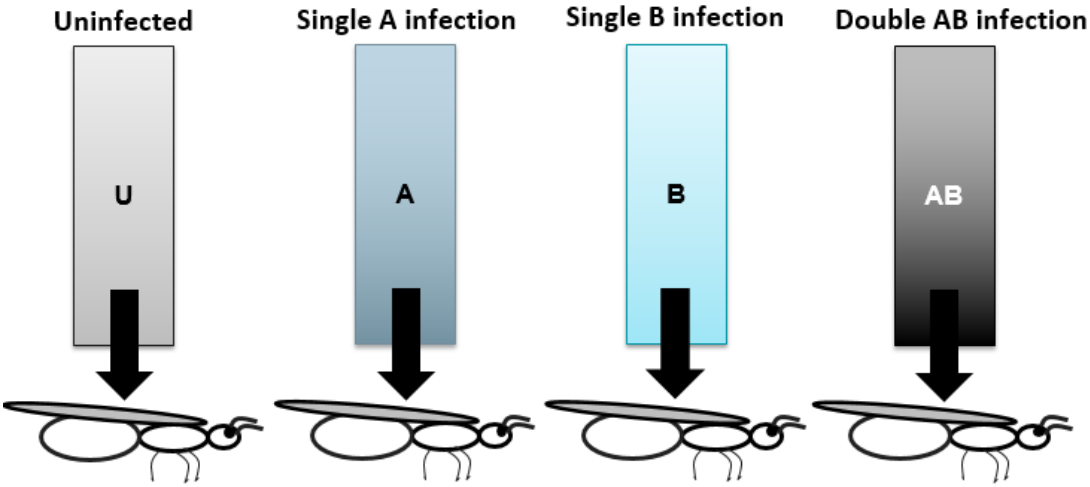


Image: Rahul Babu, Evogen lab

Figure 7: Generation of isofemale sublines

10-15 mated females were collected from each strains and transferred each female into a ria vial. Honey (external nutrition) was provided and hosted it for 48 hours with two hosts. These were kept at 25°C till emergence. The sex was identified in pupal stage (10-12th day of development) and the one's with maximum number of progenies was selected (both females and males). Individual matings of these F1 progenies were observed. Honey was provided and hosted them with two hosts. The F2 progenies obtained from this hosting are all isofemale sublines (Sublines generated from a genetically same mother).

Virgin selection: In the pupal stage of these isofemale sublines, 15 virgin females and few males were collected for the experiment.

The experimental set up is shown in figure (8).

Experimental setup

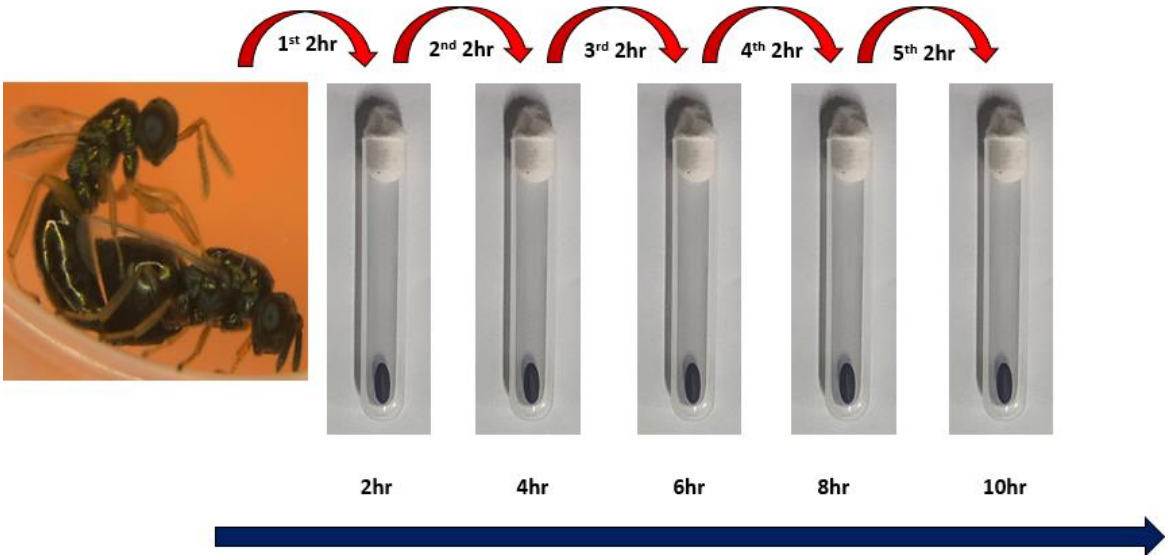


Image: Babita Ronsa (Evogen lab), Anargha Sai K K

Figure 8: Experimental setup to find the time period in which maximum female progenies are laid by a single mated female.

Individual matings of 15 virgin females collected from each infection type with the males carrying same infection were observed in ria vials. Then, the males were removed and females were kept at 25°C for 24 hours. After that, females were hosted with single *S. dux* pupa as shown in figure (8) for a constant time period of two hours, five times. All the hostings were kept at 25°C till emergence. Progeny size were counted and the graph was plotted to figure out the time interval in which maximum female progenies was obtained.

Aim (2): To identify the fecundity of a mated *N. vitripe*nnis female by hosting it at different time periods.

Assay:

Isofemale lines were generated for different infection strains (A, B, AB and uninfected).

Virgin selection: Atleast 100 virgin females and males were collected in the pupal stage and put in separate ria vials. These were kept in 25°C till one day after the emergence of female (16^{th} day of development).

Individual matings were observed by providing each female with one male. After mating, males were removed from each ria vial. Then, 30 mated females for each set of time periods were hosted with single host for 6hrs, 12hrs and 24 hrs. The progenies were kept at 25°C for emergence and counted.

Aim (3): To identify the effect of single and multiple *Wolbachia* infection on the progeny size of mated *N. vitripennis* female.

Assay:

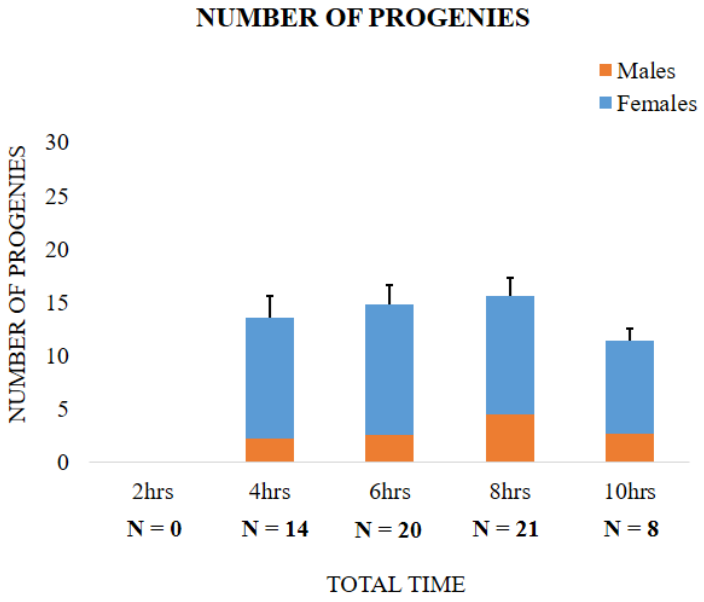
Isofemale lines were generated for different infection strains (A, B, AB and uninfected) and atleast 100 virgin females and males were collected from each strain. It was kept in 25°C till 16^{th} day of development. Individual matings were observed by providing each female with one male. The males were removed and experimental hostings of mated females were put for 24 hours with one *Sarcophaga dux* pupa. Honey (external nutrition) was not provided to females. The females were killed from each ria vials after 24 hours and it was kept in 25°C till the progenies emerge. Then, family size was counted.

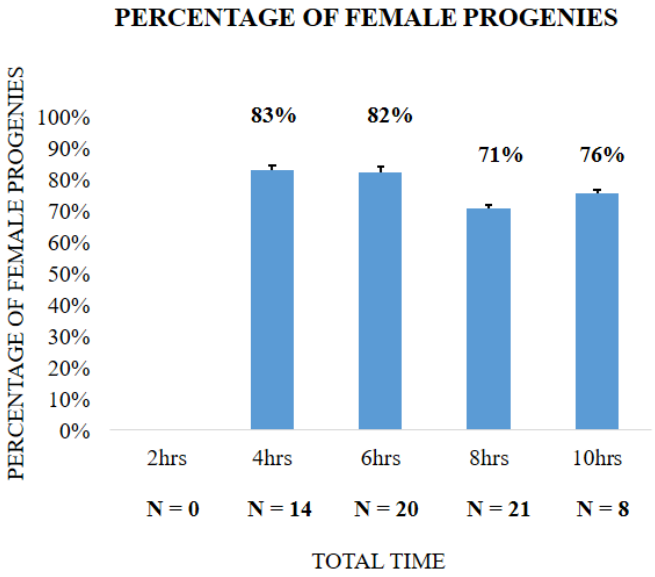
Chapter 2: Results and conclusion

7.1 Results

Aim (1): To identify the time period in which mated *N. vitripennis* females can lay maximum number of female progenies.

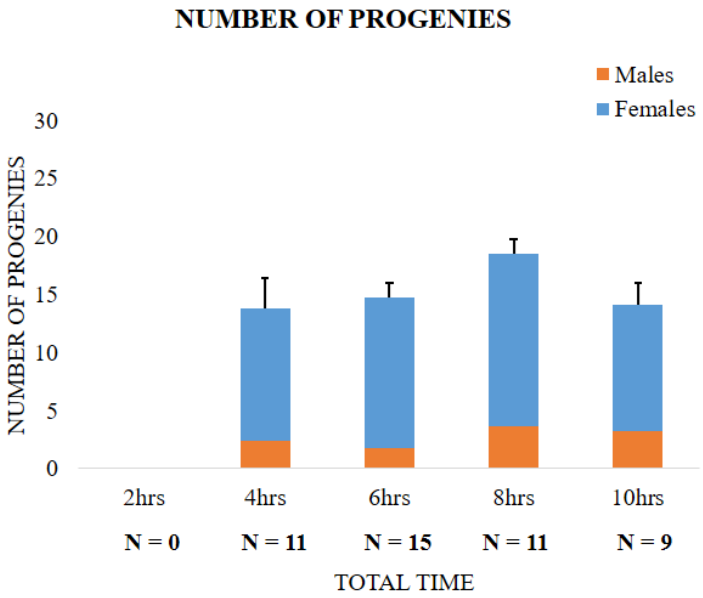
Results obtained for *Wolbachia* A-infected strain

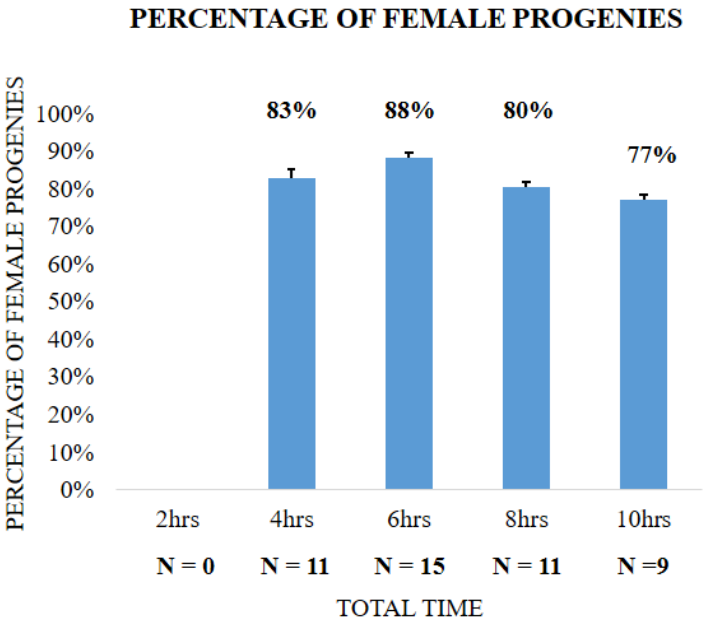




A infected strain showed almost similar trend in the total number of progenies (family size) obtained during the second and third two hours hosting. But, Percentage of female progenies was highest (83%) in the second two hours hosting.

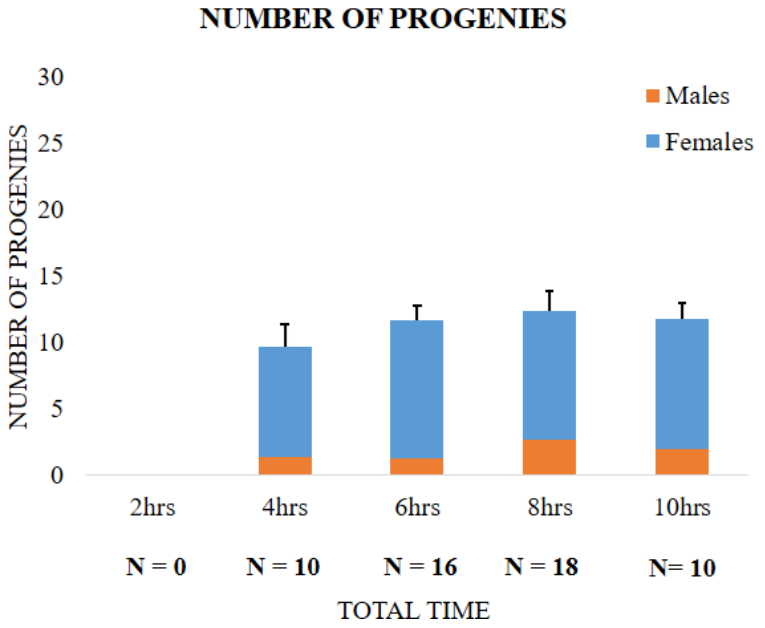
Results obtained for *Wolbachia* B-infected strain

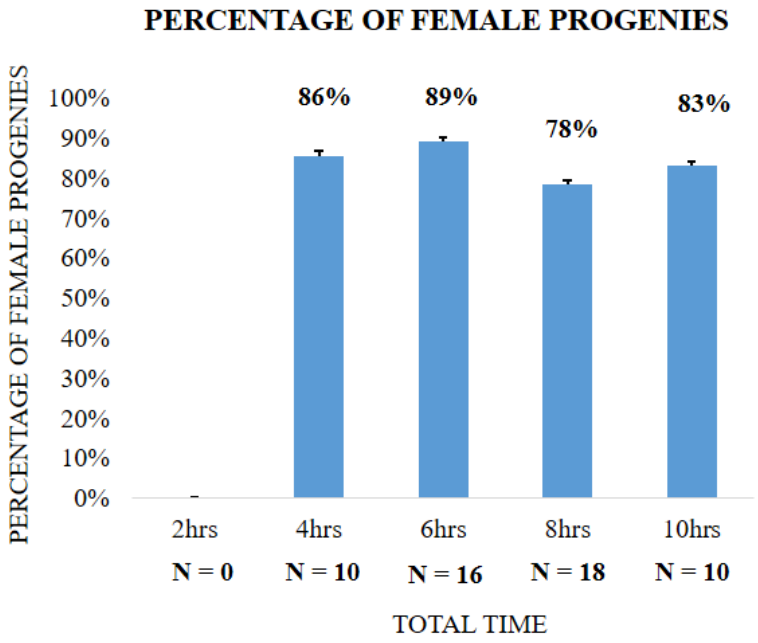




The obtained results of B-infected strains indicates, a highest family size in the fourth two hours hosting. But while considering the percentage of female progenies, it was the third two hours hosting which gave maximum females (88%).

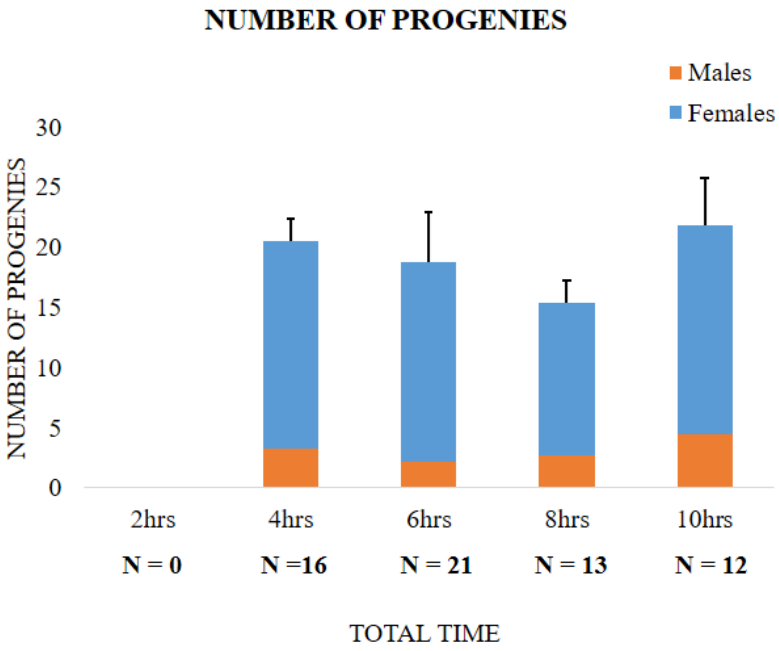
Result obtained for *Wolbachia* AB infected strain (Multiple infection)

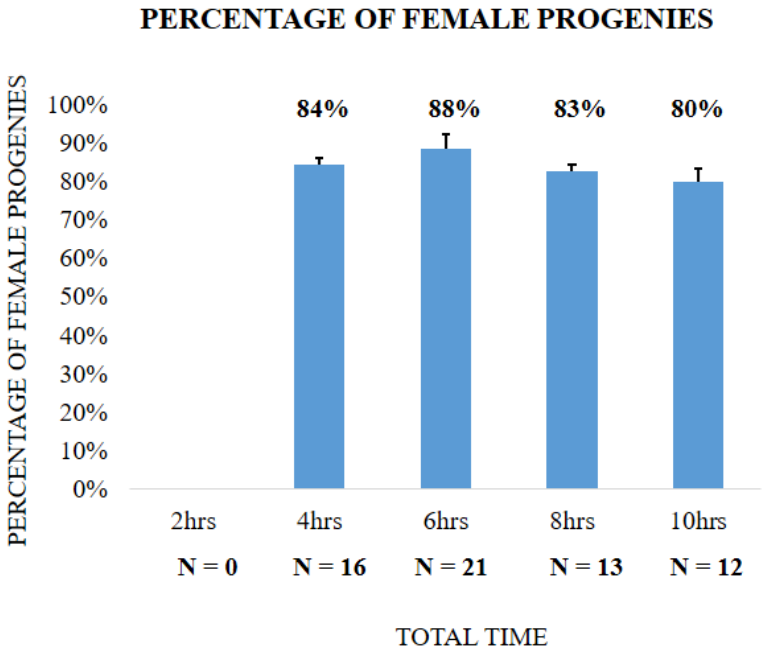




The highest percentage of female progenies was obtained in the third two hours hosting (89%).

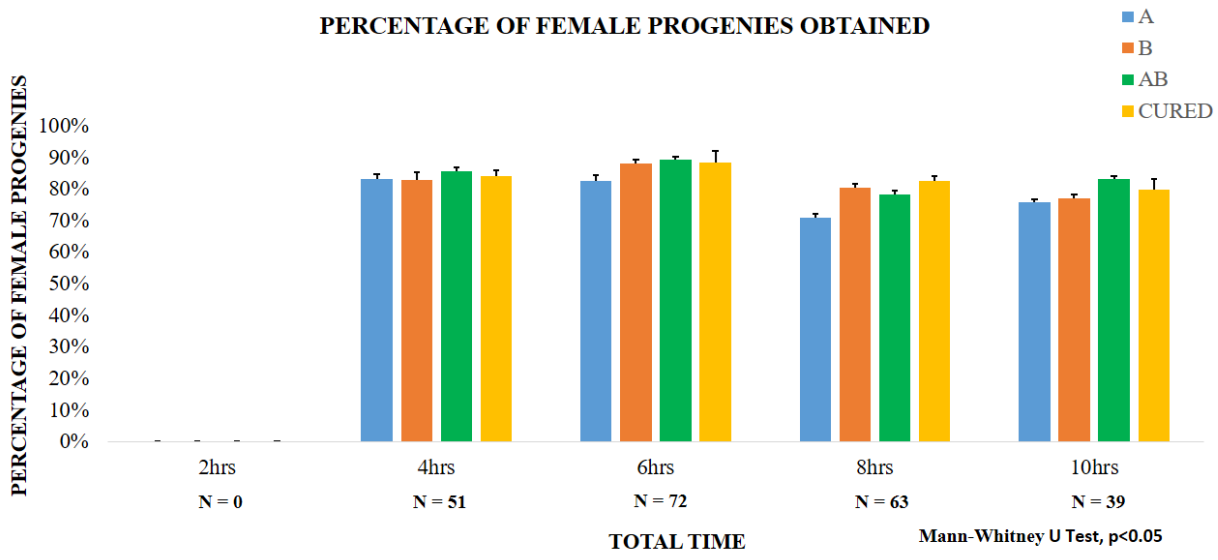
Result obtained for *Wolbachia* cured strain (uninfected)





Among all infection strains, highest family size was obtained for uninfected (*Wolbachia* cured) strain. The percentage of female progenies obtained was highest in the third two hours hosting (88%).

Summarized result of different infection



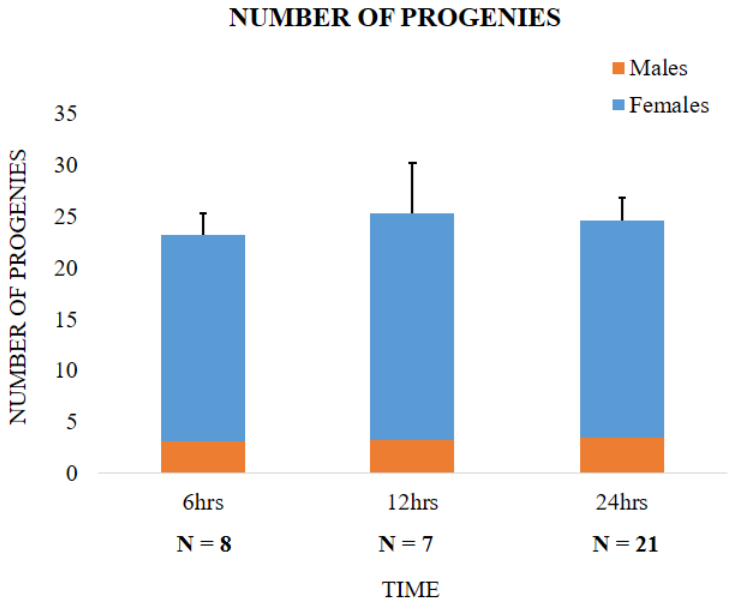
Summary of all the results obtained:

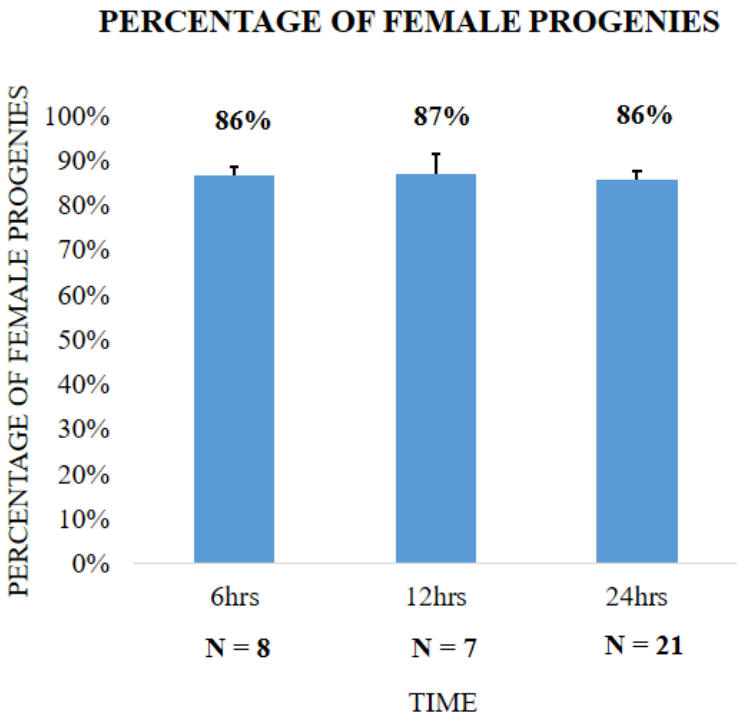
1. The first two hours hosting will not give any progenies in all infection strains.

2. The third two hours hosting was the time period (total time reaches 6 hours) in which mated *N. vitripennis* females were laying the maximum number of female progenies in almost all strains (B, AB and Uninfected). For A infected strain, it was the second two hours hosting showed highest percentage of female progenies (only 1% higher than the third two hours hosting). But when it was statistically analyzed using Mann-Whitney U test, this particular time interval (third two hours hosting) didn't show any significant difference with any other time periods.

Aim (2): To identify the fecundity of a mated *N. vitrip*ennis female by hosting it at different time periods.

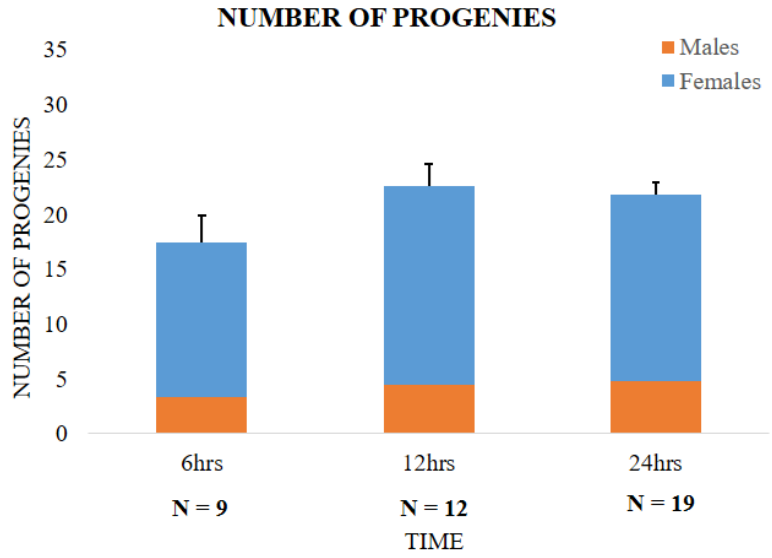
Results obtained for *Wolbachia* A-infected strain

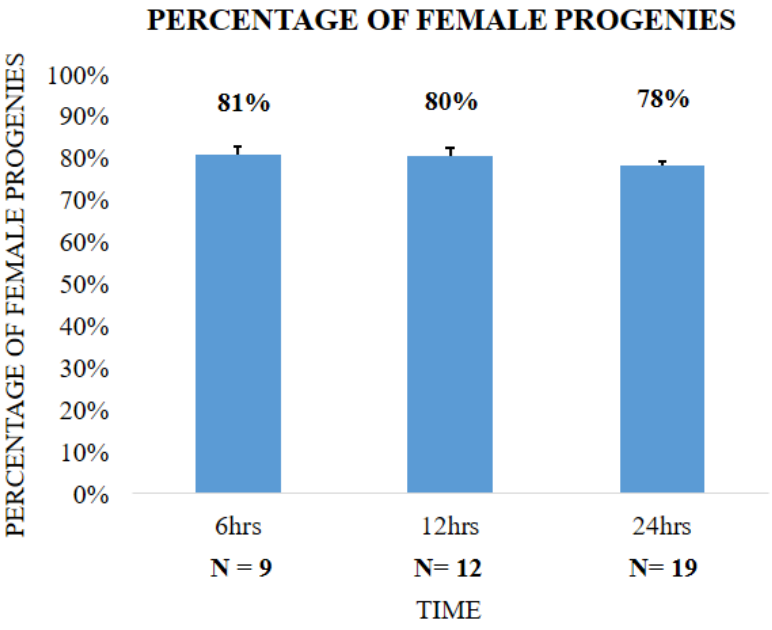




The result obtained for A-infected strain showed highest percentage of female progenies in 12 hours hosting (87%).

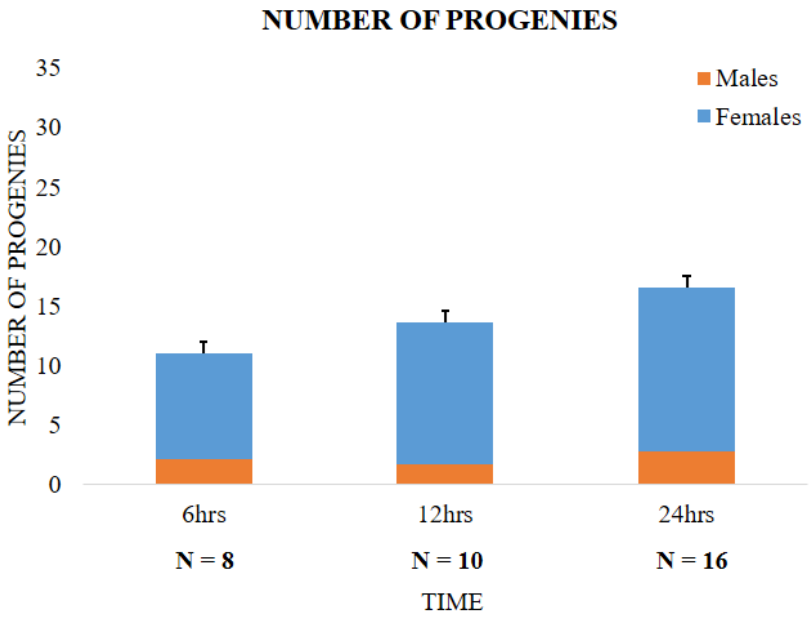
Results obtained for *Wolbachia* B-infected strain

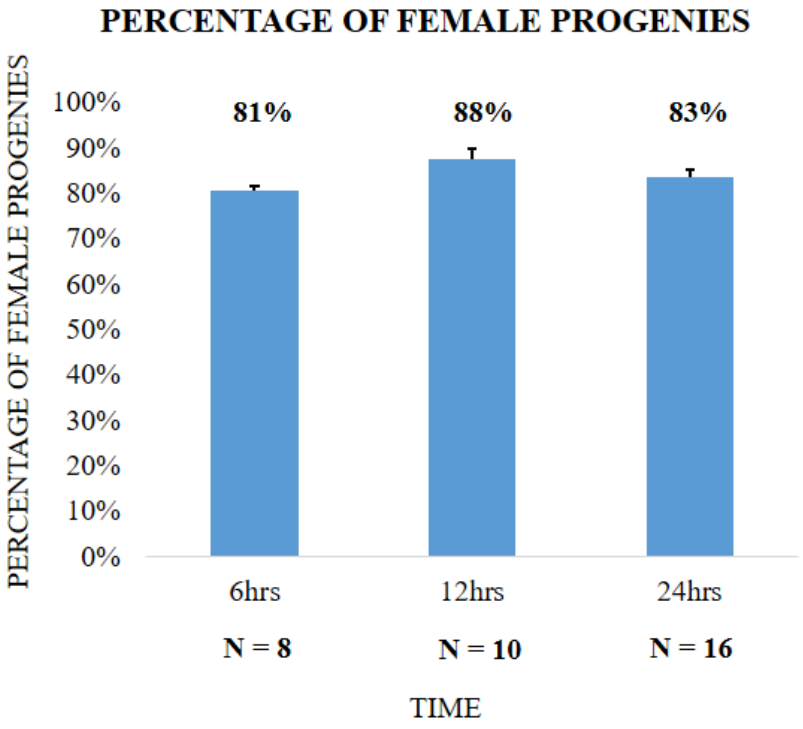




Maximum progeny size of B-infected strain was obtained in 12 hours hosting. But, maximum percentage of female progenies were obtained in the hosting for 6 hours (81%).

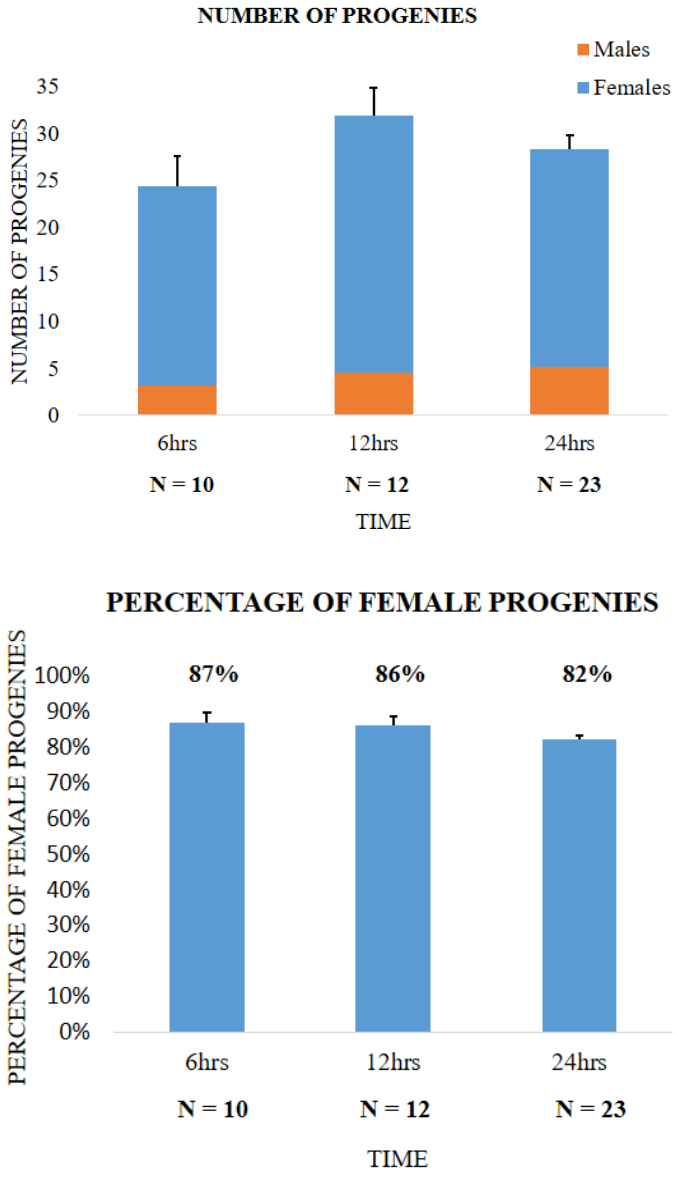
Result obtained for *Wolbachia* AB infected strain (Multiple infection)





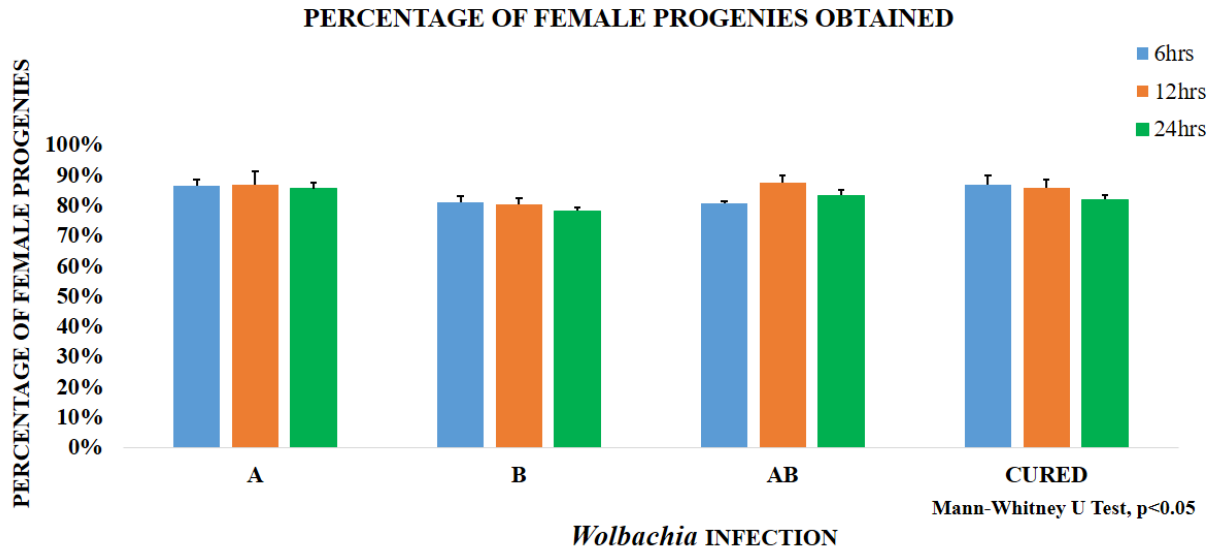
Multiple infected strain had maximum progeny size in the hosting for 24 hours whereas the female progenies were highest in the hosting for 12 hours (88%).

Result obtained for *Wolbachia* cured strain (uninfected)



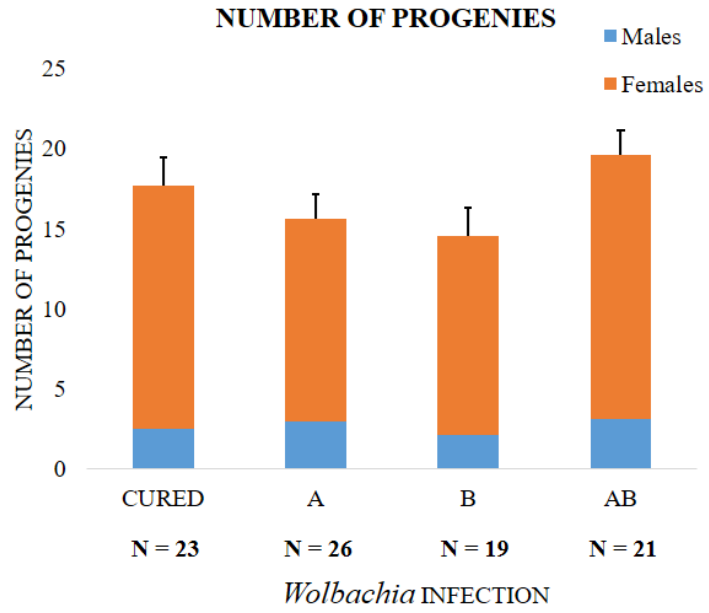
The progeny size was highest in the hosting for 12 hours and maximum female progenies were obtained in the hosting for 6 hours (87%).

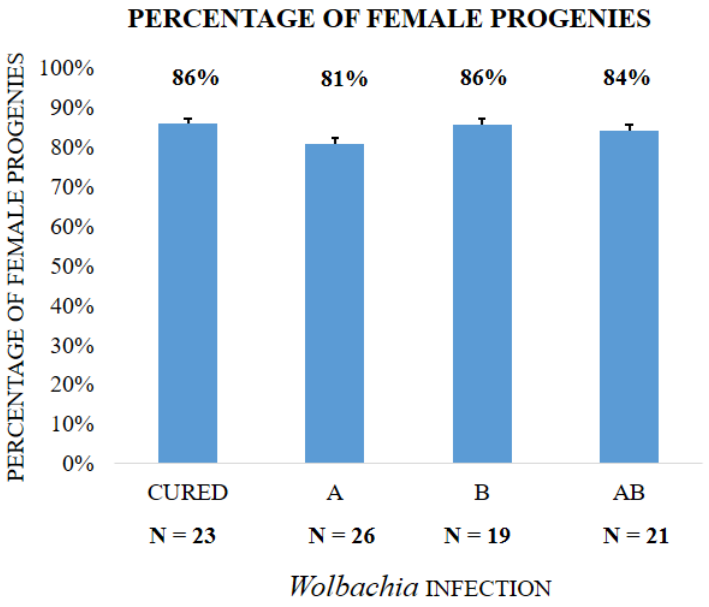
Summarized result of different infection



The results obtained for 12 hours hosting of mated females showed highest percentage of female progenies for most infection types. But the difference in female progenies obtained in the hosting for 12 hours is very negligible from other time periods. So, from these results no proper conclusion was obtained.

Aim (3): To identify the effect of single and multiple *Wolbachia* infection on the progeny size of mated *N. vitripennis* female.





In the previous studies, it was observed that the type of *Wolbachia* infection had no effect on the progeny size of *N. vitripennis* (Bordenstein *et al.* 2000). But, the obtained results of different infection strains of mated female hosting for 24 hours with single host showed highest family size for multiple infected (AB) strain and least for B infected strain. There is no significant difference between AB infected and uninfected (*Wolbachia* cured) strains.

7.2 Conclusion

Nasonia vitripennis is the model organism of this study. It was crucial to figure out a time period (window) of getting maximum fertilised eggs because a mated female can lay both fertilised and unfertilised eggs which are indistinguishable in early larval stages.

As of now from the experiments, the obtained results indicates maximum female progenies were produced in the third two hours hosting (total time reaches 6 hours), with least standard error in almost all infection types (B, AB and uninfected strains.). But it was statistically insignificant. In case of A infected strain the percentage of female progenies was more (only 1% higher) in the second two hours hosting (total time reaches four hours) compared to the third two hours. The other result obtained was first two hours won't give any progenies for all strains. The reason may be since two hours is a short period and initially, they won't be able to sting the pupa properly. While comparing with other infection strains, the family size (total number of progenies) is very less for multiple infected strain and uninfected strains had highest family size.

Any proper conclusion was not obtained from the results of experiment to figure out fecundity of a mated female for different time periods. The results indicates, there was highest percentage of female progenies in 12 hours hosting for A infected and multiple infected strains. But it was statistically insignificant.

Finally from the third experiment to identify the effect of single and multiple *Wolbachia* infection on the progeny size of mated *N. vitripennis* female, multiple infected strain showed highest family size than any other strains and B infected showed the least.

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