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# Toxorhynchites Splendens (Wiedemann) (Diptera: Culicidae) and its Efficacy to Control other Mosquitoes in Bangladesh

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# *TOXORHYNCHITES SPLENDENS* (WIEDEMANN) (DIPTERA: CULICIDAE) AND ITS EFFICACY TO CONTROL OTHER MOSQUITOES IN BANGLADESH

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Integrated Pest Management Laboratory
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June 2011

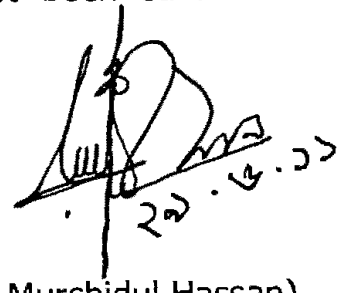
DEDICATED

TO

Professor **K. M. Nurul Huda**

# DECLARATION

I do hereby declare that the entire work to express tangibly in this dissertation towards the degree of Doctor of Philosophy is of my own investigation. The work as a whole or in part has not been submitted elsewhere for any other degree.



(Md. Murshidul Hassan)

# CERTIFICATE

This to certify that the thesis entitled "***Toxorhynchites splendens* (Wiedemann) (Diptera: Culicidae) and its efficacy to control other mosquitoes in Bangladesh**" submitted by Md. Murshidul Hassan has been supervised by me. It is his own research work carried out at the Institute of Biological Sciences, University of Rajshahi, Bangladesh and has not been submitted elsewhere for a diploma or any other degree.

29.6.2011

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# ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| *Ae.* | *Aedes* |
| *Ar.* | *Armigeres* |
| *Cx.* | *Culex* |
| DDT | Dichloro diphenyl trichloroethen |
| DMRT | Duncan's Multiple Range Test |
| d.f | Degrees of freedom |
| e.g | Exempli gratia (for example) |
| *et al* | *et alii* ( and others) |
| Fig. | Figure |
| hr/hrs | hour/ hours |
| IBSc | Institute of Biological Sciences |
| i.e. | id est (that is) |
| BS | Bamboo stump |
| BLIW | Bamboo-leaf infusion water |
| ns | Non significant |
| PP | Plastic pot |
| p:P | Prey: Predator ratio |
| RH | Relative Humidity |
| RU | University of Rajshahi |
| Sp. | Species( singular) |
| Spp. | Species (Plural) |
| *Tx.* | *Toxorhynchites* |
| T. max. | Temperature maximum |
| T.min | Temperature mixniiimum |
| WHO | World Health Organizati |
| * | 0.05 (Level of significance) |
| ** | 0.01 (Level of significance) |

# ABSTRACT

The biology of *Toxorhynchites splendens* (Wiedemann) has been observed in the laboratory. Twelve types of seasonal fruits and vegetables slices were offered as food for adults, in addition to the normal artificial diet viz 20% honey solution and 20% glucose solution in the cage and they accepted the food directly. Diurnal and nocturnal mating were also observed. Normally at night or in dark nuptial flight did not occur. But nuptial flight took place in extended photoperiod. Mating periodicity, pre-oviposition period, oviposition and fecundity were also observed. For mating the male-female ratio was 1:1, 2:1, 3:1, 6:1 and 9:1. Factorial analysis of data indicated that mating occurred more at predusk (4pm to 6pm) than at morning and it was significant at 0.05 level. Pre- oviposition period was highly significant in between ratio of males: female at 0.01 level. Pre- oviposition period were 16.33, 14.33, 7.66, 8.00 and 7.66 days with the male - female ratio of 1:1, 2:1, 3:1, 6:1 and 9:1 respectively. Pre- oviposition period decreased with the increasing of males. Statistically pre- oviposition period was significant at 0.01 level. Female also produced highest number of eggs (166.66) in ratio of male- female, 9:1. It was also highly significant at 0.01 level. But oviposition period and male- female ratio was found non-significant.

A special type of ovitrap was used for egg laying in the laboratory. The ovitrap was unique and composed of artificial and natural container. Natural container was a bamboo stump which was placed on the middle of plastic pot (artificial container) that stopped the air drafts created by females during oviposition flight, which also stopped egg from blowing out. The bamboo stump also acted as resting substratum for female during oviposition flight. Bamboo–leaf infusion water was used for rearing the mosquito in the laboratory. This was a unique rearing medium for *Tx. splendens* and prey mosquitoes.

*Tx. splendens* preferred laying eggs on second quarter of the day (12.00 pm to 18.00pm). They did not lay any egg on fourth quarter of the day

(0.00am to 6am). As an ovitrap black plastic pot with bamboo stump was preferred by *Tx. splendens* for egg laying. Statistically it was also highly significant at 0.01 level. On the basis of egg width four categories of egg i.e. W7 (0.392), W8(0.448), W9 (0.504) and W10 (0.672) were found during the experimental period. The highest number of eggs 474 out of 702 were found in group of W8(0.448).

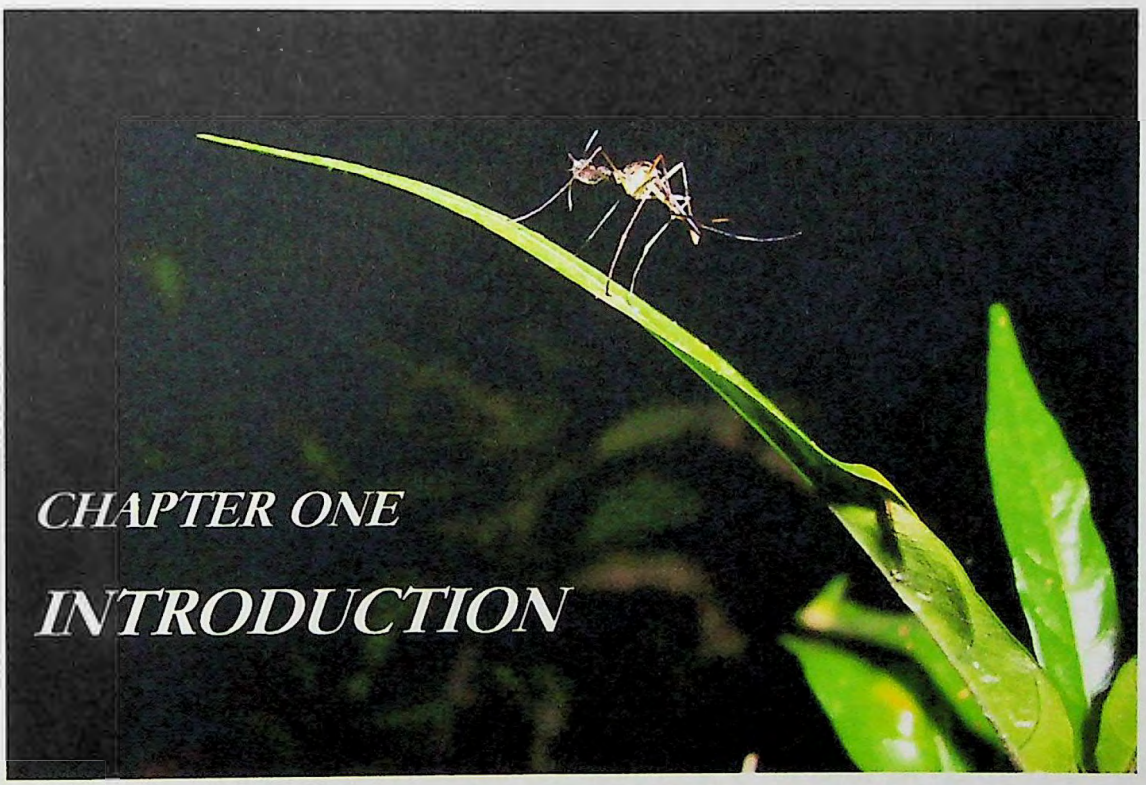
There were four larval stages of *Tx. splendens* after egg incubation. The average egg incubation period was 2.0±0.11 days. The developmental periods of larvae were, 1st instar=1.8±0.14, 2nd instar=2.34±0.17, 3rd instar = 3.15±0.13 and 4th instar = 7.7±0.33 days. The pupa attained in adult with the average of 4.31±0.17 days. *Tx. splendens* completed its life cycle within 21.47±0.52 days. Two types of prey larvae i.e. nature based prey and colony based prey were used for mass rearing of *Tx. splendens* in laboratoty.

Resting adults of *Tx. splendens* were collected from two villages (Gilagasa and Gorgogia) of Sherpur district. Adults were resting on the following trees, i.e. Bamboo, Black plum, Coconut, Hog-plum, Guava, Jackfruit, Jigar, Lichi, Mango, Pithraj, Pomelo, Rain tree, Tamarind and Shimul, during collection. The adults were found from 0 to 2.52 meter height of the trees. Total height (0 to 2.52m) was fragmented into three parts called H1 (0-84cm), H2 (85-168) & H3 (169-252cm). The seasonal abundance of the adult population of *Tx. splendens* in different height wise and month wise was observed. *Tx. splendens* were highly abundant at the height 85-168cm (H2) of all trees. Seasonal prevalence of males and females were highest in the month of March during study period.

To study the efficacy of *Tx. splendens* to control other mosquitoes in the laboratory, it was observed that the mosquito larvae of *Tx. splendens* were effectively antagonist to vector mosquitoes (*Ae. albopictus* and *Cx. quinquefasciatus*). Consumption rate of the predator increased with the increasing of food availability and this relationship was highly significant. The predator larva consumed prey larvae at the following ratios i.e. 10:1, 15:1,

20:1, 25:1 and 30:1. The predator larva consumed highest no. of II and III-instar of *Ae. albopictus* and *Cx. quinquefasciatus* at the ratio of 30:1. That was the higher rate than other consumptions. Statistically it was also significant at 0.01 level. In the prey: predator ratio (10:1) the consumption rate of prey larvae was lower. During experiment it was observed, that the predator larva killed prey larvae but not consumed them. In the prey-predator ratio, 30:1 the predator larva killed highest number of prey larvae. Developmental period of predator larvae was low in prey-predator ratio 30:1 and it was high in ratio 10:1. Pupal development of *Tx. splendens* depended on the quantity of food in the container. It was observed that the prey availability and the pupal development period always decreased when prey density was high.

The laboratory based biology was conducted at room temperature prevailed in the locality at the different seasons of Bangladesh. Sometimes the room temperature was increased with the help of room heater especially during winter season. For rearing, mating and mass rearing different types of cages (a) 35×35×35cm, (b) 35×45×35cm and (c) 65×65×65cm were used which were essentially modified froms previously used by Yap & Foo, 1984 and Horio & Tsukamoto,1985. Bamboo-leaf infusion water was used in the laboratory, it produced bamboo leaf's aroma and that allured as a source of female oviposition. This was a unique rearing medium for *Tx. splendens* and prey mosquitoes. Field collected *Culex* spp. especially *Cx. quinquefasciatus* as nature based prey and colony based prey of *Cx. quinquefasciatus* were used for mass rearing. To observed the efficacy different type of colony based mosquitoes larvae i.e. *Ae. albopictus* and *Cx. quinquefasciatus* were used routinely.



# CHAPTER ONE

# INTRODUCTION

# CHAPTER ONE

## Introduction

Mosquitoes (Diptera: Culicidae) are a potential group of insects, with about 3490 species (Harbach and Howard, 2007). Of them many responsible for the transmission of the pathogens causing some of the most life threatening and debilitating diseases of man including malaria, yellow fever, dengue, dengue haemorrhagic fever, filariasis, encephalitis etc. Among insects, the mosquitoes can be placed at the top of the list of health hazards because of their transmitting capacity of diseases and by their constant attack and painful biting irritation. However, there are some useful mosquitoes that may play an important role in medical entomology (Herms, 1961).

In the late fifties, the World Health Organization (WHO) launched a global campaign of spraying DDT to eradicate mosquitoes and gained tremendous success in many parts of the world (Gramiccia and Hempel, 1972; Sharma, 1987). Soon it was realized that the long-term residual effect of DDT had serious health hazard and mosquitoes developed resistance against it. So alternative methods of control measure like biological control method came to limelight (Chow, 1972; Wright *et al.*, 1972) because of their minimum or virtually no effect on the environment and if properly adopted it proved to be very effective, not only ecofriendly but also cost effective. In connection with the control of malaria, huge numbers of works have been done on *Anopheles* mosquitoes. Recently, works have also been done on different species of *Culex* and *Aedes* to control other mosquito-borne diseases. Mosquitoes such as *Aedes, Armigeres, Toxorhynchites* and various Culicinae genera breed in container habitats (Burton and Rudnick, 1979; Barraud, 1934; Tsukamoto *et al.*, 1987). Of them, various species of *Aedes* and *Culex* are well known as vectors (Chan *et al.*, 1971; Wolfe and Aslamkhan, 1971). Therefore, mosquitoes breeding in tree-holes (container habitats), need special attention as some of them may be dangerous. *Toxorhynchites* mosquitoes,

were suggested as effective bio-control agent for tree hole mosquito and peridomestic container breedes mosquitoes early in twenty centuery (Colledge, 1911; Buxton and Hopkin,1927). Chemical pesticides failed to give sustained control of vector population and their overuse resulted in development of resistance, elimination of natural enemies with subsequent imbalance in the ecosystem and environmental hazards besides ever-increasing cost (Price and Waldbauer, 1975; Service. 1977). To rationalize the use of insecticides with minimum impact on the environment it is now advocated to suppress the target population through integrated approach including the use of bio-control agents (Huffaker and Messenger, 1976).

Adult *Toxorhynchites* are vegetarian do not harm to human being and at the same time a deadly foe to the young of other mosquitoes (Colledge, 1911). The adult *Toxorhynchites* have an unusual life cycle that they are not capable of blood feeding and therefore, are not pests or vectors to man, and the larvae are predatory on other mosquito larvae and show 'prepupal killing' behavior (Collins and Blackwell, 2000). *Toxorhynchites* spp. are widely distributed in India, in many South East Asian countries and some island in the western Pacific (Barraud, 1934; Bonnet and Hu, 1951; Muspratt, 1951; Hu, 1955; Thurman, 1959; Nakagawa, 1963; Corbet,1964; Chan, 1968; Yasumo and Tonn, 1970; Steffan, 1975; Knight and Stone, 1977; Engber *et al.*, 1978; Furumizo and Rudnick, 1978; Focks *et al.*, 1979a, 1980, 1982; Gerberg and Visser, 1978; Bailey *et al.*,1983; GeethaBai *et al.*, 1981; Panicker and GeethaBai, 1983; Trimble,1983; Vongtangsward *et al.*, 1983; Yap and Foo, 1984; Chadee, 1985; Steffan and Evenhuis, 1985; Annis *et al.*, 1989; Rawlins *et al.*, 1991; Amalraj and Das, 1992, 1994a, 1994b, 1996a, 1998, 2005; Tikasingh, 1992; Toma and Miyagi, 1992; Yasuda, 1996; Aditya *et al.*, 2007; Focks (2007) including Bangladesh (Nasir-Ud-Din, 1952; Ameen & Talukder,1974; Rosenberg & Evenhuis, 1985; Ahmed *et al.*,1990; Hassan, 1990).

*Toxorhynchites* mosquitoes are recognized as potential biological control agents of pests and vector mosquitoes. Since the beginning of twentieth century, there have been many attempts to use them for this purpose but with low levels of success (Collins and Blackwell, 2000). It is thought that inadequate knowledge on the biology and bionomics of *Toxorhynchites* are responsible for poor success. Sufficient knowledge and intimation on the biology of each species in its own climatic and geographical zones are essential to deploy them for aforesaid purpose. Attempts to control pest and vector mosquitoes using *Toxorhynchites* mosquitoes have been made in many regions of the world (Bonnet and Hu,1951; Chan, 1968; Bailey *et al.*, 1981; Focks *et al.*,1982; Annis *et al.*, 1990; Choochote *et al.*,2003). *Toxorhynchites splendens* has been used successfully in India, where there were significant reductions in the number of *Aedes aegypti, Armigeres subalbatus*, and *Culex quinquefasciatus* breeding in domestic water containers six months after treatment began (Panicker & Geetha Bai, 1983). Second-instar *Tx. splendens* larvae also suppressed *Ae. aegypti & Ae. albopictus* in Malaysia (Chuah & Yap, 1984). In Thailand, fourth-instar *Tx. splendens* reduced the *Ae. aegypti* population( Vongtangswad *et at.,* 1983). In Uganda, *Tx. brevipalpis conradti* (Corbet, 1963 and Sempala, 1983) and in Florida, *Tx. rutilus rutilus* (Focks *et al.,* 1980, 1982) have also been considered as possible biological control agent. Focks and Boston (1979) developed a quantified mass-rearing technique for *Tx. retilus rutilus*. In this devise one can produce several thousand adults every two weeks in a laboratory of USA. Geetha Bai *et at.,* (1981) successfully colonized *Tx. spendens* in laboratory in Pondicery, India where she maintained the cyclic colony. Amalraj, *et al.* (2005) studied the biology of *Tx. splendens* and used *Ae. aegypti, Anopheles stephensi* and *Cx. quinquefasciatus* larvae as food and they found that immature development was faster with increase in food availability. Aditya *et al.*, (2006b) made a preliminary survey of larval habitat and temporal variation on mosquito diversity in the selected localities of Darjeeling town, India. They found a

positive correlation between immature population of the prey and predator (*Tx. splendens*). Rawlins *et al.*,(1991) released *Tx. moctezuma* in a Carribean Island and after one-month substantial reduction (64% males and 80% females) of *Ae. aegypti* was observed. They suggested that repeated inundative release of *Tx. moctezuma* contribute to an effective mosquito control program.

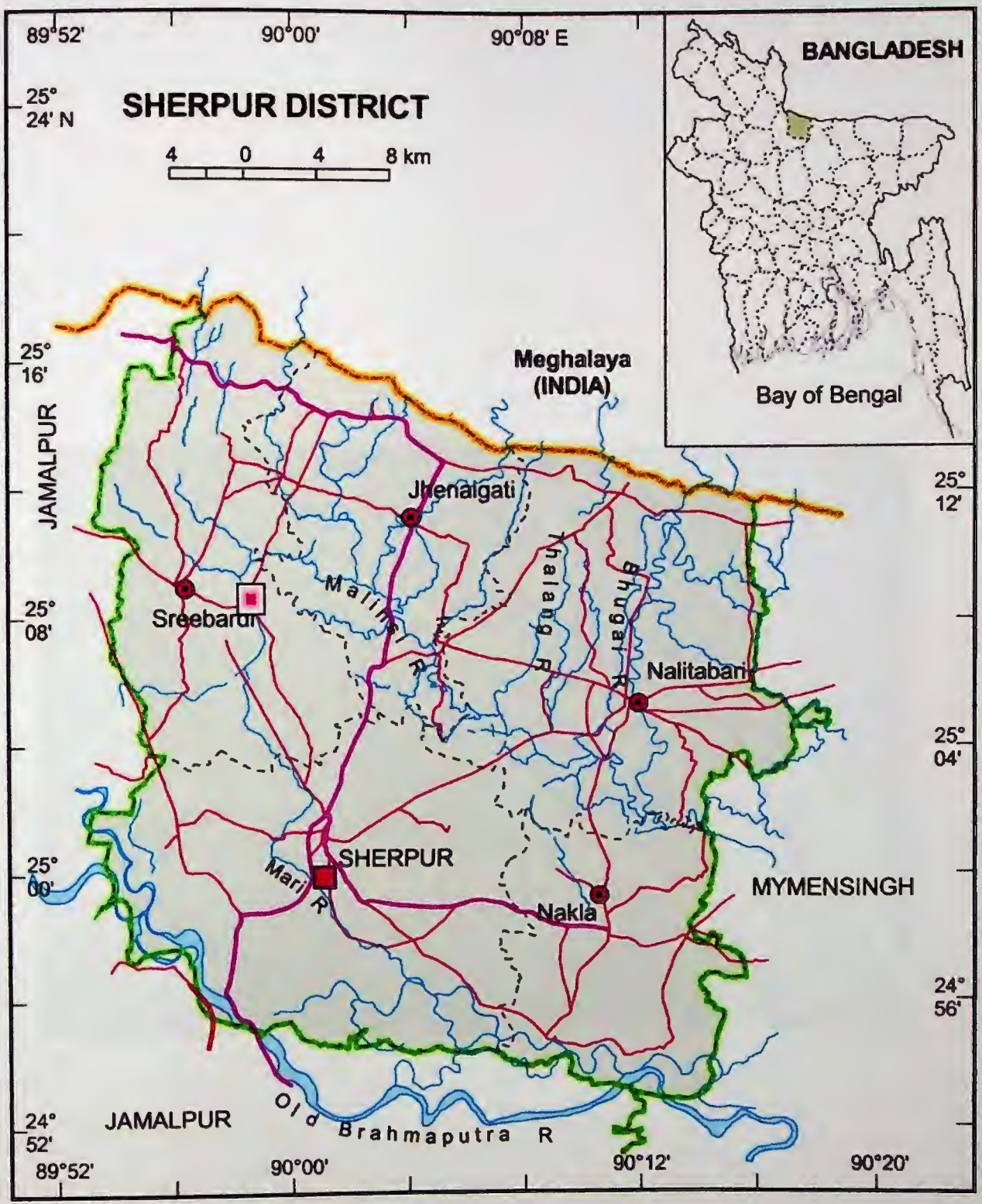
In Bangladesh, various aspects of research on mosquitoes like disease control, behavior pattern, taxonomy, toxicity, etc. have been attempted. These tasks have provided some information on the aspects related to the mosquito-borne diseases. About 100 articles have been published in the period from 1947 to 1991, a part of which listed by Elias *et al.*,(1984). Of them, only 4 were comprehensive field study on mosquitoes breeding in container habitats (Nasir-Ud-Din, 1952; Ameen & Talukder, 1974; Rosenberg and Evenhuis, 1985 and Huda & Majumder, 1985). Barraud (1934) described *Toxorhynchites* genus as *Megarhinus splendens* (= *Toxorhynchites splendens*). Extensive research work on the species of *Toxorhynchites* in our country is scanty.

An outbreak of dengue (Dacca fever) in Bangladesh caused several deaths in the summer of 1964 (Aziz *et al.*,1967). After that from 2000 to 2008, dengue diseases increased in Bangladesh (Appendix Table 1) therefore about 23370 dengue cases and 277 deaths was recorded. Of them in Dhaka City effected 21276 dengue cases and 181 deaths. *Aedes aegypti* and *Ae. albopictus* are vectors of dengue fever in Singapore (Chan *et al.*, 1971) and very common in Dhaka (Khan, 1980). Biting mosquito (*Armigeres subalbatus*), dengue and filariasis vectors are container-breeding mosquitoes (Tsukomoto *et al.*, 1987; Ahmed *et al.*,1990 & Hassan, 1990). They breed in tree-holes, bamboo stumps, coconut shell, earthenware, broken glass, tyre, cans etc. Usual control measures such as spray of chemical are difficult for their special location. *Toxorhynchites splendens* mosquitoes also breed in container

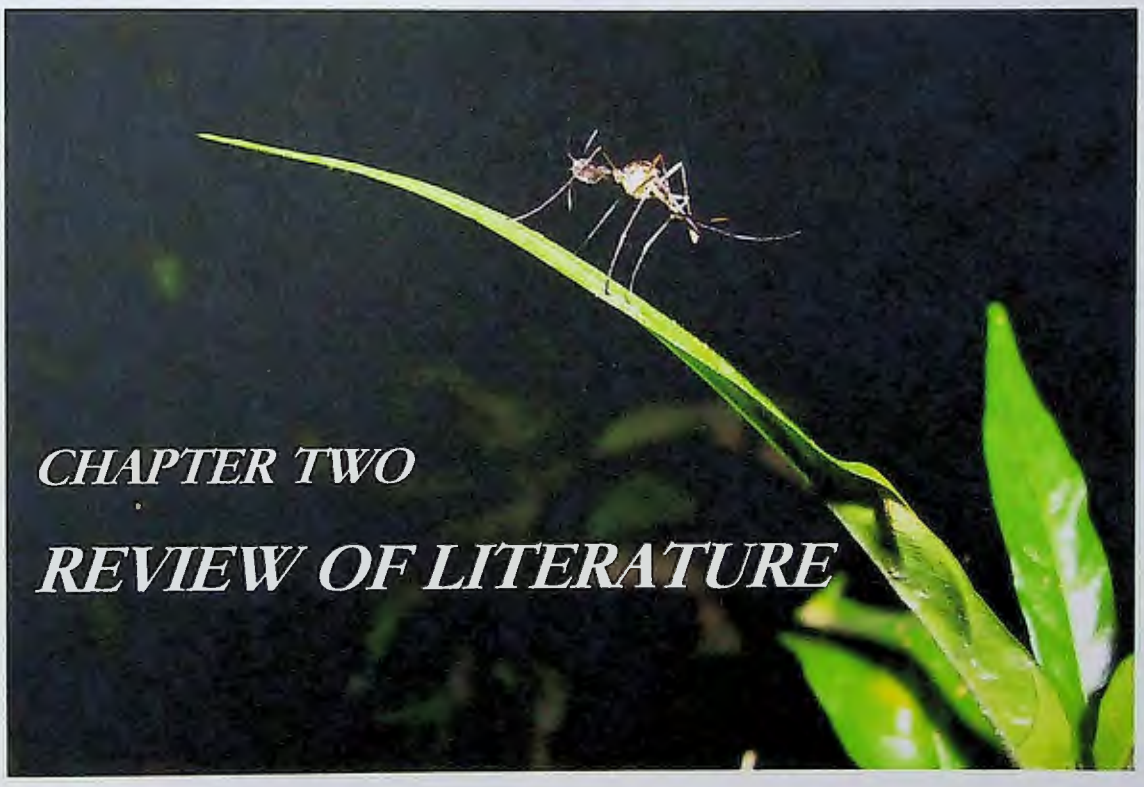
habitats and well known as biological control agents of these mosquitoes. Biological control method is fruitful because of their no harmful effect on environment like insecticides but also for cost effectiveness. Therefore, the proposed study was envisaged to detecte ecological niche and seasonal abundance; swot up the biology to evaluate the efficacy to control mosquito vectors of infectious diseases. Moreover, cyclic colony of *Tx. splendens* and prey mosquitoes (*Ae. albopictus* & *Culex quinquefasciatus*) maintained along with mass rearing in the laboratory. The researcher enthusiasm conducted investigation in the laboratory of Institute of Biological Sciences, Rajshahi and in 2 villages of sherpur districts of Bangladesh.

**Objectives**

- To study the biology of *Toxorhynchites splendens* in the laboratory.
- To determine the seasonal abundance of *Tx. splendens*.
- To evaluate the efficacy of *Tx. splendens* to control other mosquitoes in the laboratory as a biocontrol agent.
- To maintain the prey and predator mosquito colony in the laboratory and their cyclic colony.
- Mass propagation of *Tx. splendens* in the laboratory.




**Map of the study area (Gilagasa and Gorgoria Village)**



# CHAPTER TWO

# REVIEW OF LITERATURE

# CHAPTER TWO

## Review of Literature

Paiva (1910) noted that the larvae of *Toxorhynchites* were predators of a number of mosquitoes' larvae. He discovered the larvae of *Tx. immisericors* in earthen were pots round Calcutta.

Colledge (1911) noted that brush-tongued mosquito (Toxorhynchites speciosa) adults were vegetarian, no harm to human being, and at the same time a deadly foe to the young of other mosquitoes and mention its cannibalism. He suggested to use it as biological control agent as an alternative approach to the use of chemical pesticides.

Knab (1911) proved that the wrong statement of some authors about *Megarhinus* (=*Toxorhynchites*) mosquitoes biting. He identified and perfectly proved that these were non-biting mosquitoes i.e. their inability to suck blood, their mouthparts (proboscis) cannot pierce the skin, but wholly feed upon the sweet of different flowers and honey feeder.

Aders (1917) reported the insects injurious to man in Zanzibar town and collected, among others, various types of mosquitoes' species, i.e. Anophelines, Culicines, Stegomyias and *Tx. brevipalpis.* From them *C. fatigans* was the most prevalent mosquito. *Toxorhynchites* sp. predates on larvae of *A. costalis, C. fatigans, S. fasciata.* During three year survey (1913-1915) he found every mango tree with holes in the trunk with 3 to 8 larvae of *Tx. brevipalpis.* He concluded that the *Tx.* sp was of no practical value as they prey mosquitoes were rarely found in the same habitat.

Paine (1934) introduced the *Toxorhynchites* (=*Megarhinus*) mosquitoes into Fiji, pacific Island. This was the first milestone of success for establishing mosquito colony to control other vector mosquitoes. Fourth instar larvae of *Toxorhynchites* were transported from Java to Fiji (the voyage of 28days) survived lengthy period without food. From the life cycle of *Tx. splendens,*

he observed that eggs were almost round & glossy white and dropped singly on water and float on the surface film, incubated for two days. A larva of *Toxorhynchites* fed on tadpoles, tipulid, chironomus & all sorts of mosquito larvae and also showed cannibalism. Larval duration minimum was 16 days (food abundant) and maximum 134 days (food scarce), pupal duration was 6 days. Minimum developmental period egg to adult was 30 days.

Newkirk (1947) studied the *Tx. splendens* (=*Megarhinus splendens*) in New Guinea and Philippine Island abd observed its value in biological control of the mosquitoes. From field observation, larvae and pupae were collected from tree-holes, bamboo stumps, leaf axils etc. i.e. container and in one instance was found in ground pool. He observed that in New Guinea, mortality of immature stage was high (82-91%) in nature. The mortality was not due to scarcity of food; a fourth instar larva survived as long as seven weeks without food but showed cannibalism. In laboratory, 10%percent sucrose in water with a cotton wick for adult.

Muspratt (1951) described the bionomic of an African *Toxorhynchites* (= *Megarhinus*) *brevipalpis* and its possible use in biological control. Tropical and subtropical forests were the main vegetation types where the species was found. The insectary (a room 9×9×8.6 ft) was maintained at tropical heat and humidity. Oviposition was usually accomplished in flight; various in-season fruits (grapes, peaches, apples, pineapple, citrus and tomatoes) were hung up as food for adult and balls of cotton wool with honey also offered. Eggs incubation time was less than two days, larval duration 11-20 days (average 14.5 days), and pupal duration five days. Cannibalism did not occur among $4^{th}$ instars larvae but they devoured small larvae of their own species. Female layed about 85 eggs in 30 days complex. Cane sugar solution and honey mixed with water for adult diet.

Belkin (1952) reviewed the homology of the chaetotaxy of immature mosquitoes and a revised nomenclature for the chaetotaxy of the pupa and have shown that first-instar larva and pupa lack of abdominal hairs 9 & 11.

Nasir-Ud-Din (1952) recorded the mosquito species (including *Tx. splendens*) which breed in tree-holes in front of the S.M. Hall of Dacca University Campus and bamboo stumps in Malibagh, Dhaka for a period of about one year. Altogether 7139 adult mosquitoes belonging to 13 spp. were hatched out in the laboratory from the 30 samples of water from tree-holes.

Williams *et al.,* (1961) observed *Tx.rutilus septentrionalis* in the field and laboratory. Males were always found in a certain locality of a certain tree or bush. Oviposition behavior observed in the field. Female begun a vertical loop or circles some 14 to 24 inches in diameter over the water approximately the same speed and the loop has been reduced to some 4 to 6 inches when she forcibly ejected egg on the downward dive of each loop. In laboratory, mating attempts occurred after 4 pm. The longevity of male and female was 19 and 44 days respectively. Adults were offered an artificial diet consisting of 1 liter of 10% sucrose with 25 ml of extracted honey solution with Burnett's commercial food coloring(red, green, blue and yellow) was added in rearing cages(24" × 24" × 20").

Corbet and Griffiths (1963) studied the larvae of *Tx. brevipalpis conradti* and *Tx. kaimosi,* in the field and laboratory in Uganda. The larvae were obtained for experiment from regular field collection. *Tx. b. conradti* larvae need 14 days for development and *Tx. kaimosi* considerably needed more time to reach the pupal stage. The post ovarian aquatic stage was at least 3 week in each species. In laboratory pupal stage of both species required about 6 days. Fourth instar larvae of *Tx. conradti* showed no preference for either 2$^{nd}$ /or 4$^{th}$ instar larvae of *Ae. aegypti* offered as food. Also fourth instar larvae killed and devoured *Toxorhynchites* pupae as well.

Dodge (1964) described thoracic chaetotaxy of all instars with figures and observed the biology of *Tx. r. septentrionalis*. He also described chaetotaxy of abdomen in brief. He separated newly hatched 1$^{st}$ instar larvae from mature 1$^{st}$ instar larvae.

Goma (1964) described the laboratory observations on the influence of illumination on the predatory habits of two species of *Toxorhynchites* larvae. He working on *Tx. brevipulpis condradti*, *Tx. kaimosi* found that the predation on *Ae. aegypti* was considerably more active in light than in dark.

Lien (1965) described the genus *Toxorhynchites* mosquitoes of Taiwan. A key and some aspects of their bionomics and morphology were described in that with illustrations.

Chan (1968) studied biology of *Tx. splenden* in order to utilize this species for the control of dengue vectors (*Ae. aegypti* and *Ae. albopictus*) in a rural area of Singapore. The observed life history of it was: eggs hatched in 1-2 days; larval stage varied from 13.5 to 52 days, with an average of 34.6 days; pupal stages were 4 to 5 days; egg to adult was 5 to 6 weeks and predator 4$^{th}$ instar larvae withstood hunger over long periods about 30 days in the laboratory.

Yasuno and Tonn (1970) described the bionomics of *Tx. splendens* in the larval habitats of *Ae. aegypti* in Bangkok, Thailand. This preliminary investigation on ecology led to speculation on *Tx. splendens* as a predator in nature. The study was developed as parts of a life budget project on *Ae. aegypti* . *Tx. splendens* was observed to be a possible mortality factor in certain containers but not in others.

Chow (1972) summarized an alternative control activity for mosquito other than insecticides such as environmental sanitation, biological control and genetic control.

Trpis (1972) described the development of embryos, larvae and pupa of *Tx. brevipalpis* with *Ae. aegypti* as larvae as prey in relation to different constant temperatures. Embryonic and larval developmental range was 14-30°C, lower and upper threshold temperature was 13°C and 33°C respectively. Optimum developmental temperature was 29-30°C, above this temperature both in embryos and larvae development was prolonged. Although more

prey larvae were consumed and/or killed at 30-32°C than at 20-26°C. Each predator larva destroyed 154 to 358 prey larvae during entire period of larval development.

Trpis (1973) observed that the *Tx. brevipalpis* was abundant mosquito in Tanzania, it bred in tree-holes and still more extensively as prey in water filled tires and tin containers containing *Ae. aegypti* larvae and observed cannibalism. He also investigated the predator-prey relationship in the laboratory predator destroyed an average of 154 prey larvae at 26°C and 359 larvae at 32°C. He also observed that predator life cycle was almost 3 times long as the prey (the predator basics to be effective as a control agent only when the prey at all development stages).

Trpis and Gerberg (1973) described the successful colonization of *Tx. brevipalpis,* a predator on larvae of *Ae. aeggpti* and other mosquitoes, in the laboratory at 25°C. Embryonic development was 50 hours, larval and pupal development together was 27-41 days, colony cage was 15 × 15 × 15 cm and embryonated eggs were obtained 6-31 days after the adults emerged. Adults were nursing in cage with honey and water.

Ameen and Talukdar (1974) collected a larva of *Toxorhynchites* from the forest near Shalna, about 40 km away north of Dacca University campus. The detailed chaetotaxy of its pupal skin was described and identified as *Tx. splendens.*

Ramalingam and Belkin (1976) described the immature stages of *Ae. samoanus* and the status of *Toxorhynchites* in Samoa. *Tx. brevipalpis* was introduced in Samoa in 1952 and *Tx. splendens* in 1955 and these two species reintroduced in 1956 for controlling the *Ae. polynesiensis* vector of Bancroftian filariasis. An extensive collections in 1963 revealed that neither *Tx. brevipalpis* nor *Tx. splendens* occured in American Samoa instead a third species, *Tx. (T) amboiensis* was a common there. *Tx. brevipalpis* was unable to establish itself. As for the introductions of *splendens*, apparently, there was misidentification - the species actually released was *Tx. amboinensis.*

However, it was not successful as a biological control agent in suppressing the population density of any mosquito.

Bradshaw and Holzapfel(1977) described the interaction between photoperiod, temperature and chilling in dormant larvae of tree-hole mosquito *Tx. rutilus* reared in the laboratory. Photoperiod is important for initiation and maintenance of dormancy, adult response to day length so that resumed development is independent of photoperiod and behavioral process may remain active. They reared the larvae on *Tubifex* and freshly hatched normal *Artemia salina* (brine shrimp).

Crans and Slaff (1977) described the growth, behavior and induced copulation of *Tx. r. septentrionalis.* The rearing temperature was 27°C and under controlled laboratory conditions, eggs hatched in 3.0 days, 1st, 2nd, 3rd, 4th instar larva took 1.6, 2.0, 3.2 and 6.6 days respectively. Pupal period was 5.4 days. The total developmental period from egg to adult was 21.8 days for female but male completed one day sooner. Females of this species consumed 237.8 (222-245) *Ae. aegypti* larvae during their development and male consumed fewer prey than female but killed a greater number of prey.

Focks and Hall (1977) studied colonization and biological observations of *Tx. r. rutilus* in Florida under laboratory conditions. Average duration of eggs, larvae, and pupal stages were 1.6, 15.6 and 6.0 days respectively but in mass rearing condition, the duration of larval stage reduced to 11.1 days. Adult females survive for 7 weeks in cages and oviposited was an average of 1 egg per day. Fourth instar larvae survived for about 2 months without food. Females preferred to lay eggs in water previously used to rear *Ae. acggpti.* Provided the apple slices for adults.

Furumizo *et al.,* (1977) recorded the first successful colonization of *Tx. splendens* in laboratory in Malaysia and describe colony maintenance. Wild-caught adults about 145 male and 99 female were released in a 61 × 61 × 61 cm breeding cage and fed 10% honey solution, every 24 hrs wet towels were draped along three side of the cage to maintain high humidity. The insectary was maintained at 26.6 ± 4°C & RH of 78 ± 5%. They used

black plastic jar filled with tap water as ovitrap in laboratory female laying egg in air to oviposion jar and several females hovering over oviposion jar created air drafts that caused the eggs to be blown out of the jar. Prey predator ratio (10:1) was always maintained in each vial. The water of each vial was changed frequently.Resting males and females were collected from coconut tree trunks, 2-4m abouve ground.

Clausen (1978) noted that *Tx. brevipalpis* and *Tx. splendens* were predators of arthropod pest i.e. mosquitoes larvae, larvae of own species, as well as on various small insects and crustacean. *Tx. brevipalpis* bred in tree-holes and often were found in the leaf axils, where as *Tx. splendens* also bred in tree-holes and invaded rock pools, coconut husks, tins and other habitats of certain pest species.

Focks *et al.,* (1978) described laboratory rearing of *Tx. r. rutilus* on a non-living diet i.e. a tropical fish feed "Tetra Min$^{(R)}$". Larvae reared on Tetra Min$^{(R)}$ required an average of 107.5 days from egg to pupa compared to a relatively short 15.6 days for larvae reared on *Ae. aegypti.* Weights of the pupae were 29.3 mg and 50.0 mg with diet Tetra Min and *Ae. aegypti* respectively.

Furumizo & Rudnick (1978) have presented some biological observation of *Tx. splendens* in laboratory which have not been reported earlier. During the investigation they have conformed earlier findings while some observation were contrasted to their previous studies (1977). Wild adults were caught from coastal area of Malaysia. Their eggs were reared in individual vials filled with 20 ml tap water, *Aedes aegypti* and *Ae. albopictus* larvae were used as prey. For 1$^{st}$ and 2$^{nd}$ instars a 10:1 prey - predator ratio and a 15:1 ratio was kept during 3$^{rd}$ & 4$^{th}$ intars larval development. Water volume was increased up to 50 ml for the 3$^{rd}$ & 4$^{th}$ instar development. Pupae were placed in individual 15 ml plastic vials filled with 10 ml tap water. They did not regulate photoperiod in the laboratory and observed mating between 2:30 pm and sunset. During mating, in most of the cases, adults in copula fall on the cage floor. Pre-oviposition period was 5-7 days, oviposition was 27 days,

adult longevity was estimated to be 28-35 days, eggs incubation period was 48-60h, and required 21.5 days from egg to adult.

Gerberg and Visser (1978) conducted preliminary field trial in the Caribbean island St. Maarten using *Tx. brevipalpis* as a biological control agent for *Ae. aegypti.* Sixteen days after the introduction of *Tx. brevipalpis* eggs into *Ae. aegypti* breeding containers, all the houses (21) sampled no longer had *aegypti* breeding i.e. house index dropped to zero. Hartberg (1978) described the normal larval color in *Tx. brevipalpis* as mahogany-red on the dorsal half and a greyish-white on ventral surface. In laboratory newly, hatched larvae were colorless.

Burton and Rudnick (1979) described *Toxorhynchites* as large sized harmless mosquitoes. Most people were not familiar with because they are much better adopted to living in the wild than in densely populated areas, fortunately people did not notice since they had not biten and nor the females take blood meal (like vector/pest mosquitoes), both male and female live on nectar and other plant juices. For the maturation of eggs necessary protein was obtained in the larval stage, as the larvae are predaceous on other invertebrates, hence-called beneficial mosquito to man. Predacious larvae are used as biological control agent for other mosquitoes and adults are used as a laboratory research tool for isolation. *Toxorhynchites* spp. have different ecological requirement some being quite restricted in range and in breeding site but *Tx. splendens* has a wide distribution and breeding ecology.

Focks and Boston (1979) devised a mass rearing technique to produce several thousand of adult *Tx. rutilus rutilus*, in every two week in laboratory. Mass rearing method had been described in detail {in brief 7000 eggs/newly hatched larvae of *Ae. aegypti* were used as food for 1$^{st}$ instar larvae of *Tx. r. rutilus.* In addition, the culture media contained liver powder and hydrolyzed yeast (3:2). The temperature was 26-27°C and with a (13L: 9D) photoperiod}.

Focks *et al.,* (1979a) studied the field survival, migration and ovipositional characteristic of laboratory-reared *Tx. r. rutilus* in sparsely wood, 5.3-ha residential area in Gaines-ville, Florida. Oviposition was monitored for two weeks in 64 oviposition traps located in this area and surrounding woods (12.6ha).Eggs were deposited in 70% of the ovitraps, preoviposition was 6 days and lifetime egg produced per female released was 4.88 and adult survival estimated was 0.795.

Focks *et al.,* (1979b) described the susceptibility of *Tx. r. rutilus* to five adulticides currently used for mosquito control in Florida. *Ae. aegypti* was nearly twice as susceptible to malathion, naled, and fenthion as *Tx. r. rutilus* difference was observed in susceptibility to chlorpyrifos. Resmethrin was more toxic to *Ae. aegypti* than to *Tx. r. rutilus*.

Trpis (1979) described the rearing of *Tx. brevipalpis* from larva to adult on non-prey diet i.e. liver powder in water. Larvae fed on liver powder developed 3 time slower (36.2 days) than larvae fed on a pray larvae (11 days). He also gave mixed diet for larval rearing offered liver powder and prey in the early instar ($1^{st}$ - $3^{rd}$) and only liver at $4^{th}$ instar. The total developmental periods were 26.9 days. He concluded from this study that the development of *Tx. brevipalpis* larvae can be controlled by quality and quantity of food.

Padgett and Focks (1980) described the laboratory observation on the predation of *Tx. rutilus rutilus* on *Ae. aegypti* prey .The rate of consumption, killing behavior and development times as a function of container size, prey density and prey stage were presented with data and statistical analysis.

Steffan *et al.*, (1980) observed the biology of a laboratory colony of *Tx. amboinensis* and compared to published observations of the closely related species, *Tx. splendens*. Mating and oviposition behavior was similar to that of *Tx. splendens* that was diurnal. Fecundity decreased from 14.3 eggs/female/day/ (week-2) to 4.8 eggs/female(week-11) as colony aged. Adult longevity ranged from 13-107 days. Adult reared in cage size 61 × 61 ×

61 cm and fed 10% sucrose dispensed on a cotton wick. Eggs were reared in 12 ml vial and 2nd instar larvae transfer to 35 ml vial containing 20 ml of tap water. Prey: predator ratio was 5:1 and light: dark period was 24:0 and mean development time from egg to adult was 34.8 days.

Vongtangswad and Trpis (1980) found that the fourth instar *Tx. brevipalpis* larvae changed patterns predatory behavior of prey consumption as the development proceeds from moulting to pupation. After two hours of moulting, 4th instar larvae started feeding and the duration of 4th larval stadium was 8 to 10 days but with the increase of prey consumption duration decreased to 7days. Adults were maintained in wooden frame and nylon mesh cages (25 × 30 ×40 cm).

Bailey *et al.,* (1981) tested the effects of various numbers of *Tx. rutilus rutilus* larvae on *Ae. aegypti* breeding in artificial containers receiving regular imputes of *Ae. aegypti* first instars as food in New Orleans, Louisiana, USA. Pupation time for the predator was dependent on the number of prey available during the larval stage. Weekly introduction of predator larva to each container gave better overall control of *Ae. aegypti* than did a single introduction of one or two larvae per container.

Geetha Bai *et al.,* (1981) developed a laboratory colony of *Tx. splendens* in Pondicherry, India and observed the egg hatchability, immature duration, and insemination capacity, longevity of adult, gonotrophic cycle, oviposition preference, fecundity and sex ratio of the progeny. A single larva consumed on average 144 prey larvae and killed double number of prey larvae and duration from egg to adult was 16-18 days. Adults were reared in 60 × 60 × 60 cm and 30 cm cube cloths cage for experiment.

Goettel *et al.,* (1981) studied the mass rearing of *Tx. amboinensis* in laboratory in Fiji using *Tubifex* worm as good alternative food. *Tubifex* gave better result than *Culex* or *Aedes* as prey food.

Padgett and Focks (1981) designed an experiment to determine whether 4th instar *Tx. r. rutilus* preferentially consumed one stage of *Ae. aegypti* or not. Equal number of 1st instar, 4th instar and pupae prey were placed in 3.78 litre containers with one 4th instar predator. Predator ate more 4th instar (18.2) prey than pupae (12.2) or 1st instar (5.9) but they killed (without eating) more pupae than 4th instar, with no 1st instar killing observed.

Steffan and Evenhuis (1981), considered different species of *Toxorhynchites* as potential, valuable allied in main continuing battle against many mosquitoes, are pests or vectors of some of the most serious human diseases.In their review paper the authors analyses 148 papers (1901 to 1980) pertaining to the topic and also impressed on the aspects of *Toxorhynchites* spp. biology that have been stimulated further investigator to biological control of mosquitoes.

Fock *et al.,* (1982) made field experiments on the control of *Ae. aegypti* and *Cx. quinquefasciatus* by *Tx. rutilur. rutilus* biologically in a Suburban area of New Orleans, Louisiana, USA. The production of prey mosquitoes was monitored for 76 days in automobile tires, plastic buckets and painted cans in which 1-2 first instar larvae of predator were added every 10 days.

O'Flynn and Craig (1982) studied the effect of *Tx. brevipalpis* on *Ae. aegypti* in continuous breeding in laboratory, Kenya. Egg hatched 40 to 48 hours, each larva provided with 15 preys per day. Developmental duration of 4th instar larva was 10 to 12 days, pupa stage lasted 5 days. Adult were fed apple slices and honey-soaked cellucotton. These results were on the basis of 34 weeks experiment cages without *Toxorhynchites* reached to 50,000 larvae whereas with *Toxorhynchites* the peak larval density was 10,000.

Bailey *et al.,* (1983) studied the effects of indigenous *Tx. r. rutilus* on *Ae. aegypti* breeding in tire dumps in Florida. Production of prey pupae and adults were eliminated by mean levels of 1 to 5 predator larvae per time during a 10 weeks. They suggested that if large number of predator were released prior to build up of prey density, the later would be controlled.

Russo (1983) studied the functional response of *Tx. r. rutilus*, a predator on container-breeding mosquitoes. Estimates of attack rates within and across its larval instars were not significantly different. At densities of 2 and 4 predators per container, attack rates decreased with increasing predator density but with little significance.

Trimble (1983) summarized the various attributes of predaceous larvae of *Toxorhynchites* of temperate zone, the genus and fortification for use in inundative releases for the biological control of tropical container-breeding mosquitoes. He suggested that one of the three *Toxorhynchetes* species of temperate zone could be exploited to facilitate the timing of inundative releases.

Schuler and Beier (1983) studied the oviposition dynamics of *Tx. brevipalpis* and *Tx. rutilus* and potential prey species in field. The spatial distribution of predator's oviposition in relation to prey oviposition preferences was examined. The oviposition was monitored from 75 ovitraps at 25 sites in a village located near the students housing complex of Notre Dame University of Indiana.

Vongtangswad *et al.,* (1983) described the biological control of *Ae. aegypti* by using predator larvae of *Tx. splendens* mosquito in Sa-Med Island, Thailand. They suggested that it was feasible and practical to transport 4$^{th}$ instar larvae for quite a long distance by bus etc. They further suggested weekly release of 4$^{th}$ instar larvae might well suppress and possibly eradicate *Ae. aegypti* which would be ecofriendly as well as economic.

Focks (1984) studied the effects of sub-lethal dose of resmethrin on reproduction of *Tx. r. rutilus* at the established LD-90 dose for *Ae. aeggpti*. It did not reduce the daily adult survival and egg hatch of *Tx. r. rutilus*. Average fecundity was reduced from 5.6 to 2.3 eggs/female/day during the first 3-4 days of oviposition. In an IPM program where this adulticides were carried out routinely, should be done prior to release of predator to get overall better result.

Chuah and Yap (1984) carried out laboratory and field studies on a few parameters to explore the biological control potentials and efficiency of *Tx. splendens*. For longevity experiment they found that adults *Tx. splendens* were unable to survive for more than a week without food and water in the laboratory while with food and water normal adult male and female survive 21.8±2.7 and 11.6 ± 1.8 days respectively. Fourth instar larvae servived 110 ± 0.7 days in a tube with damp control without food.

Chowanadisai *et al.,* (1984) reported on the laboratory observation of biology of *Tx. splendens* in Thailand and a survey on natural enemies *Aedes* and *Culex* for a biological control. Eggs were reared individually in plastic vials, 2.5 cm in diamer and 5 cm high. Adult were maintained in cages size (60 × 60 × 75 cm). Cotton pads soaked 10% honey solution for adult diet, 2$^{nd}$ instar of *Ae. aegypti* larvae were offered as prey to *Tx. splendens* larvae. The incubation period of eggs was 50 to 57 h. Larval instars required their development 2.38±0.04, 1.24±0.26, 8.09±1.6 and 28.78±5.39 days respectively, pupal period lasted for 5.18 ± 1.06 days and adult longevity recorded was 29.55 ± 8.06 days.

Rubio and Ayesta (1984) studied the biology of *Tx. theobaldi* in the laboratory. Egg hatched within 2 days, mean larval developmental time for fed on 20 prey larvae was 14.5 days while those fed on 40 prey larvae daily was 13.0 days. Cannibalism occurred when the number of prey availability was low. From pupa mean time recorded for emergence was 49 second. Total development period from egg to adults was 20.1 days. The observation was carried out in Venezuela. The developmental sequences were filmed using two 16 mm cameras.

Yap and Foo (1984) studied the oviposition site preference of *Tx. splendens* in the laboratory using following variable: color, container type, height, water type and salinity. Female of this species preferred more attractive to artificial container: ovitrap (a drinking glass rim diam 7.5cm height 15cm coated glossy black), tincan, tire and natural container: bamboo stump,

coconut shell, tree hole used as ovitrap near ground level in the cage. Females did not touch water while ovipositing i.e. aerial oviposition. No significant preference for water type or salinity gradient was observed. Eggs were viable only in water containing less than 1% NaCl. The colony was maintained in a 40 × 40 × 40 cm copper-screen use with a plywood floor placed in an insectary with 25±1°C and 70 ± 5% RH.

Horio and Tsukamoto (1985) described the successful laboratory colonization of three Japanese species of *Toxorhynchites*. They used large size cage (60×60×75 cm) covered by a black polyvinyl sheet with small window at the top. New artificial mating technique applied and obtained enough number of eggs to sustain the colony even in smaller cages size (20×30×20 cm), and egg to adult developmental period was 19-20 days.

Ramalingam and Banu (1985) considering the importance of *Toxorhynchites* spp. for biological control of other vector and/or haematophagous mosquitoes and to detect the dengue viruses have reviewed the global position of colonization of nine species of the genus. They also in briefly reviewed the protocols of colonizing the species and used rain or tap water.

Stefan and Evenhuis (1985) dealt with classification of 36 known species of *Toxorhynhites* (*Toxorhynchites*) of Australasian, eastern Palaearctic and Oriental region. They assigned those to 7 species- groups. Taxonomic history, biology, distribution, phylogeny, taxonomic characters with key to adults, male genitalia, pupae and illustration of key characters of these species groups were provided in the paper.

Nasci (1986) described the use of tree sap as a source of plant juices by the mosquito *Tx. rutilus septentrionalis*. Normally floral and extra-floral nectaries, honeydew, fruit juices etc constitute the major source of carbohydrate, from which energy of metabolism obtained.

Russo (1986) made a comparison of predatory behavior in five species of *Toxorhynchites* (*Tx. amboinensis, Tx. brevipalpis, Tx. rutilus rutilus, Tx.*

*splendens* and *Tx. theobaldi*) larvae in laboratory, Indiana, USA. He examine the movement of the predator during the hunt, described the attack on the prey for consumptive movements and abandonment. An attack resulting in consumption was usually associated with S-Shaped posture of the predator during ingestion while an attack resulting in abandonment of the prey was accomplished by wiping of mandibles across the aciculae. He also observed that though the pattern of surplus killing varied from species to species but (never begun killing unless predators achieve the minimal weight required for pupation) it begins prior to pupation.

Smittle and Focks (1986) presented the results of radioactive labeling technique with $^{32}P$ of immature *Tx. rutilus rutilus* to produce female that deposit radioactive eggs. The radioactive labeled females and unlabeled (normal) females had similar behavioral biology. This method of radiolabeling would provide sound tools for tracking laboratory reared females release into an area with indigenous population.Pre-ovipositing days was 5 or 6.

Linley (1987) described the aerial oviposition flight of *Tx. amboinensis* from videotaped behavior. The females fly anticlockwise in a sequence of ellipse obliquely oriented to the ovitrap, until an egg is ejected on the downward flight of the final ellipse.

Annis & Rusmiarto (1988) repeated the mass rearing technique of *Tx. splendens* in laboratory in accordance with the methods used by U.S. Naval Medical research Unit of Jakarta, Indonesia as described by Focks and Boston (1979) for *Tx. rutilus rutilus*. The insectary was maintained at temperature of 24 to 29°C and 50 to 85 RH. Due to high rates of larval and pupal mortality this method of rearing was discontinued. However, they could not find the reason of high rate of mortality.

Elias *et al.,* (1988) had imported *Tx. splendens* larvae from Mi-Mi Khin of Burma (Myanmar) and reared them in the laboratory of National Institute of Preventive and Social Medicine (NIPSOM), Dhaka. On the basis of their study they published a paper depicting the biology of *Tx. splendens* that they prey

on larvae of *Ae. aegypti.* They found that average larval duration of the predator was 17.5 ± 1.28 days. During this period average 256.64 ± 20.88 and 173.5 ± 43.0 *Ae. aegypti* larvae were eaten and killed respectively at the rate of 9.91 ± 2.45 and 14.65 ± 1.19 larvae per day. They reared adults on glucose mixed with Vitamin-B complex and used a small round tin pot blacked with coaltar containing tap water for oviposition.

Annis *et al.,* (1989) made an experiment to test the efficacy of *Tx. splendens* to control the *Ae. aegypti* as an alternative to times has in household water storage container in Jakarta, Indonesia between April 1987 and April 1988. First instar *Tx. splendens* larvae were released in house hold water containers which harbor the larvae of dengue vector *Ae. aegypti.* A single 1st instar larva was released in each container. Forty-one percent of all containers in the treatment area were treated each week the average container was treated once every 2.4 weeks. *Ae. aegypti* population were suppressed but not controlled by the treatment. They hypothesized that 1st instar larvae were poor control agents due to their inability to withstand periods of starvation. In their laboratory, they found that first instar *Tx. splendens* larvae survived an average of 5.6 days without food.

Linley and Duzak (1989) made experiment on cannibalism in the laboratory, third and early fourth instar larvae of *Tx. amboinensis, Tx. splendens* and *Tx. brevipalpis,* that there larva previously starved 24 hours, they observed rapidly cannibalized eggs of their own species or ate the eggs of other species present on the water surface in small container.

Horio *et al.,* (1990) for the first time, described from field collection a wild-type strain of *Tx. splendens.* These mutants have been distinguished from wild type in both larval and pupal stage but not in adult. Genetic analysis of a new mutant larva and the effect of larval body color on cannibalism have been carried out. According to them white larvae was more cannibalistic. The mutant strain has been maintained for 53 generation in the laboratory of the University of Occupational and Environmental Health, Japan.

Linley and Seabury (1990) described the egg of *Tx. splendens* and compared with the egg of *Tx. amboinensis*. The outer chorionic cells of *Tx. splendens* egg contain uniform tubercles over the entire surface, except the micropyle. Each cell contained a single large tubercle of constant diameter (10.6 μm) surrounded by 6-11 smaller tubercles of variable diameter (5.6 μm, range 2.1-9.3 μm). The tubercles surface consisted of narrow ridges, with separating clefts originating radically at the top of each tubercle and running longitudinally down the tubercle sides. The micropyle was surrounded by a collar of large (25 μm long), fused tubercles. The egg of *Tx. splendens* was creamy white overall and rugby ball-shaped like that of *Tx. amiboinensis. Tx. ambionensis* eggs were significantly wider on the base but length and length width ratio similar to the *Tx. splendens* egg. The number of visible tubercles surrounding the large one was lower in *Tx. amiboinensis* (6.3 vs 7.7). The number of ridge around the periphery of each large tubercle (viewed on top) was also significantly lower in *Tx. amiboinensis* (21.3 vs 25.1).

Jordan and Hubbard (1991) investigated the vegetation surrounding black-painted calabash ovitraps for *Tx. moctezuma*, using seasonal deciduous forest in Trinidad, West Indies. More eggs were laid into ovitraps situated either within or directly adjacent to tree or bamboo stools.

Rawlins *et al.*, (1991) studied the effects of single introduction of first instars larvae of *Tx. moctezuma* into all potential *Ae. aegypti* oviposition sites (214) that contained water in a village of Caribbean island. Subsequent to one month 64% fewer male and 80% fewer female of *Ae. aegypti* were collected as compared to 24% fewer males, 27% fewer females were optimistic in controlled village viz where no predator were released.

Amalraj and Das (1992) observed cannibalism behavior among laboratory reared larvae of *Tx. splendens*. They found that twenty four hour starve larvae cannibalized the egg of its own and also ate eggs of other mosquito (*Ae. aegypti* and *An. stephensi*). Second and third instars consumed eggs faster than first and forth instars. The first instars larvae consumed larvae of

its own kind faster than other instars, in absence of other prey. Cannibalism rate significantly fall when prey larvae were provided.

Lane (1992) described the *Toxorhynchites auranticauda,* a new species of *Toxorhynchites* subgenus from Indonesia based on single female. The taxonomic description was based on larval and pupal exuviae of the said individual. He also provided diagrams of chaetotaxy on the pupal abdomen of *Tx. aurifluus*, *Tx. splendens* in address to the newly described species.

Miyagi *et al.,* (1992) repeatedly released the laboratory reared adults of *Tx. splendens,* during 1984, 1986 and 1987 in Minnajima a small island (0.56 $km^2$), Japan, for effective control of the target mosquitoes *Ae. albopictus* and *Cx. quinquefasciatus.* The adult release was conducted to oviposite in artificial traps which were also the breeding habitats of aforesaid vector mosquitoes. Insectaria were maintained at 25-26°C with 80% H and16:8h light: dark photoperiod. The mass culture was conducted by large cage (60×60×60 cm). From three year-experiment they suggested that in that small island approximately 250♀ and 200♂ *Tx. splendens* shoud be released per month from April to November in order to control the target mosquitoes.

Tikasingh (1992) studied the effects of *Tx. moctezuma* larval predation on *Ae. aegypti.* About fifty first instar larvae of *Ae. aegypti* were released in each of 5 water container(drum) filled with 210 liter(domestic water storage). At the beginning of $4^{th}$ week a certain number (0, 1, 2, 5 and 10) of first instar of said predator larvae were released in each drum. One or two larvae of predator stopped the output of *Ae. aegypti* adults for 1 week and five or ten larvae of predator prevented any *Ae. aegypti* emergence for up to 16 weeks. He considered that by augmentative release of *Tx. moctezuma* , *Ae. aegypti* can be controlled biologically.

Tikasingh and Martinez (1992) established a colony of *Tx. moctezuma* in Trinidad. Adult were placed in an aluminum screened cage (size 61×61cm) and maintained in an outdoor insectary. The humidity was 84 to 96% and

temperature was 23 to 30°C. Adult were offered honey soaked in a cotton pad. Three types of ovitraps were used, a cut bamboo (20 cm high × 5 cm internal diameter), a cut bicycle tire (30 cm long × 3 cm deep), and a 2-liter plastic ice cream container painted black. *Ae. aegypti* were used as prey, the mean larval development at 1:10 predator:prey ratio per day was 63.1 ± 11.3 days but development time reduced to 23.8 days when 100 prey larvae were offered. Pupal duration was 5.5 ± 0.7 days. During their three year of production only 12% larvae survived to pupation. Even then, they considered mass rearing and releasing this species for effective management of dengue vector.

Toma and Miyagi (1992) studied the biology of *Tx. splendens* in laboratory to provide a baseline for a biological control programme against *Ae. albopictus* in Minnajima Island, Japan. The laboratory has maintained at 25-26°C, 80% RH and L: D 16:8 photoperiods. The mean incubation time was 43.8 hours and number of prey larvae consumed and killed by predator larvae was 389 and 345 respectively. In mass-rearing, larval cannibalism observed during 1-3 days of post-eclosion and decreased on the 4$^{th}$ day. Pre-oviposion was 4 days, viability of eggs laid by females aged 4-14 days was high (60-90%) but decreased to less then 40% as the females aged.

Amalraj and Das (1993) studied the duel periodicity of oviposition and influence of prey on oviposition site preference by *Tx. splendens* in laboratory, in Pondicherry, India. Oviposition was diurnal with a well-defined duel peak between 11.00 and 16.00 hours and over 35% of eggs were laid at noon. Oviposition required no crepuscular light period but required a photophase. This species preferred the water containing prey larvae (*Ae. aegypti* and *Cx. quinquefasciatus*) for oviposition. They have mentioned that, oviposition periodicity differ from place to place in this species from the earlier records of fifteen publication.

Tietze *et al.,* (1993) conducted the susceptibility test on first instar *Tx. splendens* to malathion, naled and resmethrin in laboratory (JAMSRL), Panama City of Florida. The $LC_{50}$ after 24 hrs was 2.87, 69.1 and 6.23 ppb for resmethrin, malathion and naled respectively. Naled was the least toxic of the 3 compounds tested for integrated use with *Tx. splendens*.

Amalraj and Das (1994a) studied the time of death from starvation and compulsive killing without eating of prey by larvae of *Tx. splendens* in the laboratory of Pondicherry, India. The first and second instar larvae survived without food for 3 days while third and fourth instars survived for 7.8 and 14 days respectively. The corresponding instars of *Ae. aegypti*, *An. stephensi*, *Cx. quinquefasciatus* were offered, the number of prey killed but not eaten ranged from 0 to 15 per 40 prey larvae. Compulsive destroy of *Ae. aegypti* was mainly at its third instar by 9 and 10 days old predator larvae but in *Cx. quinquefasciatus* compulsive killing occured in all instars mainly by young larvae of *Tx. splendens*.

Amalraj and Das (1994b) studied the population interaction of *Tx. splendens* and *Ae. aegypti* in relation to the complexity of the breeding habitats and their initial number in the laboratory, in controlled room temperature (22°C), in Pondicherry. They introduced predator and prey in different ratios in four colony cages ($1m^3$) containing three enamel trays with different oviposition structures. Predatory prey interaction lasted for 5-9 weeks without structural complexity of the oviposition containers, but their interaction lasted for 18 weeks when there was structural complexly.

Jones and Schreiber (1994) observed the color and height effects of oviposition site preference of two predator mosquitoes, *Tx. splendens* and *Tx. rutilus rutilus* in the laboratory using eight color ovitraps affixed 0 and 1m from the cage floor. Both species preferred to oviposit in black container. Secondary preference were red and brown rather than green, blue, orange, yellow or white but *Tx. rutilus rutilus* rarely deposited in white, yellow orange and green colored container. Substantial oviposition occurred in all color containers except white and yellow container. Over 96% and more than 25% of eggs were deposited at ground level and 1m respectively. They considered *Tx. splendens* as better tools for mosquito control in urban areas where discarded containers on the ground were found but *Tx. rutilus rutilus* for tree-holes. They also suggested both species for biological control programs be developed.

Schreiber and Jones (1994) described the inoculative releases of *Tx. splendens* in urban environment in four cities (Sarasota, Tallahassee, Panama City and Panama City Beach) of Florida, USA, in 1989 and 1990. They have described the vegetation and economic condition of people which affect the incidence of *Aedes* spp. responsible for dengue. They have opined, in conclusion, that inoculative release of *Tx. splendens* would not lead to successful establishment in North and Central Florida. However, Periodic inundative release should be made in a more diffuse pattern as suggested by Fock *et al.,* (1983). They assessed predation and colonization of *Tx. splendens* in monitoring ovitraps and a variety of human discarded materials holding water.

Amalraj and Das (1996a) described the foraging behavior of frequency-dependent prey selection by larval instars of *Tx. splendens* in the laboratory, Pondicherry, India as a guideline to understanding the foraging strategies of the predator. Prey size selection by third and fourth instar predators was frequency dependent. In case of second instar predators, prey size selection was not frequency dependent and the predator preferred second instar to fourth instar prey. From their study they concluded that in a biocontrol programmed involving inundate releases, frequency- dependent predation would be more cost-effective because the number of adult releases could be reduced and secondly control of both *Ae. aegypti, An. stephensi* would be possible since both species share the same breeding habitat of the predator especially in urban and semi urban situation.

Amalraj & Das (1996b) described the toxicity of six larvicide's and five adulticides applied to *Tx. splendens* and three vector mosquitoes *(Ae. aegypti, Cx. quinquefasciatus* and *An. stephensi)* and their sublethal effect on biocontrol potential of the predator. Concentration of alphamethrin that killed 50% larvae of *Tx. splendens* was 53 and 12 times more than that which killed *Cx. quinquefasciatus* and *Ae. aegypti.* Deltamethrin was 25-132 time less toxic to adult of *Tx. splendens* in comparison to vector mosquito and synthetic pyrethroids owing to their higher safety margin can be used in an integrated vector management.

Sahlen (1996) described the ultrastructure of eggshells of eight species under four mosquito genera including *Tx. splendens* using transmission electron microscope. Endochorion of *Tx. splendens* varies from the rest. *Anopheles* spp. lack exochorion of its ventral side. The endo- and exochorion of *Toxorhynchites* were fused and contain numerous large empty spaces.

Yasuda (1996) studied the larval ecology including the structure of the predator mosquito larva in relation to prey community structure. He found that the larvae living in each pool were of same instars and 86% were of fourth instars. Females of *Tx. towadensis* laid a number of eggs into one oviposition cup irrespective of the existence of conspecific larvae in the laboratory. The number of prey species decreased with an increase of predator larvae.

Ribeiro (1997) described a new species of toxorhynchitine mosquito from Macau, China as a *Toxorhynchites (Toxorhynchites) macaensis* on the basis of the examination of a type series of 6 ♂ and 7♀ similarities of the new species with the other species of the *Splendens* group, to which the new taxon belongs, were discussed and separate keys to identification of male and female of all the species of the group, were provided. The paper also provided five diagrams with male genitalia viz. (1) Tergum IX, paratype, (2) Paraproct, paratype, (3) Basal mesal lobe, holotype, (4) Gonostylus, paratype, (5) Aedeagus, parameres, basal pieces, holotype.

Amalraj and Das (1998) estimated the predation by instars of *Tx. splendens* on aquatic stage of prey larvae, *Ae. aegypti* in laboratory. The aim of the study was to assess the inter relationship between *Tx. splendens* and its prey *Ae. aegypti*, incorporating variables such as prey size, foraging area and water temperature by using component analysis techniques. They found that predator kept its attack rate constant irrespective of the size of the foraging area and the predator instars may search for prey in some situations though they were basically ambush predators. The predator at relatively high water temperature (32°C) shows high attack rate. They estimated functional

response such as attack rate and handling time when presented with increasing densities of any given size and class of prey.

Collins and Blackwell (2000) made a review accumulate the current knowledge on the general biology and taxonomy of *Toxorhynchites* spp.. It also described the adult *Toxorhynchites* spp. have an unusual life cycle that they are not capable of blood feeding and therefore, they are not pest and vectors to man, but the larvae are predatory on the other mosquito larvae. They have affirmed the potentialities of *Toxorhynchites* spp. as biological agents for the control of other vector mosquitoes. But the desired rate of success was not up to the mark. This was they opined, due to the lack of derailed knowledge on the biology of *Toxorhynchites* sp. with a desire to avoid chemical pesticide for mosquito control, the authors have reviewed 163 publication for this endeavor.

Choochote *et al.,* (2003) colonized *Tx. splendens* in the laboratory (at 27 ± 2°C, 70-80% RH and 12D:12L) using artificial mating technique for adult and autogenous *Ae. togoi* larvae as a food in Thailand. This method of rearing enabled a reduction in the large size cage (60 × 60 × 75 cm) to small size cage (30 × 30 × 30 cm) for small laboratory. The 5 day old male was proved to be the most suitable age for artificial mating. Further, they have offered to provide autogenous *Ae. tagoi* subcolony for other researchers who mass-rear *Toxorhynchites*. Prey larvae used was *Ae. togoi* (adults of being non-blood feeder and/or autogenous) instead of *Ae. aegypti / Ae. albopictus* and *Cx. quinquefasciatus*. Suitable adult age for artificial mating ranged from 3-6 days, with 65-90% mating and 100% insemination success. *Ae. togoi* larvae consumed by associated stage of *Tx. splendens* were found 8.55 ± 2.74, 9.75 ± 3.86, 27.10 ± 78.10, 61.30 ± 10.62 respectively. Larvae of *Tx. splendens* reared in individual plastic container having size 4.5 cm in diameter, 6.5 cm in depth and containing 50 ml deionized water. They have been maintaining the colony since 1987. This species of mosquito is a very good host for studying dengue hemorrhagic virus and lymphatic filarial.

Larval developmental duration of *Tx. splendens* fed on 2nd instar larvae of *Ae. aegypti* was longer (40.49 days) than autogenous *Ae. tagoi* prey (16.95 days).

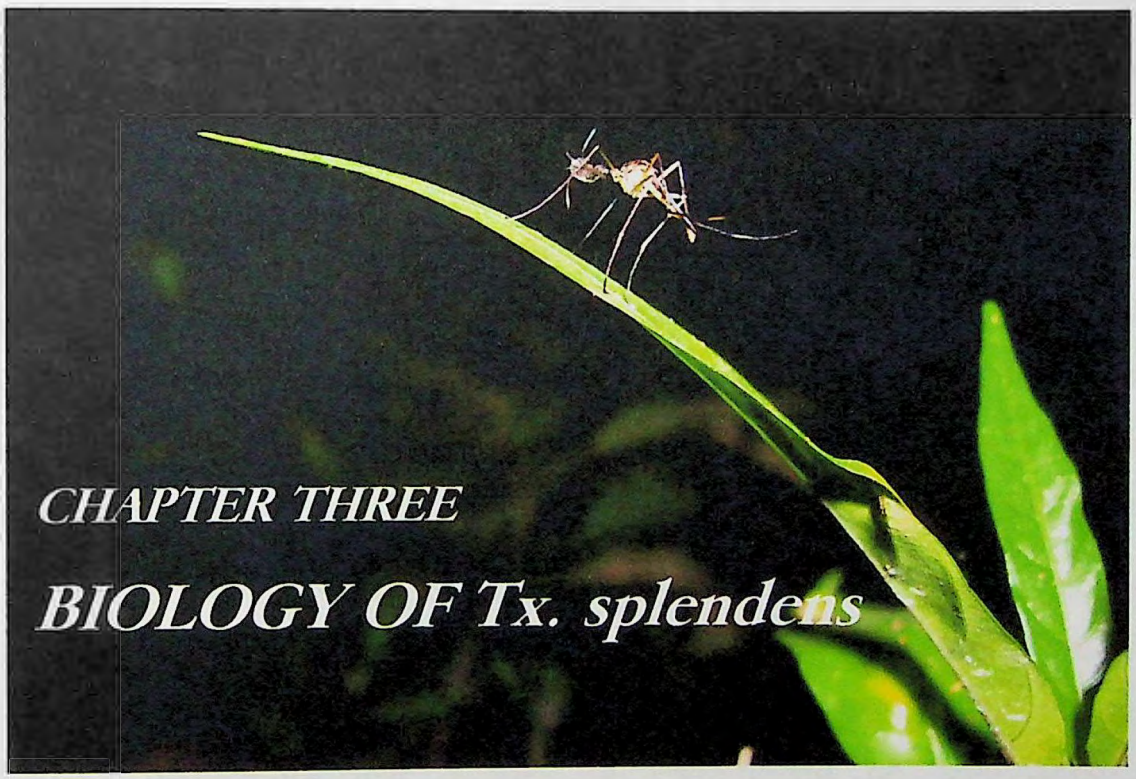
Amalraj *et al.*, (2005) studied the food utilization of *Tx. splendens* in the laboratory condition. They offered different larval instars of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. Effect of food on immature development of *Tx. splendens* was significantly faster with increase in food availability.The relationship between immature duration and food availability were higher when offered 1st and 2nd instars of prey than late instars. Increase in consumption rate resulted on immature duration. They considered an adaptive character of the predator as this result in increase in population turnover in a reduced time when the prey is abundant. This facilitates mass rearing of the predator in large number. The ability of the predator to survive of low feeding rate was an appreciable adaptation in tide over the period of the low prey availability.

Aditya *et al.*, (2006a) studied the predation potential of *Rhantus sikkimensis* (an aquatic dytiscid beetle) and larvae of *Tx. splendens* on 4th instar larvae of *Cx. quinquefasciatus*. Ten to twelve hour starved predator individual were released before every experiments. The rate of predation larvae by *R. sikkimensis* and *Tx. splendens* ranged 21.56 to 86.89 and 0.67 to 34.22 *Cx. quinquefasciatus* larvae per day respectively, depending on the prey and predator densities.

Aditya *et al.*, (2006b) made a preliminary survey of larval habitat and temporal variation on mosquito diversity in the selected localities of Darjeeling town, India for a qualitative and quantitative assessment of mosquito distribution during 2003-2004. They have categorized aquatic bodies into six types depending on the size and structural complexity that might accounted for observed variation in the species composition of the larval habitat. In those habitat they encountered six species under four genera, including, *Tx. splendens*. They found a positive correlation between immature population of the prey and predator.

Aditya *et al.,* (2007) studied the predatory efficiency of the sewage drain inhabiting larvae of *Tx. splendens* on Cx. *quinquefasciatus* and *Armigeres subalbatus* larvae in the laboratory. The rate of predation depended on age of the predator, density of prey and prey types. The number of *Ar. subalbatus* larvae consumed by a predator larva was ranges from 0.50±0.71 to 16.40±2.01 while for *Cx. quinquefasciatus* 0.20±0.42 to 20.40±1.43 per day. In presence of predator pupation rate of prey varied to control was minimum of 0.20±0.42 to maximum of 12.20±2.30 pupa/day.

Focks (2007) considered on *Toxorhynchites* mosquitoes as biocontrol agents of other mosquitoes. She dealt with pros and cons of different aspects of their biology holistically with special a reference to mass culture and release; factors limitation to success and their possible remedial measures were also suggested.



# CHAPTER THREE

# BIOLOGY OF *Tx. splendens*

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# Biology of *Tx. splendens*

## 3.1 Introduction

*Toxorhynchites splendens* is a harmless mosquito, destroy the nuisance, and vector mosquitoes in human community (Colledge, 1911; Steffan and Evenhuis,1981). Although mentionable works on its biology in neighbouring country were done (Aditya *et al.*, 2007; Amalraj and Das, 1998, 2005; Annis and Rusmiarto, 1988; Chan, 1968; Geetha Bai, 1981) and other parts of the world (Muspratt, 1951; O'Flynn *et al.*, 1982; Rawlins *et al.*,1991; Robert *et al.*, 1983; Russo,1983; Focks *et al.*, 1980,1983; Focks, 2007). In order to establish *Tx. splendens* for control of other mosquitoes of Bangladesh, it is very essential to study its biology in Bangladesh agoecological condition. Therefore, an attempt was initiated to study different developmental stages and fuctional behavior of all stages, as well as pre-oviposition, oviposition, insemination rate, fecundity, egg hatchability, duration of developmental stages, sex ratio of progeny, preference of egg laying, longevity of adult and seasonal abundance.

From literatures review on *Toxorhynchites* mosquito found many works in labotatory and a few in field study. Moreover, inundative resealed produce mass population to control other mosquitoes in field-labotatory path indicated that there was a lack of imformation on abundance of *Tx. splendens* and seasonality. Such information is necessary earlier than any successful biological control program using *Toxorhynchites* approved.

## 3.2 Material and Methods

### 3.2.1 Study Area

To study the efficacy of *Toxorhynchites splendens* for control of other mosquitoes two spots in Sherpur district namely: a) Gilagasa village b) Gorgoria village and one in c) Rajshahi University campus were chosen. The geographic position of Sherpur District is N 25°5'0" and E 90° 5' 0" and

Rajshahi University campus is N 24°5'0" and E 89° 5' 0". Two villages are considered as rural environment and the Rajshahi University campus is a mixture of urban and semi urban areas. Descriptions of the spots are given below:

Sherpur district is located at the northern part of Bangladesh from Dhaka City. Gilagasa and Gorgoria are adjacent villages in Sribordi upozila of this district and situated at the north-west corner, which is about 16 km away from the district head quarter. Tow villages having about 442 hectare and 75 hectare of land respectively. Villages are mainly plain land with some ponds, ditch, a canal (khal) at western side and a highway runs at southern side. Most of the lands are used for cultivation and residence. Most of the houses have primary or secondary vegetation and bamboo bush. Tree trunks, the bamboo stump and tree holes served as the mosquito-resting place and breeding ground. The villages are free from urban pollution and quite serene in nature.

Rajshahi is the northwestern division of Bangladesh, it also one of divisonal city. Rajshahi University campus is located on the northwestern side in Rajshahi City Corporation, having about 303.8 hectare. The campus is too busy on the northern side a Dhaka-Rajshahi highway, close to river Padma. A railway line pass through on its northern part. It is a plain land area with some ponds, ditches and cultivated land. The area is differentiated into faculty buildings, hall of residence, school, medical center, stadium, play grounds, residential quarters. The campus is almost free from pollution. The primary and secondary vegetation growing with scattered bushy quiet place.

Investigation was made on this mosquito from July 2007 to January 2010 in Sherpur District and Rajshahi University campus and to detecte its ecological niche from Sherpur District of Bangladesh. The laboratory colony was set up in the Rajshahi University campus. Laboratory based investigation on different aspects biology was conducted in at normal environmental condition.

The climate of the study areas is sub-tropical with summer and monsoon rains. Three main seasons are distinguishable, viz, the cool dry winter from November to February, the hot dry summer from March to May and the hot wet rainy season from June to October (Ahmad, 1968).

### 3.2.2 Colony

#### 3.2.2.1 Preparation of Cage

Three types of cases used in this study, Cage type-A, Cage type-B and Cage type-C. Each cage has two parts one was iron frame with 10cm four stands and another was mosquito' net box with a tubular net opening was 50-90cm diameters and 50-80 cm long from a lateral side. Sizes of frame were 40×40×40cm, 40×50×40cm and 70×70×70cm and mosquito' net box sizes were 35×35×35cm, 35×45×35cm and 65×65×65cm for Cage type-A, Cage type-B and Cage type-C respectively. Neting boxes were embed into the frames and tighten with frame nooks by box's lashes. The cages sizes were followed with some modifications described by Yap and Foo, 1984 and Horio and Tsukamoto, 1985. Each cage was placed on small plastic pot with tap water as an ant guard.

#### 3.2.2.2 Preparation of Ovitrap

Ovitraps used in this study was consisted of two parts: a plastic pot and a bamboo stump. A plastic pot rounded with 4cm high and 11cm diameter in different colors i.e. black, red green, blue and white. Bamboo stump open at one end about 10cm high with 6cm diameter and inside the stump was blackish. Bamboo stumps were placed in the middle of the plastic pots. Bamboo stumps and plastic pots contained bamboo-leaf infusion water. Water level was 3cm in plastic pot and filled full in bamboo stump. The containers were placed in middle or corner of the cages for egg laying.

#### 3.2.2.3 Preparation of Bamboo-leaf infusion water

This was prepared in large earthenware manger with 54 cm diameter at rim . Two kilogram of dry bamboo leaves were placed in the manger containing

40 liters of tap water.This was kept for two weeks for partial decompostion. After two weeks this water was ready for use. More water and leaves were added subsequently to get more infusion water.

### 3.2.2.4 Preparation of Predator Colony

A laboratory colony of this mosquito was initiated during August, 2007. A total of 20 fourth instar larvae were collected from bamboo stump of Gilagasa (12) and Gorgoria (8) with the help of aspirator. All sample brought to the laboratory of Rajshahi University campus from Sherpur district about 450km bus journey. To avoid cannibalism larvae were reared individually in a white plastic vial (35 mm Fuji film capsule) 3cm in diameter and 5cm in height, with a capacity of 27 ml water. These larvae were fed on larvae of *Cx. quinquefasciatus* as prey. Twenty adult emerges from the above-mentioned larvae rearing in bamboo-leaf infusion water. Out of these 20 emerged adults six were males and fourteen were females. In the laboratory environment, all these rearing processes was carried out separately and the corresponding exuviae of the fourth instar larvae and pupae collected from vials were preserved in 70% alcohol for identification. The adults, thus obtained, were placed in rearing cages for future study.These adults were stock colony. Colony was started in Cage type-C.

Search was made in Rajshahi University campus to find out the presence of *Toxorhynchites* sp. adult and larvae from the habitats where they should be. Then different sized earthen pitchers (Kalsi) were used in the campus as a trap(Plate 1) for the collection of *Toxorhynchites* eggs or larvae. Pitchers were filled with 1/3 tap water and with some dry leaves of bamboo,mango or jackfruit.

### 3.2.2.5 Preparation of Prey Colony

Two mosquitoes (*Ae. albopictus* and *Cx. quinquefasciatus*) larvae were used as a prey and experimental tool of *Tx. splendens* in the laboratory. These two vector mosquitoes were very common in Bangladesh (Khan, 1980 and

Ahmed, *et al.* 1986). Prey colony was maintained in cage type-B. From RU campus, wild caught fourth instars larvae of *Ae. albopictus* and *Cx. quinquefasciatus* were reared in bamboo-leaf infusion water in plastic pots to get adults. Egg of prey mosquitos were allowed to lay in white plastic pots including bamboo-leaf infusion. These prey eggs reared in plastic bowl (diameter 20 cm and 8 cm deep containing 1.5 litres water). Eggs of *Ae. albopictus* were stored in dry condition for 9-10 days or month.

### 3.2.2.6 Preparation of food for predator and prey adults

Adults' diets in the rearing cages consisted of two types of artifical food a) 20% honey solution (20 ml honeys +80 ml $H_2O$) and b) 20% glucose solution (20 ml glucose + 80 ml $H_2O$). These solutions were soaked in cotton balls for predator and prey adults. These soaked balls hanged up in each cage by wire or put on plastic petridish. In addition, twelve types of natural seasonal fruits or vegetables slices were offered as food for adults. Every two days fruits and vegetables slice and after every $4^{th}$ days, honey solution and glucose solutions were changed.

A young pigeon was placed in the pigeon iron-box and then this box was in prey colony of *Ae. albopictus* and *Cx. quinquefasciatus* of blood meal for adults.

### 3.2.2.7 Preparation of food for predator and prey larvae

Prey of $1^{st}$, $2^{nd}$, $3^{rd}$ instar larvae produced in laboratory were given to predator larvae as food. In addition laevae collected from their natural habitat were also used as food. Usually $1^{st}$- $2^{nd}$ and $3^{rd}$ instar prey were given to $1^{st}$-$2^{nd}$ and $3^{rd}$-$4^{th}$ instars respectively.

Prey larvae diet was prepared from yeast and liver dust of goat in the ratio 2:3 (Focks and Boston, 1979). These dry ingredients were mixed and stored, in 1kg container, in an arid place until required. Dry ingredient of 100g was mix together with one litre of water in covered container and then contents

shaken; from mixture about 35ml was added in a round plastic bowl containing 5 liter water of bamboo-leaf infusion water for prey larvae.

Studies carried out in the field laboratory with an area 325 squire feet, with 8 windows, a door, located in research field of the Institute of Biological Sciences (IBSc), University of Rajshahi, Bangladesh. Studies have been generally conducted at room temperature, usually 24°C to 33°±2°C (day-night) and relative humidity 75-85%. Photoperiods was extended upto midnight (18L:6D) with an electric light (100w bulb) if needed. To keep the room temperature above 24°C a room heater was used during winter.

### 3.2.3 Life cycle

#### 3.2.3.1 Mating and Oviposition

##### 3.2.3.1.1 Diurnal

Mating was a prerequisite phenomenon for male and female. In the laboratory, freshly emerged pupae from laboratory were isolated according to their sex and a known number of adults of the same age proportionally 1:1, 2:1,3:1,6:1 and 9:1 male: female were released into Cage type-A. To observation of mating periodicity with different male-female ratio usually was done from April to October at the morning from 6am to 8am and at the dusk from 4pm to 6:30pm with three replication upto pre-oviposition period and extended photoperiod. The females were started oviposition as early as after the occurrence of insemination. Pre-oviposition and oviposition period were studies in three replication was recorded. Ovitraps were provided in the mating cages to record fecundity. The numbers of eggs laid in each cage by a female was counted every day. Eggs were collected from ovitrap and transferred to water filled petrideshes with the help of salt spoon. The records were maintained cagewise for further study. The study included a. fecundity, b. insemination rate or egg hatchability, and c. sex ratio. This process continues unlit a female completed her egg laying or death. Collected eggs were measured in mm with the help of Stereo bionocular

microscope. The mating and oviposition behavior of gravid females in started egg laying to an ovitrap in cages also observed.

During this the cotton balls soaked with 20% honey or glucose solution and different type of fruits and vegetables were provided as food for adults. Illuminations, temperature and humidity were same as mention earlier.

The unfertile eggs were found in very old colony Cage type-A where the age of males and females were unknown and or males and females very old and incapable of mating, however females layed eggs.

#### 3.2.3.1.2 Nocturnal

During night mating behavior was observed in the laboratory in Cage type-C of about 90 pairs male and female. Study was carried out for two and half hours duration from 8:30pm to 11:00pm under extended photoperiods as mention earlier. Mating of this group observed seven days. Different color of ovitraps were provided in the cage for eggs collection.

### 3.2.3.2 Preference of egg laying

Preference of egg laying by adult was observed in Cage type-C. In the laboratory, 90 pair adults of known age were released for color preference of ovitraps. Ovitraps used were five pairs color plastic pots viz black plastic pot,red plastic pot, green plastic pot, blue plastic pot, and white plastic pot. A bamboo stump was placed in one of each colored pot. These were filled with bamboo-leaf infusion water. All of these ovitraps were placed in Cage type-C. Plastic pot were round with a diameter of 11 to 18cm in height 3.5 to 7cm.The bamboo stump with inner wall blackish open at one end, with a diameter of 6cm in height 10 cm. The females were allowed to lay egg on the ovitraps (plastic pots/bamboo stump).Observations were made at six hours interval i.e. first quarter of day (6.00-12.00am), second quarter of day (12.00-18.00pm), third quarter of day (18.00-24.00pm) and fourth quarter of day (00.00-6.00am). This observation continued eighteen conjucative

days. Studies conducted under room temperature and relative humidity 75-85%. A 100w bulb was used from dusk to midnight to extend photoperiod.

### 3.2.3.3 Larval stage

The eggs were reared in two ways simply in a plastic vial and in mass in a plastic bowl. The single rearing plastic vials were used (35mm Fujifilim capsule 3×5cm with a capacity of 27ml water). Larva of observed in fertile egg from split the eggshell.First-second and third-fourth instar larvae of *Tx.splendens* provided with prey larvae of *Cx. quinquefasciatus* first/second and third instar respectively for their development. The observation from first instar to adult emergence was made. Feeding behavior, killing behavior (without eating), movement and consumption duration on a prey was observaed.

An experiment on cannibalism was made. In this experiment, 26 fertile eggs were pleaced in a mass bowl and no food was provided. The observation was continued upto adult emergence.

Fourth instar larvae in bamboo stump was observated in desiccate condition. Full-grown fourth instar larvae were placed separately in bamboo stumps and then bamboo-leaf infusion water gradualy dry up. After few days water was added to observe in the laboratory.

### 3.2.3.4 Pupal stage

Pupal stage appeared from 4$^{th}$ instar larva in the similar environment. Fresh bamboo-leaf infusion water was added to maintain water level up to 18ml in each vial. A small piece of net and rubber band was used to cover the opening of each vial to prevent the adult escape and duration of each pupa was recorded. Mass reared pupae were kept in a plastic bowl (20cm diameters, 8cm deep containing 1.5 liter water) with fresh bmboo-leaf infusion water in Cage type-C for emergence and duration was noted.

### 3.2.3.5 Adult stage

After pupal stage adult emerged from pupa in Cage type-C and diets consisted of cotton soaked with 20% honey or diluted glucose and different type of seasonal fruits and vegetables slices. Adults were maintained in Cage type-A or Cage type-C to study their cyclic colony.

The longevity of the reared adults with different nutrient was recorded for mated individuals. Fifty four pairs of newly emerged (0 to 24 hours old) adult *Tx. splendens* were released in three Cage type-A, with 18 pairs each cage. The experiment was completed with the death of all adults in the cages.

During 2009, laboratory produced mosquitoes were released in Rajshahi University Campus in large numbers.

### 3.2.3.6 Mass-rearing

Mass rearing was made from two methods: one was based on Focks & Boston (1979) methods with some modification (colony based prey) and the second was indigenous methods (nature based prey). In order to mass rearing of *Tx. splendens* sufficicent food (prey) was necessary. For the purpose of getting sufficient food *Cx. quinquefasciatus* was mass reared as per (Focks & Boston, 1979) whele *Culex* larvae/other were collected from their natural habitat and offered as *Tx. splendens* as prey for mass rearing the mater.

Mass rearing was carried out in the laboratory following Focks & Boston (1979) with modification due to laboratory equipment limitation. A round plastic bowl (33 cm diameters, 11cm deep) and containing 5 liter of bamboo-leaf infusion water was used (first rearing bowl). Roughly, 2500 first instar of larvae and eggs of *Cx. quinquefasciatus* from laboratory colony were used as a prey. Prey larvae diet was followed 3.2.2.7. One hundred 0 to 24 hour old *Tx. splendens* eggs were placed in a first rearing bowl. Another second rearing bowl was set up with 5 liter bamboo-leaf infusion water with 2500 first instar larvae or eggs of *Cx. quinquefasciatus* and added prey diet. Prey

larvae in second rearing bowl mostly fourth instar on day 4, then pump out rearing water and prey larvae were added to the first rearing bowl. Bamboo-leaf infusion water was replaced after every 4th and prey larvae was added every 3rd days. In this way predator larvae nursing with additional prey were continued. Rearing bowl were examined daily with magnifying glass.The dead larvae–pupae and live larvae–pupae were countedand recorded.Pupae were cleaned with bamboo-leaf infusion water and placed rearing Cage type-A until adult emerged. The cannibalism of larvae and pupation of predators observed for that experiment and this experiment repeated seven times.

Indigenous methods for mass rearing, was used daily collected prey larvae from natural habitat Rajshahi City Corporationa were *Cx. quinquefasciatus* associated with *Anopheles*, *Armigeres* and *Culex* spp.. Sorting procedure prepared after the collection: *Anopheles, Armigeres* larvae pick up by dropper. Rest of larvae and pupae then cleanse, put on 30×30cm mosquito net covered on 20cm diameter and 8cm deep bowl containing 1.5-liter tap water, here 1st, 2nd, 3rd and early 4th instars larvae go down to bowl water through mosquito net and residue on net was pupae and full-grown 4th instars larvae. And than used tea-sieve for straining bowl water with larvae, than used metallic sieve (560 mesh) set on similar size bowl with fresh bamboo-leaf infusion water, and than larvae of tea-sieve put on metallic sieve for larval grading into 1st- 2nd instars and 3rd-4thinstar larvae. Larvae in bowl water were 1st-2nd instars and residue in metallic sieve was 3rd - 4th instar larvae. Those 1st - 2nd instars larvae used as prey larvae in fiest rearing bowl for *Tx. splendens* larvae. Rest of the procedure was following method one.

### 3.2.3.7 Seasonal abundance

The seasonal abundance on the adult population of *Tx. splendens* and its resting behavior was studied in the two villages (Gilagasa and Gorgoria) of Sherpur district of Bangladesh. Usually fruit and timber trees grow arround the villagers' dwelling. There many common plants fruit trees, timbers,

bushes and vegetations peridomestic areas of villages in Bangladesh situation. Within this areas cattlesheds, latrines, etc are omnipresent. On these vegetations the adults took rests.

Field collections at Gilagasa and Gorgoria villages carried out during January 2008 to December 2009. Resting adult collected from trees trunks of zero to 2.52 meter height above the ground. This height fragmented into three parts called H1, H2, H3 and the area of each fragmented height was H1=0-84cm, H2=85-168cm, H3=169-252cm. A man used plastic vial (3cm in diameter and 5cm in height) to collect resting adults from different heights of tree trunks in between 10:00 to 11:30 am. After collection plastic vial was covered with cap and then was transfered to cage. Each collection was recorded on the basis of tree name and sample was collected on triplicate method. Collection was done every 15 days aftet. Monthly collection of these adult mosquito were recorded tree wise. From numbers of type tree resting adults (male & female) were collected in the both of villages, but only the 6 types of tree i.e. mango,jackfruit,hog plum,tamarind, shemul and phitraj in the village of Gilagasa and in the village of Gorgoria from the 4 types of tree i.e. mango,jackfruit, shemul and phitraj were found through out the years, 2008 and 2009.

The collected mosquitoes were identified using appropriate keys (Ameen & Talukdar, 1974; Barraud, 1934; Belkin, 1962; Harbach and Knight, 1980; Knight and Stone, 1977; Lane, 1992; Lee *et al.*, 1988; Steffan and Evenhuis, 1985; Thurman, 1959).The temperature, relative humidity, rainfall and sunshine of collected areas were obtained from Bangladesh Meteorological Department (Climate Division) Agargaon, Dhaka 1207.

## 3.3 Results and Discussions

### 3.3.1 Colony cages

The colony cage was three types, Cage type-A (frame: 40×40×40cm & net box:35×35×35cm) (Plate 2), Cage type-B (frame: 40×50×40cm & net box:

35×45×35cm) (Plate 3) and Cage type-C (frame: 70×70×70cm & net box: 65×65×65cm) (Plate 4). (Geetha Bai *et al.*, 1981 were used 60×60×60 cm and 30 cm cube cloths cage for *Tx. splendens* in India). This is deliberate to *Tx. splendens* and prey colony due to temperate environment and various experiment conducted in room tempareture i.e. Cage type-A (35×35×35cm) for experiment; Cage type-B (35×45×35cm) for prey colony and Cage type-C (65×65×65cm) for *Tx. splendens* colony and other studies but Elias *et al.*, (1988) not mention the cage size in their experiment.

### 3.3.2 Ovitrap

This type of ovitrap (Plate 5) coalesces of artificial and natural container. Ramalinggam, 1979 stated in Malaysia *Tx. splendens* breeds in both artificial and natural container. According Yap and Foo, 1984 *Tx. splendens* show attractiveness to artificial container than to natural bamboo stump as ovitrap. Females found two options for oviposition—one was the bamboo stumps water and the other was plastic pots on which the bamboo stumps were placed both contained bamboo-leaf infusion water. Females deposited eggs on either container during the down stoke flight. Eggs were found to bounce(due to hydrophobic nature) when dropped from the water surface and blow out or about the water surface of the container. The bamboo stumps were also acted as resting substratum for female during oviposition flight. Furumizo *et al.*,(1977) mention eggs were laying in black plastic jars (diameter 8cm, height 9cm) in large cages when several number of females hovering over the oviposition jar at the time created air drafts that caused the eggs to be blown out of the jar and landing on the floor paper of the cage. To solve the air drafts over ovitrap bamboo stump on the middle of plastic pots stopped the air drafts creation which stopped egg from blowing. Newkirk (1947) used petridish as an ovitrap; Chowanadisai *et al.*, (1984) black plastic cups (10×15cm); Elias *et al.*, (1988) provided a small round tin black pot and Geetha Bai *et al.*, (1981) served bamboo stumps open at one end as ovitraps of *Tx. splendens* in India. Focks and Hall (1977) used pair of

black oviposition jars for *Tx.r.rutilus* and Steffan *et al.*, (1980) a black cup for *Tx. amboinensis*. Tikasingh and Martinez (1992) used three types containers for oviposition ; a cut bamboo 20cm high × 5 cm diameter,a cut bicycle tire 30cm long × 3cm deep and a 2-liter plastic ice container . This is one type of ovitrap which is very first time consider as a new tool that prevents egg loss and provides resting substratum of *Tx. splendens* . It also performs as an unique device with bamboo leaves infusion water to collect egg.

### 3.3.3 Bamboo-leaf infusion water

Bamboo-leaf infusion water (Plate 6), basic principle was larvae of *Tx. splendens* caught in bomboo stump in the study villages. (Burton and Rudnick, 1979 mention that *Tx. splendens* breeds in bomboo stump; Hassan, 1990 collected this mosquito from bomboo stump in chittagong city and Ahmed *et al.*, (1990) in Modhupur forest & Thakurgaon and Nasir-Ud-Din, 1952 in Dacca). Tap water and bamboo leaf in the manger after two weeks became deep reddish yellow color and produced bamboo leaf's aroma that allured as a source of oviposition. This was a unique rearing medium for *Tx. splendens* and prey mosquitoes in the laboratory. Focks and Hall (1977) used oviposition jars each with well or colony water for *Tx.r.rutilus* ; Elias *et al.*, (1988) & Geetha Bai *et al.*, (1981) chosen tap water in laboratory study for *Tx. splendens*; Ramalingam and Banu (1985) used rain or tap water. Different types of water such as distilled water, tap water, rainwater, overnight-seaoned tap water, seasoned tap water with *Ae.aegypti* larvae, seasoned tap water with *Tx. splendens* eggs, culture water for *Ae.aegypti* , seasoned tap water of varying salinities ranging from 0.1 to 40%(saturation) NaCl were used in oviposition site preference of *Tx. splendens* by Yap and Foo (1984).

### 3.3.4 *Tx. splendens* colony

Colony of *Tx. splendens* were maintained in the small (35×35×35cm) and large cages (65×65×65cm) in the laboratory of IBSc, RU. (Plate 2, Plate 4).

### 3.3.5 Prey colony

*Ae. albopictus* and *Cx. quinquefasciatus* are two vector mosquitoes in Bangladesh incremented the dengue and filarial diseases respectively (Khan, 1980 and Ahmed *et al.*, 1986). These two mosquitos' larvae were used as a prey and experimental tool of *Tx. splendens* in the laboratory. Their colony was maintained in the Cage type-B (Plate 3).

### 3.3.6 Food for adult of *Tx. splendens* mosquito

Twelve types of seasonal fruits and vegetables slices (Table 1, Plate 7-16) were offered as food for *Tx. splendens*. In this observation adults are flew directly to the fruits and vegetables slices to take their meal and rest there. This may be considered as a diversification of feed for *Tx. splendens*. Muspratt, (1951) made similar observations but used various in-season fruits like graps, peaches, apples, pineapple, citrus and tomatoes for *Tx. brevipalpis* and Paine (1934) provided banana, papaya, raisins for adults *Tx. splendens*. Focks and Hall (1977) provided apple slices for *Tx.rutilus rutilus* adults. Williams *et al.,* (1961) mixed Burnett's commercial food coloring (red, green, blue and yellow) with 1 liter of 10% sucrose solution to which 25 ml of extracted honey for *Tx.rutilus septentrionalis*.

Cotton ball soaked 20% honey solution and 20% glucose solution for adult nutritoin was used in laboratory colony as had been done by many earlier investigators (Chan, 1968; Focks and Hall, 1977; Furumizo *et al.,* 1977; Geetha Bai *et al.,*1981; Knab, 1911; Trpis and Gerberg, 1973; Vongtangswad and Trpis, 1980; Williams *et al.,* 1961). Newkirk, (1947) and Steffan *et al.,* (1980) offered 10% percent sucrose in water; Elias *et al.,*1988 mixed glucose with Vitamin-B complex and Muspratt (1951) used cane sugar solution and honey mixed with water for adults.

### 3.3.7 Mating

**3.3.7.1 Diurnal** Mating of *Tx. splendens* generally occurred in flight. During mating active male and female began flying with humming in nuptial flight. Where males intercepted the females in flight, both fell downward and separated in air before touching cage floor; in most cases mating pair fall on the floor of the cage due to their claspers grasping. After separation from claspers both copula rested for a moment on the cage floor then male flied to the top of the cage roof and rushed for another female for mating. Resting female on the floor cage flew to side net wall of the cage took rest or made another nuptial flight. Gravid females, after successful completion of mating took more rest on side net wall of lower part of the cage and became ready for oviposition. Egg laying females completed oviposition into ovitrap which was placed on floor cages, abruptly she discard oviposition in addition to made another nuptial flight to mate with active male. She returned for oviposition as usual. Sometime active males forcibly made an attempt to copulate with a resting female or ovipositing female but rarely succeed. In several occasions they were perceived to be in copula whilst in air flight with their heads in face to face direction. Sometime two active males grasped each other and similar behavior was also found in two gravid females but sepateted each other instantaneously. Mating pairs fall downward or some adults in copula fall on the cage floor in a similar way.Similar incidence was reported for *Tx. amboinensis* (Steffan *et al.*, 1980) and *Tx. splendens* (Furumizo and Rudnick,1978). Mating of *Tx. splendens* generally occurred in flight Furumizo and Rudnick(1978); in *Tx. r. rutilus* Focks and Boston(1979); in *Tx. brevipalpis* Muspratt(1951); in *Tx. amboinensis* Steffan *et al.*, (1980). Effect of mating at morning (6am to 8am) or at predusk (4pm to 6:30 pm) in different ratio of male with single female (1:1,2:1,3:1,6:1 and 9:1) data was subjected to statistical analysis of RCBD (One Factor Randomized Complete Block Design), ANOVA (Table 2) and DMRT (Table 3). According to data analysis the relation in between mating at dusk (4pm to 6:30 pm) and male-

female ratio are highly significant at 0.05 level but the relation in between mating at morning (6am to 8am) and male-female ratio are non significant (Table 3). Mating occurred at predusk in 6:1 ratio (male: female) is highly significant at 0.05 level then other ratio 9:1, 3:1,2:1, 1:1 (Table 3) (Fig. 1). Mean of mating intensity was observed at dusk than morning (Table 3). Similar mating observation was made by Furumizo and Rudnick (1978) observed, (mating took place, in the laboratory, between 2.30pm to sunset). GeethaBai *et al.*,(1981) mentioned that when male to female ratio were 5:1 and 6:1, all females were inseminated but higher male to female ratio in rate of insemination decreased.

### 3.3.7.2 Nocturnal

Nuptial flight of *Tx. splendens* was not observed after sunset. However, when photoperiod was extended after sunset by lighting the room with 100w bulb the adults performed nuptial flight and females also oviposit as during daytime.Similar observation was reported by (Furumizo and Rudnick,1978) (*Tx. splendens* oviposited at night in lighted insectary). This physical property induced males and females' movement to increased mating intensity in the cage. Interesting observation recorded that all mosquitoes, inside the cage, after a nuptial flight were suddenly set off quiet rest for few minutes on the cage wall and none was found to fly, this happened about for four to five minutes, if undisturbed. Abruptly a female— brake the quietness in the cage to start flying with humming, at the same time resting active males performed nuptial flight along with other females. This nuptial flight was durable for more than five to six munites. Typically mating occurred among 5 to 9 pairs. Following this nuptial flight, all mosquitoes in the cage proceeded for resting about four to five munites and after that, impulsively a female call to (humming) braked, the quietness in the cage and started flying at the same time once for nuptial flight. This cyclic order nuptial flight and mating need about two and half hours. During seven days calculated mating was time 113.42 ± 3.62 and in conjucative days eggs were 177.14 ± 14.46 (Table 4).

### 3.3.8 Pre-oviposition period

Pre-oviposition period was presented Table 2 and Table 3 within ratio of male- female 1:1, 2:1, 3:1, 6:1 and 9:1. Data were subjected to statistical analyses of RCBD (One Factor Randomized Complete Block Design), ANOVA (Table 2) and DMRT (Table 3). There are significant interactions in pre-oviposition period between male-female ratio. Pre-oviposition occurred in 1:1, 2:1, 3:1, 6:1 and 9:1 male-female ratio, mean days were 16.33, 14.33, 7.66, 8.00, 7.66 respectively. Pre-oviposition period at 1:1 ratio was highly significant at 0.01 level i.e. male female took more days mating in Cage type-A before egg laying. But ratio 3:1, 6:1 and 9:1 statisticaly similar at 0.01 level significant i.e. males ratio rised to a female in Cage type-A the pre-oviposition period droped (Table 3) (Fig. 1). Paine (1934) noted a pre-oviposition was 5-28 days in his out door studies for *Tx. splendens* without mentioning male: female ratio. Newkirk (1947) from a single mating reported a pre-oviposition period was 12 days; Furumizo and Rudnick,(1978) observed that pre-oviposition period was 5-7 days and Elias *et al.*, (1988) was 13.3 days for *Tx. splendens* without stated the male: female ratio. Pre-oviposition period of 5-10 days in *Tx. amboinensis* (Steffan *et al.*, 1980); in *Tx. rutilus rutilus* was 6 days (Focks *et al.*,1979a) and 5-6 days (Smittle and Focks,1986). Geetha Bai *et al.*, (1981) found pre-oviposition as 4 days at 5:1 or 6:1 male: female ratio for *Tx. splendens*. In the present experiment it has been observed that enhanced males : female ratio in mating less time was needed and vice-versa. This phenomenon was not reported by earlier workers.

### 3.3.9 Oviposition

Archetypal of the egg laying character of *Tx. splendens* mosquito was found aerial that the female urge to egg laying in full swing flies up and down to create anti-clockwise in a sequence of oriented elliptical ring in the region of the ovitrap, until an egg was ejected on the downward flight of the final (egg) ellipse. All through the sequence particularly complex but dependable changes come out in the ellipses, female increase rapidly in size of ellipse for

about the first quarter of the sequence, and kept on relatively large until the final quarter, when female made smaller the ellipse. Ellipses increasingly flatter through most of the sequence, but with some upturned of this movement presently previous to and during the egg ellipse. The position of the main axis to horizontal diminished gradually, as did the average flight speed (excepting the egg ellipse) and distance from the center of the ovitrap. Individual ellipses were composed of two stages, downflight (lower and towards the ovitrap center) and upflight. Flight speed in each stage passed in the course of accelerative and decelerative phases. In the large ellipse; acceleration, maximum speed, and deceleration were bigger in upflight than downflight. In the egg ellipse, accelerating in downflight considerably exceeded that in all preceding ellipses, the female's abdomen arched forward on the downward flight to the water surface, finally a single egg was forcibly ejected from the air on to the water surface of ovitrap.Similar observation made by Williams *et al.*, (1961) in *Tx. r. septentrionalis* that the loop has been reduced to some 4 to 6 inches in diameter,the female forcibly ejects an egg on the downward dive of each loop. Furumizo and Rudnick (1978) observed, as female neared the ovitrap and would continue this flight pattern until an egg was discharged into the container.This time fore and hind legs spread forward and backward raise respectively with a swings project motion to keep body balance giving rise to an undulate buzzing. Similar behavioral oviposition reported by Linley, 1987 in *Tx. amboinensis*. He made an intensivestudy of nuptial flight behavior use video tape and subsequently analysed the different dimention of flight. But the present observation was based on naked and ordinary video. The aerial behavior of the female in the course of oviposition has been described by Paine (1934), Newkirk (1947) and Furumizo and Rudnick (1978) in *Tx. splendens* and in *Tx. amboinensis* (Steffan *et al.*, 1980). The female in the course of oviposition never touch the water surface directly before or for the period of oviposition. Related observation stated by Furumizo and Rudnick (1978) in *Tx. splendens* and in *Tx. amboinensis* (Steffan *et al.*, 1980).

Oviposition period was focused on Table 2 and Table 3 at the level of male-female ratio 1:1, 2:1, 3:1, 6:1 and 9:1. According to data analysis of RCBD, ANOVA (Table 2) and DMRT (Table 3) it was found that the relation in between oviposition period and male-female ratio non significant. But mean days of oviposition period i.e 26.33, 33.66, 28.33, 46.00 and 48.33 of 1:1, 2:1, 3:1, 6:1 and 9:1 male and female ratio respectively. More male to a female in mating the oviposition period was increased.

Fecundity with male and female ratio were 1:1, 2:1, 3:1, 6:1 and 9:1 shown in Table 2. From the data statistical analysis of RCBD, ANOVA (Table 2) and DMRT (Table 3) was done. According to data analysis the relation in between fecundity (166.66) and sex ratio (9:1) was highly significant at 0.01 level. Mean of egg according to sex-ratio (1:1, 2:1, 3:1, 6:1 and 9:1) was 50.66, 100.33, 90.66,118.00 and 166.66 respectively (Fig.1). A female mosquito mate with more active males fecundity was raised. The fecundity 100.33, 90.66 and 118.00 statistically are very similar to the sex ratio of 2:1, 3:1 and 6:1 respectively. Elias *et al.*,(1988) observed 135.0 (63-240) eggs and GeethaBai *et al.*, (1981) reported the same as 97.8 (83-116) eggs in *Tx. splendens*.

### 3.3.10 Preference of egg laying

Preference of egg laying is shown in Table 5 & Table 5a. Egg laying period of *Tx. splendens* was diurnal; generally, a good number of the eggs were laid between 6:00am to 12:00am hours (First quarter of the day) and 12:00am to 18:00pm(Second quarter of the day). At extended photoperiod by artificial light *Tx. splendens* was found to oviposit at night between 18:00am to 24:00pm hours (Third quarter of the day) and nocturnal oviposition never happened between 0:00am to 6:00pm hours (Fourth quarter of the day) and highly significant at 0.01 level (Table 5a)(Fig. 2). Moreover, in laboratory a unique type of ovitrap with bamboo-leaf infusion water was used for oviposition where more than 36.88%, 40.95% and 22.16% eggs laid at first, second and third quarter of day (Table 5a). However, 90 pairs of males and

females in extended photoperiod were capable for oviposition under an electrical 100w bulb upto 12:00pm. Similar observation was recored by Furunizo & Rudnick (1978) and they also reported on severral occasions *Tx. splendens* ovipositing at night in both the darkened and lighted insectary. Paine( 1934) reported that *Tx. splendens* ovipositing only in the afternoon. Amalraj and Das(1993) did not found *Tx. splendens* to oviposit at night and mentioned the water containing prey as preferred oviposition site and during 11:00 to 16:00 hours over 35% of eggs were laid at noon. Steffan *et al.*, (1980) did not observe any night time oviposition in *Tx. amboinensis*. It was observed the ovitrap black plastic pot with bamboo stump is responsible for highest egg laying capacity of *Tx. splendens*. At the same time the ovitrap white pot showed the lowest value (Table 5b) (Fig. 3). Simultaneously it was found that the *Tx. splendens* laid maximum number of eggs in the black plastic ovitrap with bamboo stump on first,second and third quarter of the day and *Tx. splendens* laid minimum number of eggs in the white plastic ovitrap respectively(Table 5c) (Fig. 4). It was also found that on the basis of DMRT analysis the result of ovitrap black plastic pot & ovitrap red plastic pot with bamboo stump on the first quarter of the day and second quarter of the day are statistically very similar at 0.01 level (Table 5c).

The relation in between types of ovitrap and egg laying period, types of ovitrap and the capacity of egg laying were shown in Table 5, Table 5a, Table 5b and Table 5c respectively. From statistical analysis (RCBD, ANOVA, DMRT) of data it was found that the relation in between types of ovitrap and egg laying period, types of ovitrap and the capacity of egg laying were highly significant at 0.01 level (Table 5a,5b,5c) (Fig. 2,3,4). The ovitrap black plastic pot with bamboo stump was responsible for highest egg laying attitude of *Tx. splendens* and highly significant at 0.01 level in first quarter of the day, second quarter of the day and in extended photoperiod condition by artificial light it was also significant on third quarter of the day (Table 5b) (Fig 3). The mean value were 58.500, 64.167 & 33.333 no. of eggs.

*Tx. splendens* females preferred the color black plastic pot with bamboo stump for oviposition. Secondary preferences were black plastic pot and red plastic pot with bamboo stump.These were statistically very similar and significant at 0.01 level in different quarter of the days (Table 5c). Yap and Foo (1984) and Jones and Schreiber (1994) stated that similar observation for oviposition site preference of *Tx. splendens* females were recored in black color container. But they did not use bamboo stump in their containers.

### 3.3.11 Egg

The eggs of *Tx. splendens* mosquito were large, typically creamy white in color and oval in shape (Plate 17).Outer surface of the egg shell covered with a wrinkle papilliform tubercles decoration to show the egg a granular in appearance. Usually, eggs hydrophobic in nature i.e its outer surface was water repellant.The eggs were adherent to one another on water surface. During the experimental period a number of 702 eggs were examined. The eggs were categorised into four groups on the basis of egg width. The groups were named by W7, W8, W9 and W10. The measurement of the egg of different groups were 0.392 mm (W7), 0.448 mm(W8), 0.504mm (W9) and 0.672 mm (W10). Different eggs length were found in each group. In other way the collected egg were grouped in eight categories on basis of their length, i.e L9 (0.504 mm), L10 (0.560mm), L11 (0.616mm), L12 (0.672mm), L13 (0.728mm), L14 (0.784 mm), L15 (0.840 mm) and L16 (0.897 mm). From 702 eggs, four types of egg length were measured in group W7 and (W9), eight types of egg length were measured in group W8 and single type of egg length were measured in group W10 (Table 6). Linley and Seabury (1990) described the eggs of *Tx. splendens* was 517.0 micro meter length and 388.6 micro meter width under scanning electron microscope.

Fresh eggs were floating horizontally high on the water surface but after 28 to 30 hour eggs that floated horizontally dived beneath the water surface.

Eggs from water surface bounced when dropped and eggs drive or slide with a puff, or airflow or water with slight disturbances. One or more days old eggs become adapted to the water surface and became immobile. The freshly dropped eggs could not be picked up with needle but older eggs (28-36 hours) could be picked up with needle. Fertile egg shells (28-36 hours) became yellowish.First instar larvae splitted the eggshell into two halves and attached at one end. The splitted cup-shaped egg shell floating on water surface turned into S-sheped structure at hatching time. The immerged tiny first instar larvae went into bottom of the water container. The infertile eggs shell turned blackish and did not form splitted S-sheped structure(plate 18) but the shellsplitted into different shaped pieces and floated on water surface (plate 19).

### 3.3.12 Larval stage

Individual rearing of egg was used plastic vial (plate 20). A larval bristle was visible through the eggshell of a fertile and ripe egg (36 to 46 hours old). Very tiny yellowish first instar larva of *Tx. splendens* came out by spliting the eggshell and within a second it dived to bottom of water. In this condition, through transparent body wall very prominent heart beat was observed under microscope. After about half an hour, its transparent body turned opaque(plate 21). At this stage they became active swimmer— float to the surface, aerial respiration were conducted with siphon.The head capsule, saddle and siphon gradually darkened and sclerotized. It has four larval instars. Each instar of *Tx. splendens* larvae devoured on prey mosquito larvae. The behavioral epitome of *Tx. splendens* larvae is opportunistic. They did not move in search of prey—they ambushed prey. Steffan *et al.*, (1980) observed the similar behavior for *Tx. amboinensis*. In small container, a predator larva ambusing or rested on water surface by siphon or rest almost parallel to water surface similar to Anopheline due to its tiny siphon (Furumizo and Rudnick 1978, found similar observation). Prey larvae were wriggling close to the predator larva in rearing container. Wriggling of prey

larvae occurred left or right side, the head of predator turned into the left or right side and moves to a horizontal position when predator ready to eat. Some time preys were found cleaning the predator body. The larva rested at a 45°angle with the surface of the water (Steffan and Evenhuis, 1981) and the 1st instar larvae orbit with help of siphon. When preys were wriggling within range of predator, then using sharp mandibles to grasps the prey and coarse mouth brushes to secure prey, attacking their prey laterally or any location of the body (Breland, 1949; Furumizo and Rudnick, 1978) but sometime nibbling failed to grasps. When 1st instar larvae captured small or large size prey the head was trembling and the siphon was still to balance the body. After a few minutes the larvae chewed the prey. Sometime the larvae goes to bottom immediately after capturing the prey. During this stage(1.8 days), each larva consumed 24.70±0.72 no. of prey of 1st instar (*Cx. quinquefasciatus*). At this stage the corresponding larvae were resting on water surface. The first bite of larva was set by one to the nearer one of them, it happens in the condition of low density of prey. It may happen when two or three predator larvae came within biting range. It is called cannibalism and found in all instars. Sometime clash was seen when two or three larvae were close together. Similarly the 2nd instar larva(2.34 ± 0.17 days) fed 23.86±0.65 no. of 2ndinstar pery. Third and fourth instar larvae showed unusual consumption in their developmental period (3.15 ± 0.13 and 7.7 ± 0.33 dats) were 40.46±2.46 and 115.54 ±2.33 consumption respectively (Table 7). Depending on the location of the prey, *Tx. splendens* larvae may bend toward their prey or extend their head in order to reach their target(Plate 22). Similar observation stated by Linley, 1990 in *Tx. amboinensis* and *Tx. brevipalpis*. *Tx. splendens* larvae in the laboratory has been shown to have strong direct effects on prey abundance in container.Similar observation was made in *T. rutilus* both in the field and in the laboratory.(Bradshaw and Holzapfel, 1983; Lounibos, 1979;Focks *et al.*, 1980; O'Flynn and Craig, 1982;Hubbard *et al.*,1988; Yasuda, 1996 and Lounibos *et al.*, 1997). Prey were captured from (any depth of water)

surface, bottom or middle of container water but Steffan *et al.*, 1980 stated that preys were captured from surface or bottom water.The larval stages are blind. The preys were captured without photoreceptor. There are three school of thought regarding capture— killing or eating and or killing without eating a. With the help of mechanoreceptor (Steffan and Evenhuis 1981), b. With the help of chemoreception (Barber and Hirsch 1984) and c. With the help of a probe (Lounibos *et al.*, 1987).

Generally a predator grasped a prey instar for consume , but a fourth instar larva of *Tx. splendens* grasped at a time even two fourth instar prey of *Ae. albopictus*. Generally consumption duration on a prey depended on kind of prey and size/instar of prey. Fourth instar of an *Ae. albopictus* and a *Cx. quinquefasciatus* larva was engulfed by a predator larva (Table 8) within 1.68±0.31 and 15.17±1.20 minutes (range 0.50-2.49 and 12.50-20.84) respectively. Consecutively three fourth instar of *Ae. albopictus* larvae were comsued by a predator within 20:55 minutes. Steffan *et al.*,(1980) pointed out that a prey was comsued within minutes but did not mention larval category. All larval instars of *Tx. splendens* killed many prey larvae without eating. Typically killing activity was found just before larval ecdysis. It might be larval metabolic and physical structure changed and ingestion power went down and wriggling by prey larvae arised agitation when killing activity was induced i.e killed the prey without eating. Chan (1968) observed similar behavior in *Tx. splendens* in Singapore.Massive killing behavior in laboratory by a fourth instar of predator was found due to introduced more prey larvae (above 35 to 40 in Fujifilm vial).The massive killing by fourth instar larvae ensured their future food security. After massive killing of prey larvae, the predator with the help of abdomen dead larvae were dumped into heaps at the bottom corner of the container. In the cleaner part of the bottom the predator larvae lived. At the same time, there appeared a transparent yellowish creamy layer above the water surface of container probably for keeping the water cool, thus delayed decomposition

of the dead prey. This killing might have been induced due to long duration of predator larvae in short space and short developmental duration of prey larvae and more erratic movement.

The experimental cannibalism test was conducted in the massbowl from 26 starved instar larvae. After 72 days only one-4$^{th}$ instar larvae of *Tx. splendens* survived to pupation i.e. all other larvae eaten by their brothers. Only one male *Tx. splendens* adult was obtained from all avove mentioned larvae. Cannibalism between *Toxorhynchites* larvae is common (Steffan and Evenhuis, 1981). Colledge (1911) in *Tx. speciosa*; Paine(1934) in *Tx. splendens*; Olinger (1957) in *Tx. r. rutilus*; Goma, (1964); Trpis, (1973) in *Tx.brevipalpis*; GeethaBai *et al.*, (1981), Elias *et al.*,(1988) , Amalraj and Das, (1992) and Collins & Blackwell, 2000 observed cannibalism in *Tx. splendens* . Total duration of all larval stage was found to be 15.15 ± 0.49 (range 13.2-17.3) days. Chan, (1968) found the developmental duration of all larval stage as 34.6 (13.5-52.0) days and GeethaBai *et al.*, (1981) found the same as 10-15 days in *Tx. splendens*. *Tx. brevipalpis conradti* need 14 days for development (Corbet and Griffiths, 1963). The variation of the duration of different life stage obtained in the study might be due to nutrition intake. The variation in the speed of development might also be due to the density of prey larvae, the size of the prey larvae offered, the temperature, the container size and photoperiod.

Fourth instar larvae of *Tx. splendens* survived 33.20±2.46 days (range 26-39) (Table 9) in bamboo stump moisture with out food and water. It may be diapause that larva adopted a long time in bamboo stump desiccate.To complete the 4$^{th}$ instar larval ecdysis 2.89±0.30 minutes (range 2.10-4.05) were require (Table 10).

### 3.3.13 Pupal stage

Fourth, instar larva molted to pupa. Before molting full-growth larva usually stopped eating and movement became sluggish. The pupae came out spliting notal skin. A dorso-lateral cleave appeared on notum of the larva

which bend body like arch and then abdomen muscle contract to thorax region slowly, when head came out through the cleave. At first two trumpet were visible then head appeared and finaly a knob like pupal head emerged. Abdomenal muscle contract to push larval exuviae with head capsule sclerite to the back and then hollow exuviae was pulled down over the pupal abdomen and feebly attach with pupal paddle.Pupal abdomen curved toward the pupal head, at last pupa jerk the abdomen swimm through water the cuticular exuviun discarded. A pupa had a knob like cephalothorax (Head) with two-trumpet and arc shape abdomen with two paddles alongside body with bristles or hairs, it swimmed in water up and down or slight angle by paddles (Plate 23 a-b). Duration of pupal stage was 4.31 ± 0.17 (range 4.0-6.0) days (Table 7). To complete the pupal ecdysis 4.71±0.68 minutes (range 3.25 - 7.42) were needed (Table 10), where as Rubio and Ayesta (1984) recorded pupa mean time for emergence was 49 second in *Tx. theobaldi*. Banks (1908) found pupal period as 5-6 days. Paine (1934) found the pupal duration as 5.7 (4-9) days in Fiji. Elias *et al.*, (1988) observed the same as 5.9 (5.0-6.7) days, Chan, (1968) found 4.6 (4.12) days, GeethaBai *et al.*,(1981) found 4-6days in *Tx. splendens* whereas Furumizo and Rudnick (1978) reported the pupal duration as 12.4 days.

### 3.3.14 Adult stage

Adults came out from puparium on water surface. A fully-grown pupa after 4 to 6 days appeared on water surface turned out head and abdomen straight alongwith water surface and paddles projected up to attach any floating substance in water or rim of water pot (Plate 23c-d).Under this circumstance part of the knob and paddles were exposed out of water. Pupa in straight line jerked its body several times to push abdomen toward knob, finally cephalothorax splited at dorso-frontal ecdysial line making an Y-form split and an imago emerged slowly; at first dorsal apotome splitted out. This time paddles acted upright the imago in puparium on water surface.The contraction of body muscles, the front-middle legs and wings came out from

puparium and spread with proboscis at a time on water surface and within few moments the hind leg popped out. A new born *Tx. splendens* floating on water surface for few minutes and after that it flew to cage wall (Plate 25).

Cyclic colony of *Tx. splendens* was maintained in the laboratory for thirteen generations. Sex-ratio of adult was observed in $F_3$, $F_6$, $F_8$ and $F_9$ generation, single female laid egg was 103, 134, 168 and 199 respectively (Table 11) (Fig. 5). All eggs were reared separately in plastic vial. Sex-ratio was found 47.68% males and 52.32% females in four generations and hatchability, insemination rate was 100%.

Duration life cycle from egg was 21.47 ± 0.52 (19.3-24.9) days (Table 11). According to Elias *et al.*, (1988) the period was the same as 23.4 (20.3-26.8) days. Chan (1968) the period was 39.2 (17.5-57.0) days. Geetha Bai *et al.*, (1981) reported the duration of egg to adult as 16-18 days while Furumizo and Rudnick (1978) observed 21.5 days and Paine (1934) found minimum developmental period egg to adult was 30 days for *Tx. splendens*.

Longevity of adults *Tx. splendens* were found 94.16±4.32 (range 3 - 150) days and 87.85 ±6.00 (range 5 - 151) days for male and female respectively (Appendix Table 2) due to proper care of adults with natural and artificial food. Chuah and Yap (1984), found mean survival values for adults *Tx. splendens* male given food and water, water only or no food and water were 21.8±2.7, 6.0±0.1, and 6.0 ±0.4 days respectively. GeethaBai *et al.*, (1981) mentioned the average survival time of *Tx. splendens* as 14 days (range 4-22) and 13 days (range 2-27 ) for female and male respectively. In Fijian condition the adults longevity was at least three weeks (Paine,1934). Furumizo and Rudnick (1978) estimated in *Tx. splendens* was 28-35 days; Trpis and Gerberg (1973) found in *Tx. brevipalpis* was 42 days; Steffan *et al.*,(1980) in *Tx. amboinensis* 120 days but individual longevity was not recorded. In dry condition(cool dry winter from November to February and hot dry summer from March to May), females could holding the eggs, the longevity,it may long upto monsoon (rainy season from June to October)

(Collines and Blackwell, 2000 stated that *Toxorhynchites* spp. mosquitoes oviposit during the rainy season but not during the dry season).

### 3.3.15 Mass-rearing

The larvae & pupae of *Tx. splendens* survived in mass-rearing (Plate 24) was shown in (Table 12) (Fig. 6). From mass-rearing in nature based and colony based prey were used and total duration of larvae of *Tx. splendens* were found 14.85±0.14 and 14.48±0.15 days, the survival rate of adults was 65.57±0.94 and 68.28±1.58 and egg to adult was 20.55±0.17 and 21.17±0.18 respectively. But individual rearing in plastic vial was 15.15±0.49 days and egg to adult duration was 21.47±0.52 days, it might be undersized space and limited food (Table 10) i.e. in order to mass rearing of *Tx. splendens* sufficicent food (prey) and laboratory condition were prerequisite phenomena. Jones (1993) suggests that *Toxorhynchites* spp. larvae are well adapted for feeding on mosquito larvae. Cannibalism in both methods at all stages of predator larvae. With in both methods 2$^{nd}$ instar shown maximum number cannibalism then fellowed 1$^{st}$ instar, 2$^{nd}$ instar, 3$^{rd}$ instar and 4$^{th}$ instar. Focks and Boston (1979) devised a mass rearing technique to produce several thousand of adult *Tx. rutilus rutilus* in every two week in laboratory. Annis & Rusmiarto (1988) repeated the technique for *Tx. splendens* in laboratory. Due to high rates of larval and pupal mortality this method of rearing was discontinued and they could not find the reason of high rate of mortality. Heating water and maintain temperature of developing bowl's were not follows as like as Focks & Bostom (1979). Laboratory was maintained room temperature (25-33°) and 76-85% RH. Laboratory produced mosquitoes, *Tx. splendens* were released (plate 26) in the Rajshahi University Campus in large numbers but no further investigation carried out.

### 3.3.16 Seasonal abundance

Resting adult of *Tx. splendens* were collected from two villages named Gilagasa and Gorgoria(plate 27) (plate 28) under the district of Sherpur. Adults were resting on the following trees, i.e. Bamboo (*Bambusa vulgaris*), Black- plum (*Syzgium cumini*), Coconut (*Cocos nucifera*), Hog-plum (*Spondias pinnata*), Guava (*Psidium guajava*), Jackfruit (*Artocarpus heterophyllus*), Jigar(*Lannea coromandelica)*, Lichi(*Litchi chinensis*), Mango (*Mangifera indica*), Pithraj (*Aphanamixis polystachya*), Pomelo (*Citrus maxima*), Rain tree (*Albizia procera*), Tamarind (*Tamarindus indicus*) and Shimul (*Salmalia malabaricum*) during collection. The trees were basically fruit and timber type and located arround the house-hold of the villages. The adults of *Tx. splendens* were found at the different height of the trees. Plastic vial (35mm Fujifilim) were used to collect the adults and all of them were found from 0 to 2.52 meter height of the trees. The collecton period was January, 2008 to December, 2009.The adults were collected on resting condition through out the year of 2008 and 2009 from the following trees i.e. mango, jackfruit, hogplum, tamarind, shemul and phitraj. From the ecological view above trees may be considered as ecological niche for this mosquito (male and female, both),because the mosquitoes were available on above trees. Similar findings were reported by several workers. Aders(1917) stated that different species have been obtained from holes in mango trees in association with *Tx. brevipalpis* in Zanzibar. Furumizo *et al.*, (1977) collected resting males and females of *Tx. splendens* with sweep net from the coconut palm (2-4 meter above the ground). Williams *et al.*, (1961) carried out a field study on abundance of *Tx. r. septentrionalis*. They worked in a small valley called Neotoma, in Ohio.They found the males of a certain locality are resting on a certain tree(backberry) or bush and are always to be found there. Not a single female was seen.

The seasonal abundance of adult population of *Tx. splendens* in different heightwise and monthwise were presented in the following Table 13, 13a ,

13b,13c,14,14a,14b,14c, 15,15a,15b,15c, 16, 16a,16b and 16c.For statistical analysis, all collected data were processed and ANOVA,DMRT, and RCBD (Two Factor Randomized Complete Block Design) were done.

According to the Table 13a it was found that highest no. of resting male collected on the following trees such as mango, jackfruit, hogplum, tamarind, shemul and phitraj at the height of 85 to 168cm (H2) of tree trunk in the village Gilagasa during the period of January to December,2008. Similar observation was reported from the village Gilagasa during the period January to December,2009 and that was presented in Table 14a.

In another Village- Gorgoria the highest no. of resting males were found on the mango, jackfruit and shemul trees at the height of 85 to 168cm (H2) during the experimental period 2008. A slight change was marked in case the abundance of male of *Tx. splendens* in resting condition and that was the highest on phitraj trees at the height of 00 to 84 cm (H1) during the period of January to December, 2008 in the village of Gorgoria(Table 15a). Similarly in the village Gorgoria the abundance of male of *Tx. splendens* were highest on mango, jackfruit, shemul and phitraj trees at the height of 85to 168cm (H2) for the year 2009 (Table 16a).

The abundance of female *Tx. splendens* in resting condition was shown also in Table 13a.It was the highest on mango, jackfruit, and phitraj trees at the height of 85 to 168cm (H2) during the year, 2008 in the village of Gilagasa. A change was marked and that was the highest abundance on hogplum and tamarind trees at the height of 169 to 252 cm (H3). In case of shemul tree the abundance of female was found at two height i.e 00 to 84 cm(H1) and 169 to 252 cm (H3) during the year, 2008 in the village of Gilagasa. In the village Gilagasa the abundance of female were highest on mango, jackfruit, shemul and phitraj trees at the height of 85 to 168cm (H2) for the year,2009 and that is presented in Table 14a.There was a change for the abundance of female that was the highest on tamarind tree at the height of H3 and hogplum trees at the two height i.e. H2 & H3 respectively.

In another village Gorgoria the abundance of female *Tx. splendens* in resting condition was also shown in Table 15a.The highest abundance of female were found on mango, jackfruit and shemul trees at the height of 85 to 168cm (H2) during the experimental period 2008. No abundance of female was available on phitraj tree in the village Gorgoria for the year, 2008.Similar observation was recored for the abundance of female in the village Gorgoria during the year,2009(Table 16a).

The Table 13b showed monthwise abundance of *Tx. splendens* (male and female) in the village Gilagasa for the year,2008. The males and females were abundantly found on mango, jackfruit, hogplum, tamarind, shemul and phitraj trees in the month of March, 2008 (Fig. 7). Beside this, the males were also abundant on mango, jackfruit, hogplum, tamarind and phitraj trees in the month of January, August, April, June and April,2008 respectively. Incase of females, they were also abundant on mango, shemul and phitraj trees in the month of April, August and April,2008 respectively.

The Table 14b showed monthwise abundance of *Tx. splendens* (male and female) in the village Gilagasa for the year,2009. The males and females were abundantly found on mango, jackfruit, hogplum, tamarind, shemul and phitraj trees in the month of March,2009(Fig. 8). Beside this, the males were also abundant in the month of January & April on mango tree; January on jackfruit tree; February & April on hogplum tree; January & April on tamarind tree; April on shemul and phitraj trees in the year,2009. Incase of females, they were also abundant on mango, shemul and phitraj trees in the month of April, August and April,2009 respectively.

The Table 15b showed monthwise abundance of *Tx. splendens* (male and female) in the village Gorgoria for the year,2008. The males and females were abundantly found on mango, jackfruit, shemul and phitraj trees in the month of March,2008 (Fig. 9). Beside this, the males were also abundant in the month of April on mango,jackfruit and phitraj tree and February & April on shemul tree in the year,2008. Incase of females, they were also abundant

on mango and jackfruit tree for the month of April and on shemul tree for the month of February & April.There was no abundance of females on phitraj tree in the year, 2008.

The Table 16b showed monthwise abundance of *Tx. splendens* (male and female) in the village Gorgoria for the year,2009. The males and females were also abundantly found on mango, jackfruit and shemul trees in the month of March,2009(Fig. 10). Beside this, the males were also abundant in the month of April on mango trees. But only males were found on phitraj tree in the month of March and no females were observed on phitraj tree through out the year,2009.

'Two Factor Randomized Complete Block Design analysis' were performed mosquito resting on trees trunk at different height (H1, H2 & H3), with respect to abundance of *Tx. splendens* in respect to months. Abundance peak uauall found in the month of March in both years for males and females. In Bangladesh,month of March is a dry season or end of the winter so abundance peak was establish in the month of March. In this data, females were less caught and resting frequency or diversity not like males, it may be females were busy in egg laying or search for oviposition site. *Toxorhynchites* spp. mosquitoes oviposit during the rainy season but not during the dry season (Collines and Blackwell, 2000). From this view end of winter or before beginning of rainy season females were less busy in oviposit and resting around the males, consistently they were completed their mating and waiting for monsoon.

Literatures review on *Toxorhynchites* mosquito reveals many works in labotatory and a few in field study. Inundative released (mass reared) of *Tx. splendens* revealed unsucceful stories. In some case a little success as reported 1934 by Paine could not be successfully adopted elsewhere.Very little information on the distribution,density seasonal abundance, ecological niche on the resting places etc on *Tx. splendens* are available. Biological control of mosquito with predators and other biocontrol agents would be a

more-effective and eco-friendly approach, avoiding the use of synthetic chemicals and concomitant damage to the environment. Manipulating or introducing an auto-reproducing predator into the ecosystem may provide sustained biological control of pest populations. In order to achieve an acceptable range of control, a sound knowledge of various attributes of interactions between a pest population and the predator. Such information i.e abundance of *Tx. splendens* in its seasonality is indispensable for successful biological control program.

**Table 1** Different types of fruit and vegetable given to adult *Tx. splendens* as nutritoin

| No. | Common (Native) name of Plants | Scientific name |
|---|---|---|
| 1 | Mango(Amm) | *Mangifera indica* |
| 2 | Jack fruit(Khathal) | *Artocarpus heterophyllus* |
| 8 | Love apple (Jamrul) | *Syzygium samarangense* |
| 4 | Radish (Mula) | *Raphanus sativus* |
| 5 | Cucumber (Sasa) | *Cucumis sativus* |
| 6 | Carrot (Gajor) | *Daucas carota* |
| 7 | Jicama( Shakalu) | *Pachyrhizus erosus* |
| 8 | Banana ( Kala) | *Musa paradisiaca* |
| 9 | Water melon (Turmus) | *Citullus lanatus* |
| 10 | Sweet potato(Mistialu) | *Ipomoea batatas* |
| 11 | Pumpkin(Mistikumra) | *Cucurbita maxima* |
| 12 | Sugarcane(Ankh/Kushor) | *Saccharum officinarum* |

**Table 2** ANOVA TABLE of mating, pre- oviposition, oviposition and fecundity in different ratio of male with single female

| Source | Degrees of Freedom | Mean Squares | | | | |
|---|---|---|---|---|---|---|
| | | Mating | | Pre-oviposition | oviposition | Fecundity |
| | | 6am to 8am | 4pm to 6pm | | | |
| Replication | 2 | 8.600 | 8.067 | 34.200 | 317.267 | 333.267 |
| Factor | 4 | 7.933 | 32.333* | 52.933** | 306.267 | 5363.067** |
| Error | 8 | 2.933 | 7.233 | 7.033 | 142.767 | 755.017 |

* Level of significant = 0.05, ** Level of significant = 0.01

**Table 3** Effect of mating, pre-oviposition, oviposition and fecundity in different ratio of *Tx. splendens* male with single female

| No. of Replication | Ratio of male:female | Mating periodicity | | Pre-oviposition days | oviposition days | Fecundity |
|---|---|---|---|---|---|---|
| | | Morning 6am to 8am | Dusk 4pm to 6pm | | | |
| 3 | 1:1 | 3.333 | 7.000 $^{c}$ | 16.333 $^{a}$ | 26.333 | 50.667 $^{b}$ |
| | 2:1 | 5.333 | 11.000 $^{abc}$ | 14.333 $^{ab}$ | 33.667 | 100.333 $^{ab}$ |
| | 3:1 | 6.000 | 9.667 $^{bc}$ | 7.667 $^{b}$ | 28.333 | 90.667 $^{ab}$ |
| | 6:1 | 6.667 | 15.333 $^{a}$ | 8.000 $^{b}$ | 46.000 | 118.000 $^{ab}$ |
| | 9:1 | 7.667 | 13.667 $^{ab}$ | 7.667 $^{b}$ | 48.333 | 166.667 $^{a}$ |
| LSD | | - | 5.064 | 7.266 | - | 75.28 |
| Level of significance | | ns | 0.05 | 0.01 | ns | 0.01 |

**Fig. 1** Effect of mating, pre-oviposition, oviposition and fecundity in different ratio of *Tx. splendens* male with single female

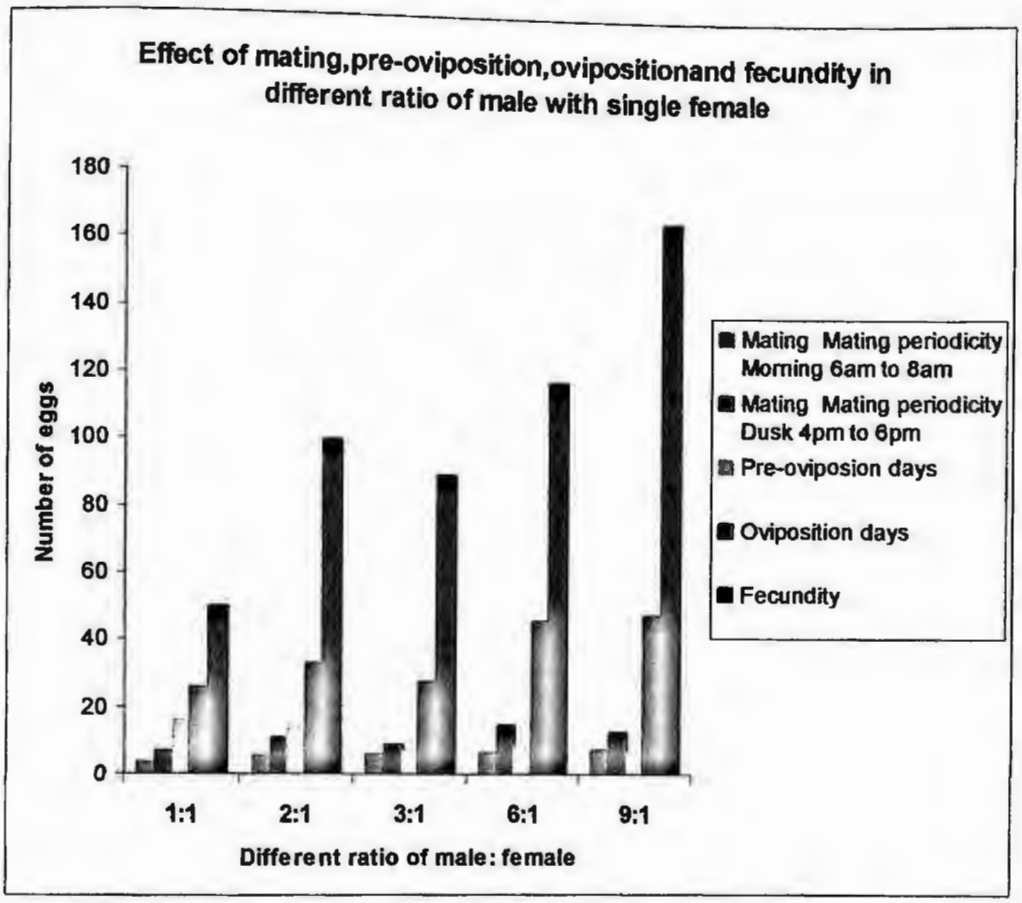



**Table 4** Nocturnal mating observed in Cage

| Replication (Day) | Mating Mean ± SE | Egg laying Mean ± SE |
|---|---|---|
| 7 | 113.42 ± 3.62 | 177.14 ±14.46 |

**Table 5** ANOVA T A B L E

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 1 | *Replication* | *17* | *4444.267* | *261.427* | *9.6293* | *0.0000*** |
| 2 | Factor A | 2 | 3345.211 | 1672.606 | 61.6081 | 0.0000** |
| 4 | Factor B | 9 | 122628.148 | 13625.350 | 501.8710 | 0.0000** |
| 6 | AB | 18 | 8374.196 | 465.233 | 17.1362 | 0.0000** |
| -7 | Error | 493 | 13384.511 | 27.149 | | |

** Level of significant = 0.01

**Table 5a** Egg laying period of *Tx. splendens*

| No. of replications | Egg laying period | Mean of eggs (%) |
|---|---|---|
| 18 | First quarter of day (6 am - 12 am) | 11.372 [a] (36.88%) |
| | Second quarter of day (12 pm-18 pm) | 12.628 [a] (40.95%) |
| | Third quarter of day (18 pm - 24 pm) | 6.833 [b] (22.16%) |
| | Fourth quarter of day (00am - 6am) | 0.000 |
| LSD | | 1.420 |
| Level of significance | | 0.01 |

**Fig. 2** Egg laying period of *Tx. splendens*

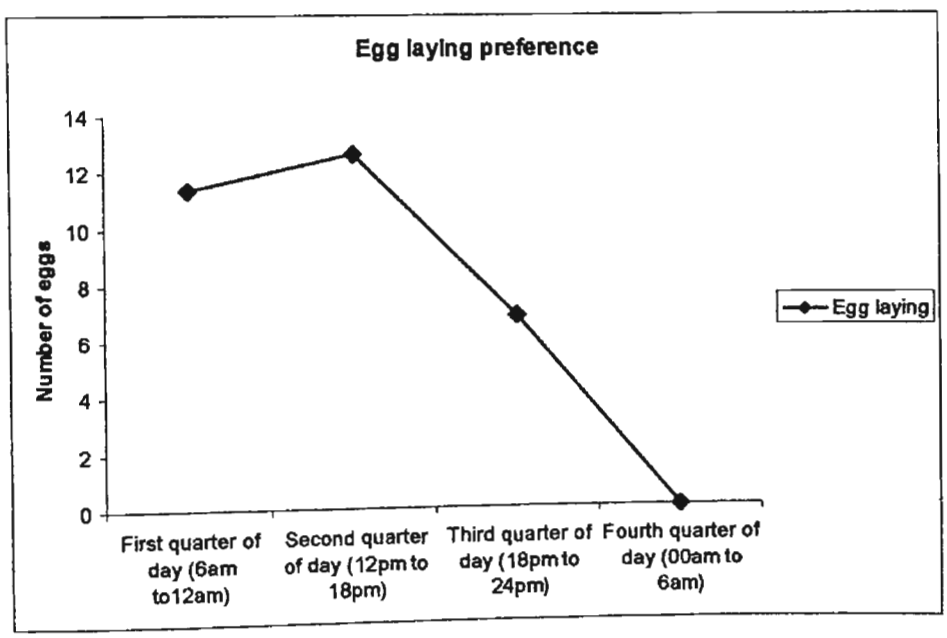


**Table 5b** Egg laying of *Tx. splendens* to different colors plastic pots/with bamboo stump in Cage

| No. of replications | Different colors of plastic pot/ with bamboo stump | Mean of eggs |
|---|---|---|
| 18 | Black pot with bamboo stump | 52.000[a] |
| | Black Pot | 15.852[b] |
| | Red pot with bamboo stump | 17.463[b] |
| | Red Pot | 4.981[c] |
| | Green Pot with bamboo stump | 5.407[c] |
| | Green Pot | 1.370[d] |
| | Blue Pot with bamboo stump | 2.870[cb] |
| | Blue Pot | 1.204[d] |
| | White Pot with bamboo stump | 0.944[d] |
| | White Pot | 0.685[d] |
| LSD | | 2.593 |
| Level of significance | | 0.01 |

**Fig. 3** Egg laying of *Tx. splendens* to different colors plastic pots/with or without bamboo stump in Cage

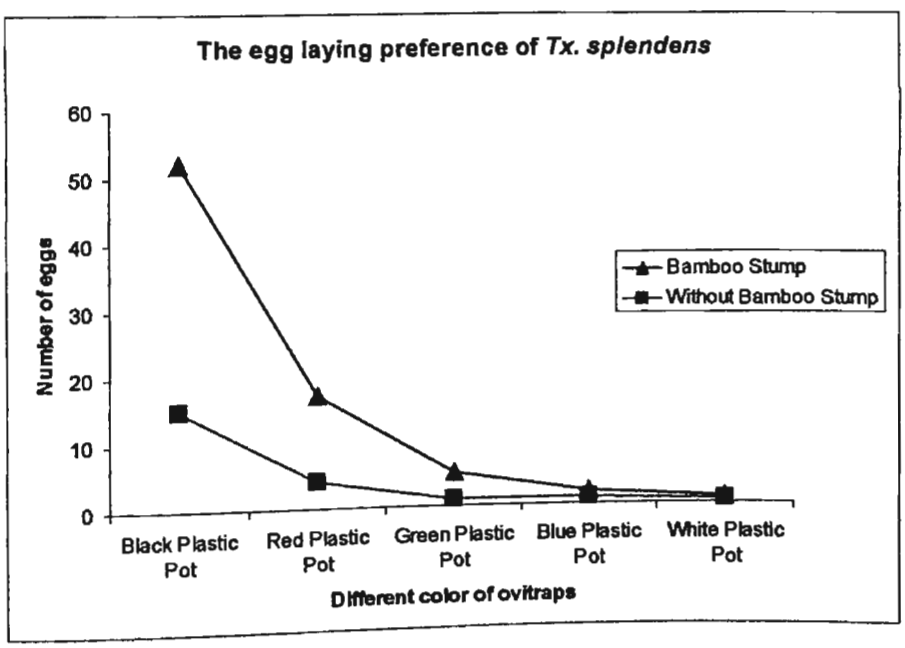


**Table 5c** Egg laying preference at different quarter of days to different colors plastic pots/with bamboo stump in the Cage

| No. of Replications | Different plastic pots/ with bamboo stump | First quarter of the day (6am-12am) | Second quarter of the day (12pm-18pm) | Third quarter of the day (18pm-24pm) | Fourth quarter of the day (00am- 6am) |
|---|---|---|---|---|---|
| 18 | Black plastic pot with bamboo stump | **58.500** $^{b}$ | **64.167** $^{a}$ | **33.333** $^{c}$ | **0.000** |
| | Black plastic pot | **17.722** $^{d}$ | **19.556** $^{d}$ | **10.278** $^{ef}$ | **0.000** |
| | Red plastic pot with bamboo stump | **19.444** $^{d}$ | **21.556** $^{d}$ | **11.389** $^{e}$ | **0.000** |
| | Red plastic pot | **5.833** $^{fghi}$ | **6.389** $^{fg}$ | **4.000** $^{ghi}$ | **0.000** |
| | Green plastic pot with bamboo stump | **5.222** $^{ghi}$ | **5.889** $^{fgh}$ | **3.833** $^{ghi}$ | **0.000** |
| | Green plastic pot | **1.389** $^{ghi}$ | **1.556** $^{ghi}$ | **1.167** $^{ghi}$ | **0.000** |
| | Blue plastic pot with bamboo stump | **2.889** $^{ghi}$ | **3.333** $^{ghi}$ | **2.389** $^{ghi}$ | **0.000** |
| | Blue plastic pot | **1.111** $^{ghi}$ | **1.667** $^{ghi}$ | **0.833** $^{hi}$ | **0.000** |
| | White plastic pot with bamboo stump | **0.889** $^{hi}$ | **1.278** $^{ghi}$ | **0.667** $^{hi}$ | **0.000** |
| | White plastic pot | **0.722** $^{hi}$ | **0.881** $^{hi}$ | **0.444** $^{i}$ | **0.000** |
| LSD | | 4.491 | 4.491 | 4.491 | - |
| Level of significance | | 0.01 | 0.01 | 0.01 | - |

**Fig. 4** Egg laying observation at diurnal-nocturnal period to different colors plastic pots/with bamboo stump in Cage

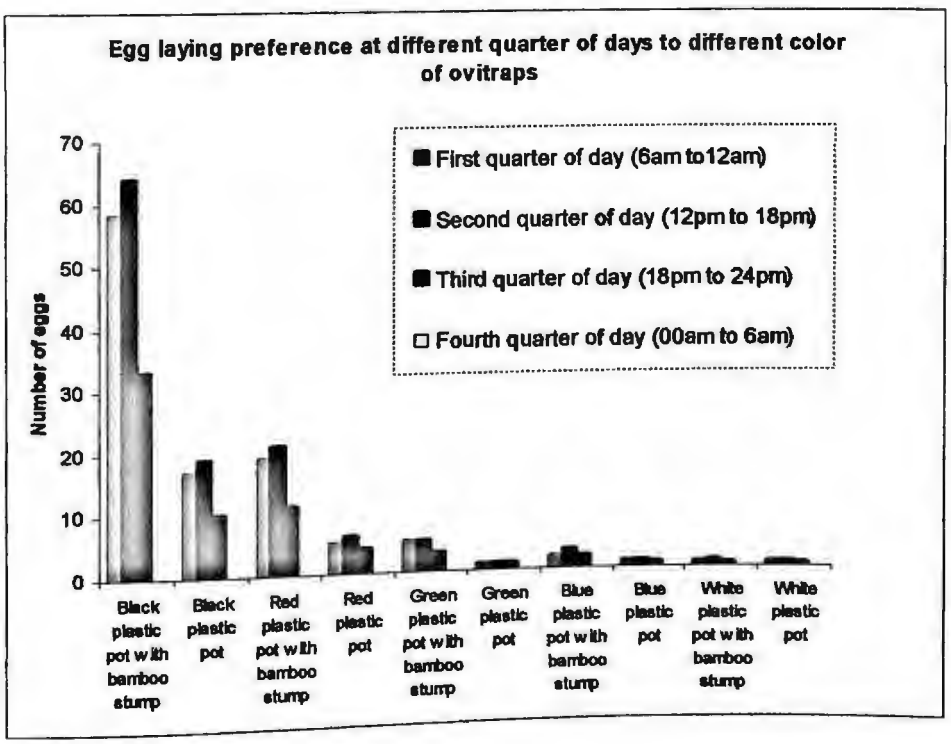


**Table 6** Measurement of the eggs of *Tx. splendens*

| Length (mm) | Width(mm) | | | |
|---|---|---|---|---|
| | W7 (0.392mm) No. of Egg | W8 (0.448 mm) No. of Egg | W9 (0.504 mm) No. of Egg | W10 (0.672 mm) No. of Egg |
| L9 (0.504) | 10 | 22 | - | - |
| L10(0.560) | 12 | 34 | - | - |
| L11(0.616) | 29 | 75 | - | - |
| L12(0.672) | 27 | 143 | 23 | - |
| L13(0.728) | - | 129 | 41 | - |
| L14(0.784) | - | 41 | 51 | - |
| L15(0.840) | - | 17 | 13 | - |
| L16(0.897) | - | 13 | - | 22 |
| Total | 78 | 474 | 128 | 22 |

**Table 7** Laboratory observations on the life cycle of *Tx. splendens*

| No. of Replication | Stage | | Duration of development Mean ± SD (Range) Days | Consumption (25:1 p:P ratio) |
|---|---|---|---|---|
| 30 | Egg incubation | | 2.0 ± 0.11(1.6-2.8) | - |
| | Development of larvae | 1st instar | 1.8 ± 0.14(1.6-3.0) | 24.7±0.72 |
| | | 2nd instar | 2.34 ± 0.17(1.8-3.5) | 23.86±0.65 |
| | | 3rd instar | 3.15 ± 0.13(2.8-4.1) | 40.46±2.46 |
| | | 4th instar | 7.7 ± 0.33(6.5-9.3) | 115.5±2.33 |
| | | Total | 15.15 ± 0.49(13.2-17.3) | 204.36±3.25 |
| | Development of pupa | | 4.31 ± 0.17(4.0-6.0) | - |
| | Egg to adult | | 21.47 ±0.52(19.3-24.9) | - |

**Table 8** Duration of consumption for prey

| Replication | Prey larvae ($4^{th}$ instar) | Duration(Minute) Mean±SE (Range) |
|---|---|---|
| 6 | *Ae. albopictus* | 1.68±0.31 (0.50-2.49) |
| | *Cx. quinquefasciatus* | 15.17±1.20 (12.50-20.84) |

**Table 9** Larvae of *Tx.splendens* in bamboo stump survive in desiccate condition

| Replication | Survival days in bamboo stump Maen (range) |
|---|---|
| 5 | 33.20±2.46(26-39) |

**Table 10** Duration of ecdysis on fourth instar larva to pupa and pupa to adult

| No. of Replication | Duration of ecdysis on larva to pupa Mean±SE (Minute) | Duration of ecdysis on pupa to adult Mean±SE (Minute) |
|---|---|---|
| 6 | 2.89±0.30 | 4.71±0.68 |

**Table 11** Observation of hatchability, insemination rate and sex-ratio in the laboratory

| No. of Repications | Fecundity Egg (number) | Hatchability (%) | Insemination rate(%) | Sex-ratio | |
|---|---|---|---|---|---|
| | | | | ♂ | ♀ |
| $F_3$ | 103 | 100 | 100 | 50 | 53 |
| $F_6$ | 134 | 100 | 100 | 59 | 75 |
| $F_8$ | 168 | 100 | 100 | 82 | 86 |
| $F_9$ | 199 | 100 | 100 | 97 | 102 |
| Total | 604 | 100 | 100 | 288 (47.68%) | 316 (52.32%) |

**Fig. 5** Observation of hatchability, insemination rate and sex-ratio in the laboratory

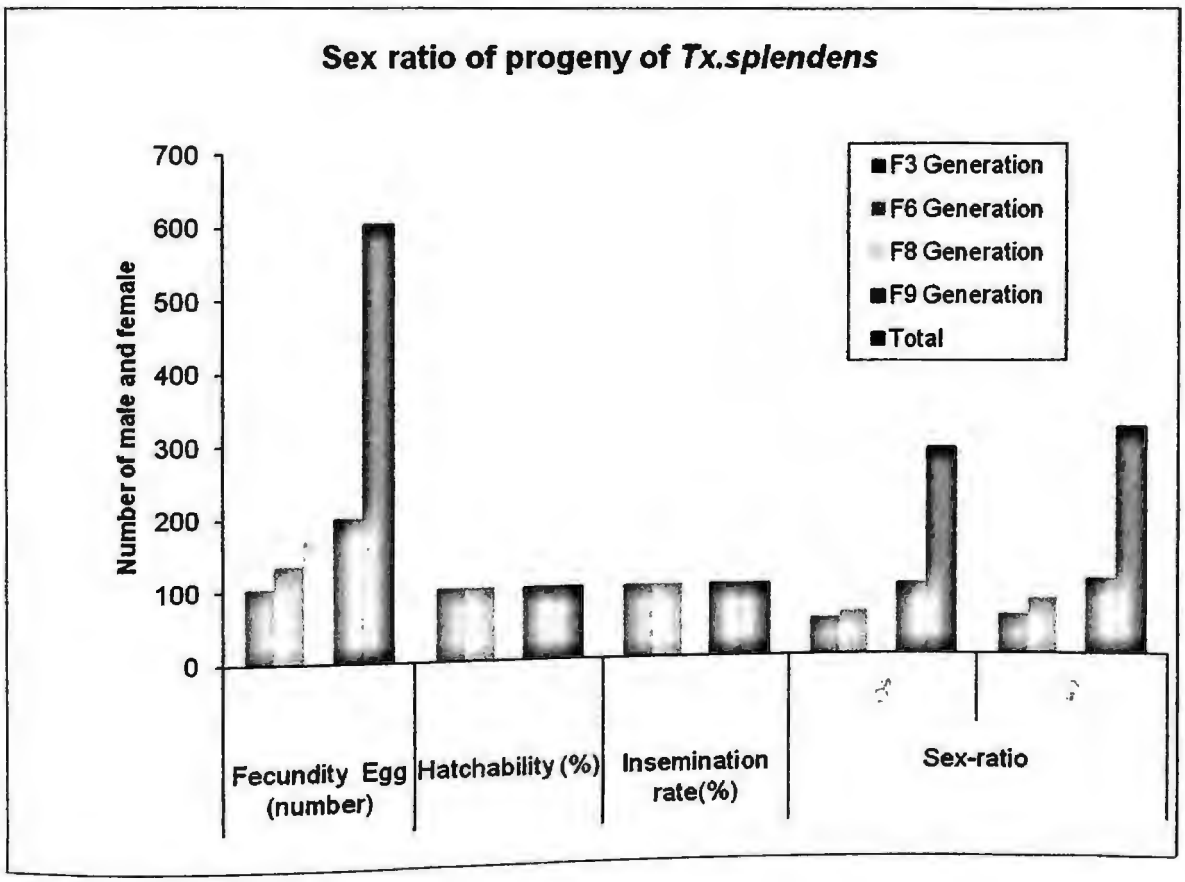


**Table 12** Mass rearing of *Tx. splendens* developed in prey

| Prey larvae used | Replication | Number of Egg | Hatchability | Larval duration days | Cannibalism | | | | Survival rate of pupa | Survival rate of adult | PUpal duration | Total development egg to adult days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | I Instar | II Instar | III Instar | VI Instar | | | | |
| Nature based prey | 7 | 100 | 100 | 14.48± 0.15 | 8.85± 1.10 | 15.0± 0.76 | 6.57± 0.35 | 2.42± 0.42 | 67.14± 1.18 | 65.57± 0.94 | 4.11± 0.37 | 20.55± 0.17 |
| Colony based prey | 7 | 100 | 100 | 14.85± 0.14 | 9.42± 3.56 | 14.85 ±0.96 | 5.28± 0.83 | 1.42± 0.48 | 69.0± 1.56 | 68.28± 1.58 | 4.34± 0.14 | 21.17± 0.18 |

**Fig. 6 Mass rearing of *Tx. splendens* developed in prey**

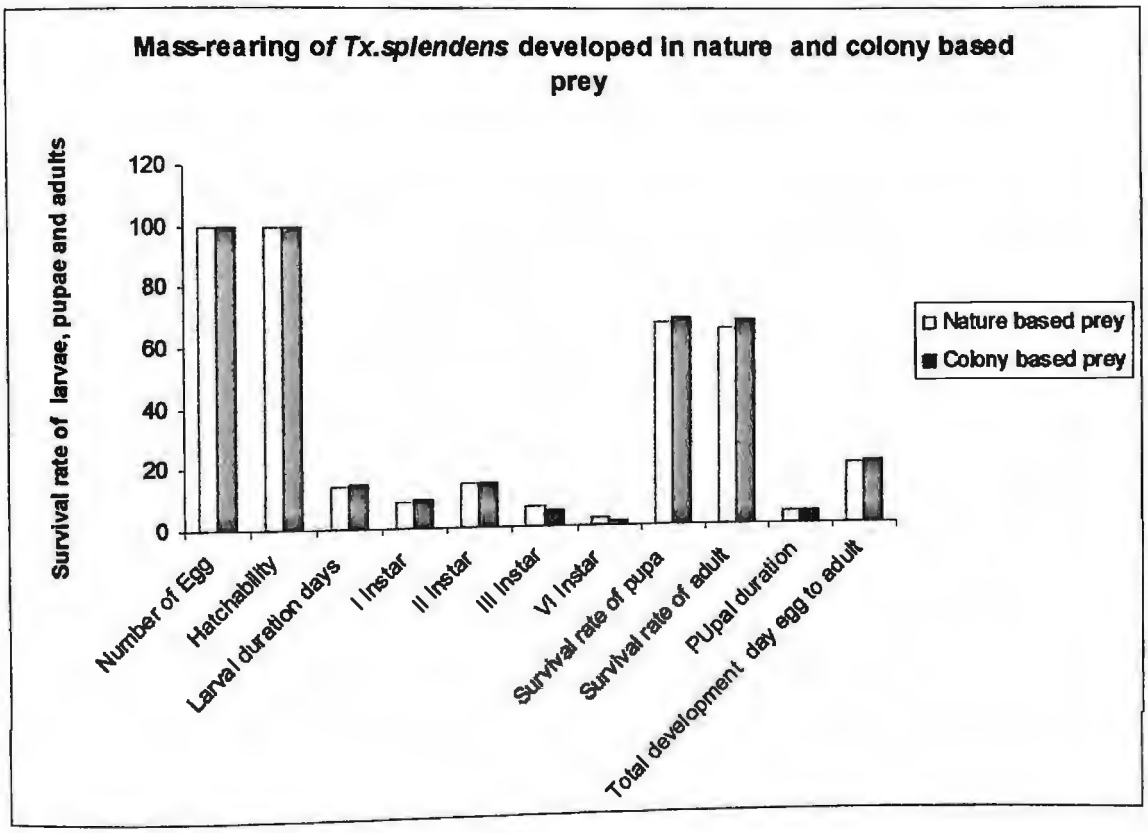


**Table 13** ANOVA Gilagasa 2008

| Source | Degrees of freedom (d.f) | Mean Square | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mango | | Jackfruit | | Hog plum | | Tamarind | | Shemul | | Phitraj | |
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| Replication | 2 | 1.120 | 0.065 | 6.676 | 0.926 | 8.620 | 0.398 | 7.898 | 0.120 | 3.731 | 0.148 | 2.111 | 0.037 |
| Factor A | 2 | 7.343** | 0.481** | 5.954** | 0.343* | 3.370** | 0.259* | 3.509** | 0.037 | 5.009 | 0.065 | 0.361** | 0.009** |
| Factor B | 11 | 0.633* | 0.211** | 0.535** | 0.411** | 0.875** | 0.108* | 2.029** | 0.151 | 0.562** | 0.047 | 0.667** | 0.017** |
| AB | 22 | 0.565** | 0.098 | 0.489** | 0.141* | 0.320 | 0.047 | 0.186* | 0.017 | 0.272 | 0.024 | 0.149** | 0.019 |
| Error | 70 | 0.263 | 0.084 | 0.162 | 0.078 | 0.192 | 0.055 | 0.193 | 0.035 | 0.131 | 0.034 | 0.159 | 0.018 |

* Level of significant = 0.05, ** Level of significant = 0.01

**Table 13a** Resting of *Tx. splendens* (Males & Females) on different height of different tree trunks in Gilagasa village 2008

| No. of Replication | Height | Mango | | Jackfruit | | Hog plum | | Tamarind | | Shemul | | Phitraj | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 3 | H1 (00 - 84cm) | $0.389^{b}$ | $0.028^{b}$ | $0.361^{c}$ | $0.056^{b}$ | $0.278^{c}$ | $0.000^{b}$ | $0.194^{b}$ | 0.000 | 0.194 | 0.028 | $0.278^{c}$ | 0.028 |
| | H2 (85 - 168cm) | $1.250^{a}$ | $0.250^{a}$ | $1.167^{a}$ | $0.250^{b}$ | $0.889^{a}$ | $0.056^{b}$ | $0.889^{a}$ | 0.083 | 0.333 | 0.000 | $0.889^{a}$ | 0.083 |
| | H3 (169 - 252cm) | $0.583^{b}$ | $0.083^{ab}$ | $0.667^{b}$ | $0.139^{a}$ | $0.611^{b}$ | $0.167^{a}$ | $0.306^{b}$ | 0.028 | 0.139 | 0.028 | $0.472^{b}$ | 0.028 |
| LSD | | 0.3201 | 0.1809 | 0.2512 | 0.1313 | 0.2735 | 0.1102 | 0.2259 | - | - | - | 0.245 | 0.120 |
| Level of significance | | 0.01 | 0.01 | 0.01 | 0.05 | 0.01 | 0.05 | 0.01 | NS | NS | NS | 0.01 | 0.01 |

**Table 13b** Month wise resting of *Tx. splendens* (Males & Females) on different tree trunks in Gilagasa village 2008

| Month | Mango | | Jackfruit | | Hog plum | | Tamarind | | Shemul | | Phitraj | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| January 2008 | 1.222$^{a}$ | 0.000f$^{c}$ | 0.889$^{ab}$ | 0.111$^{b}$ | 0.778$^{ab}$ | 0.111$^{ab}$ | 0.444$^{abc}$ s | 0.000 | 0.000$^{b}$ | 0.000 | 0.667$^{bc}$ | 0.000$^{b}$ |
| February 2008 | 0.778$^{ab}$ | 0.111$^{c}$ | 0.889$^{ab}$ | 0.000$^{b}$ | 0.667$^{abc}$ | 0.111$^{ab}$ | 0.667$^{abc}$ | 0.000 | 0.333$^{b}$ | 0.000 | 0.667$^{bc}$ | 0.000$^{b}$ |
| March 2008 | 1.222$^{a}$ | 0.556$^{a}$ | 1.222$^{a}$ | 0.778$^{b}$ | 1.222$^{a}$ | 0.333$^{a}$ | 0.889$^{a}$ | 0.222 | 0.889$^{a}$ | 0.111 | 1.889$^{a}$ | 0.444$^{a}$ |
| April 2008 | 0.778$^{ab}$ | 0.222$^{b}$ | 1.000$^{ab}$ | 0.222$^{b}$ | 0.889$^{ab}$ | 0.222$^{ab}$ | 0.556$^{abc}$ d | 0.111 | 0.556$^{ab}$ | 0.000 | 0.889$^{b}$ | 0.111$^{b}$ |
| May 2008 | 0.556$^{b}$ | 0.111$^{c}$ | 0.444$^{b}$ | 0.000$^{b}$ | 0.556$^{bc}$ | 0.111$^{ab}$ | 0.556$^{abc}$ d | 0.000 | 0.000$^{b}$ | 0.000 | 0.444$^{bc}$ | 0.000$^{b}$ |
| June 2008 | 0.778$^{ab}$ | 0.111 | 0.556$^{b}$ | 0.111$^{a}$ | 0.556$^{bc}$ | 0.000$^{b}$ | 0.778$^{ab}$ | 0.111 | 0.333$^{b}$ | 0.000 | 0.333$^{bc}$ | 0.000$^{b}$ |
| July 2008 | 0.444$^{b}$ | 0.000$^{c}$ | 0.444$^{b}$ | 0.000$^{b}$ | 0.111$^{c}$ | 0.000$^{b}$ | 0.222$^{cd}$ | 0.000 | 0.000$^{b}$ | 0.000 | 0.222$^{c}$ | 0.000$^{b}$ |
| August 2008 | 0.556$^{b}$ | 0.000$^{c}$ | 0.889$^{ab}$ | 0.000$^{b}$ | 0.333$^{ab}$ | 0.000$^{b}$ | 0.111d | 0.000 | 0.000$^{b}$ | 0.111 | 0.222$^{c}$ | 0.000$^{b}$ |
| September 2008 | 0.889$^{ab}$ | 0.111$^{c}$ | 0.667$^{ab}$ | 0.111$^{b}$ | 0.111$^{c}$ | 0.000$^{b}$ | 0.333$^{bcd}$ | 0.000 | 0.111$^{b}$ | 0.000 | 0.333$^{bc}$ | 0.000$^{b}$ |
| October 2008 | 0.444$^{b}$ | 0.000$^{c}$ | 0.556$^{b}$ | 0.111$^{b}$ | 0.667$^{abc}$ | 0.000$^{b}$ | 0.111$^{d}$ | 0.000 | 0.222$^{b}$ | 0.000 | 0.222$^{c}$ | 0.000$^{b}$ |
| November 2008 | 0.556$^{b}$ | 0.111$^{c}$ | 0.667$^{ab}$ | 0.111$^{b}$ | 0.556$^{ab}$ | 0.000$^{b}$ | 0.333$^{bcd}$ | 0.000 | 0.111$^{b}$ | 0.000 | 0.444$^{bc}$ | 0.000$^{b}$ |
| December 2008 | 0.667$^{b}$ | 0.111$^{c}$ | 0.556$^{b}$ | 0.222$^{b}$ | 0.667$^{abc}$ | 0.000$^{b}$ | 0.556$^{abc}$ d | 0.000 | 0.111$^{b}$ | 0.000 | 0.222$^{c}$ | 0.000$^{b}$ |
| LSD | 0.4822 | 0.3368 | 0.5024 | 0.3486 | 0.5469 | 0.2205 | 0.4518 | - | 0.4977 | - | 0.5484 | 0.2335 |
| Level of significance | 0.05 | 0.01 | 0.01 | 0.01 | 0.01 | 0.05 | 0.01 | ns | 0.01 | ns | 0.01 | 0.01 |

***Fig. 7*** Month wise resting of *Tx. splendens* (Males & Females) on different tree trunks in Gilagasa village 2008

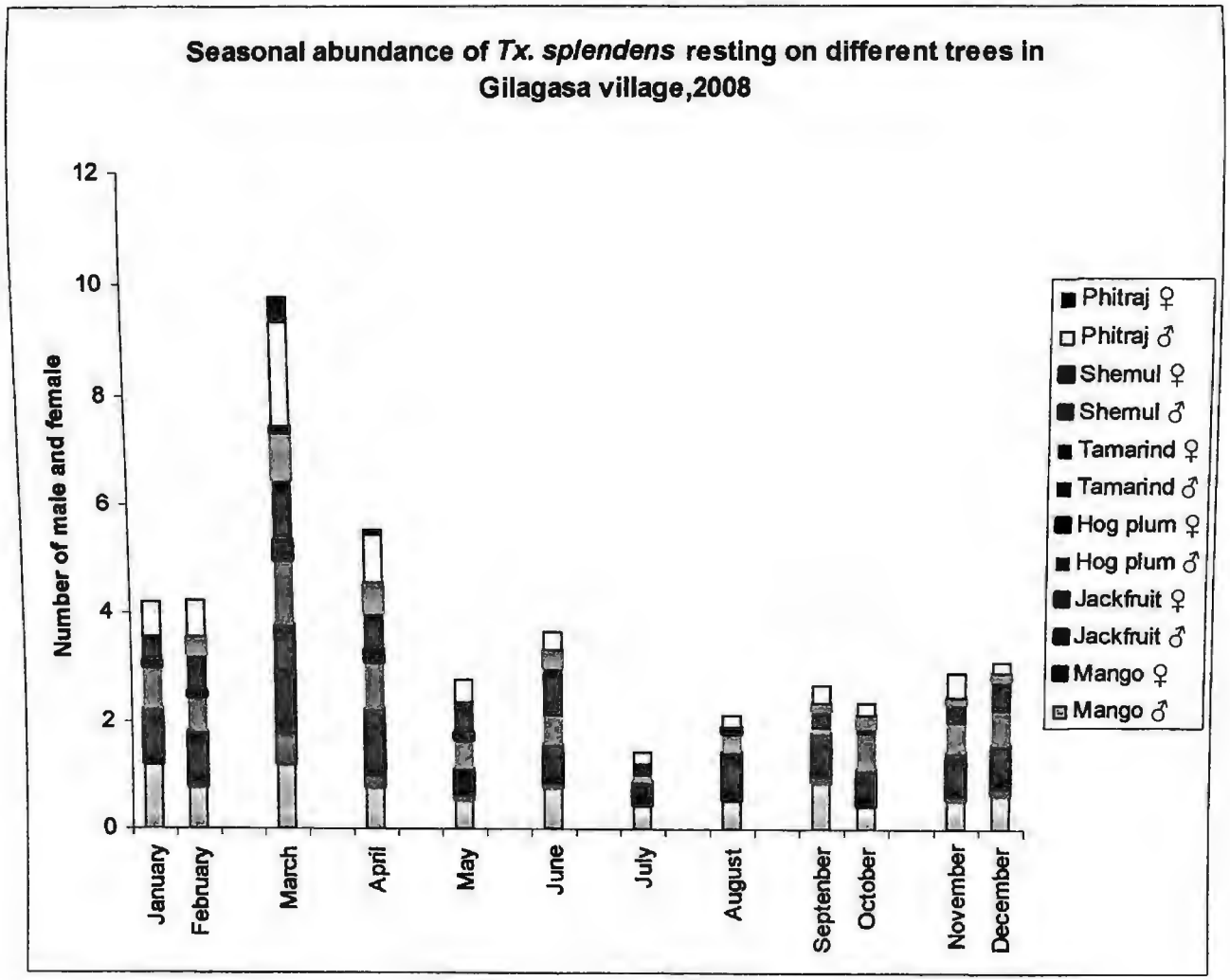


**Table 14** ANOVA Gilagasa 2009

| Source | Degrees of freedom (d.f) | Mean Square | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mango | | Jackfruit | | Hog plum | | Tamarind | | Shemul | | Phitraj | |
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| Replication | 2 | 2.898 | 0.704 | 5.898 | 1.694 | 6.370 | 0.287 | 3.528 | 0.083 | 0.528 | 0.083 | 3.528 | 0.037 |
| Factor A | 2 | 5.454** | 0.843* | 5.454** | 0.444* | 1.620** | 0.037 | 1.861** | 0.028 | 0.194 | 0.028 | 3.583** | 0.037 |
| Factor B | 11 | 0.979** | 0.636** | 0.858** | 0.616** | 0.835** | 0.151** | 0.730** | 0.083 | 0.859** | 0.043 | 0.730** | 0.017 |
| AB | 22 | 0.343 | 0.287 | 0.302 | 0.091 | 0.297 | 0.037 | 0.174 | 0.028 | 0.053 | 0.018 | 0.199 | 0.017 |
| Error | 70 | 0.289 | 0.199 | 0.308 | 0.142 | 0.247 | 0.049 | 0.194 | 0.045 | 0.071 | 0.026 | 0.137 | 0.018 |

* Level of significant = 0.05, ** Level of significant = 0.01

**Table 14a** Resting of *Tx. splendens* (Males & Females) on different height of different tree trunks in Gilagasa village 2009

| No. of Repication | Height | Mango | | Jackfruit | | Hog plum | | Tamarind | | Shemul | | Phitraj | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 3 | H1 (00 - 84cm) | $0.417^{b}$ | $0.056^{b}$ | $0.444^{c}$ | $0.056^{b}$ | $0.333^{b}$ | 0.028 | $0.167^{b}$ | 0.000 | 0.111 | 0.000 | $0.194^{b}$ | 0.000 |
| | H2 (85 - 168cm) | $1.167^{a}$ | $0.333^{a}$ | $1.222^{a}$ | $0.278^{a}$ | $0.750^{a}$ | 0.083 | $0.611^{a}$ | 0.028 | 0.250 | 0.056 | $0.778^{a}$ | 0.056 |
| | H3 (169 - 252cm) | $0.611^{b}$ | $0.083^{b}$ | $0.861^{b}$ | $0.167^{ab}$ | $0.472^{ab}$ | 0.083 | $0.472^{a}$ | 0.056 | 0.139 | 0.028 | $0.278^{b}$ | 0.000 |
| LSD | | 0.335 | 0.209 | 0.346 | 0.177 | 0.310 | - | 0.274 | - | - | - | 0.231 | - |
| Level of significance | | 0.01 | 0.05 | 0.01 | 0.05 | 0.01 | ns | 0.01 | ns | ns | ns | 0.01 | ns |

**Table 14b** Month wise resting of *Tx. splendens* (Males & Females) on different tree trunks in Gilagasa village 2009

| Month | Mango | | Jackfruit | | Hog plum | | Tamarind | | Shemul | | Phitraj | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| January 2008 | 0.889$^{ab}$ | 0.111$^{b}$ | 1.222$^{ab}$ | 0.000$^{b}$ | 0.667$^{abc}$ | 0.000$^{b}$ | 0.667$^{ab}$ | 0.000 | 0.000$^{c}$ | 0.000 | 0.222$^{bcd}$ | 0.000 |
| February 2008 | 0.556$^{b}$ | 0.000$^{b}$ | 0.889$^{ab}$ | 0.111$^{b}$ | 0.778$^{abc}$ | 0.000$^{b}$ | 0.111$^{b}$ | 0.000 | 0.111$^{c}$ | 0.000 | 0.333$^{bcd}$ | 0.000 |
| March 2008 | 1.556$^{a}$ | 0.889$^{a}$ | 1.556$^{a}$ | 0.889$^{a}$ | 1.000$^{a}$ | 0.444$^{a}$ | 1.111$^{a}$ | 0.333 | 1.000$^{a}$ | 0.222 | 1.111$^{a}$ | 0.111 |
| April 2008 | 1.111$^{ab}$ | 0.444$^{ab}$ | 0.889$^{ab}$ | 0.444$^{ab}$ | 0.778$^{abc}$ | 0.111$^{b}$ | 0.333$^{b}$ | 0.000 | 0.222$^{c}$ | 0.000 | 0.667$^{ab}$ | 0.111 |
| May 2008 | 0.556$^{b}$ | 0.222$^{b}$ | 0.667$^{b}$ | 0.222$^{b}$ | 0.333$^{abc}$ | 0.111$^{b}$ | 0.333$^{b}$ | 0.000 | 0.556$^{b}$ | 0.111 | 0.444$^{bcd}$ | 0.000 |
| June 2008 | 0.778$^{b}$ | 0.111$^{b}$ | 0.889$^{ab}$ | 0.111$^{b}$ | 0.556$^{abc}$ | 0.111$^{b}$ | 0.444$^{b}$ | 0.000 | 0.000$^{c}$ | 0.000 | 0.111$^{cd}$ | 0.000 |
| July 2008 | 0.444$^{b}$ | 0.000$^{b}$ | 0.556$^{b}$ | 0.111$^{b}$ | 0.333$^{abc}$ | 0.000$^{b}$ | 0.222$^{b}$ | 0.000 | 0.000$^{c}$ | 0.000 | 0.444$^{bcd}$ | 0.000 |
| August 2008 | 0.444$^{b}$ | 0.000$^{b}$ | 0.667$^{b}$ | 0.111$^{b}$ | 0.222$^{bc}$ | 0.000$^{b}$ | 0.222$^{b}$ | 0.000 | 0.000$^{c}$ | 0.000 | 0.444$^{bcd}$ | 0.000 |
| September 2008 | 0.667$^{b}$ | 0.000$^{b}$ | 0.556$^{b}$ | 0.000$^{b}$ | 0.111$^{c}$ | 0.000$^{b}$ | 0.333$^{b}$ | 0.000 | 0.000$^{c}$ | 0.000 | 0.000$^{d}$ | 0.000 |
| October 2008 | 0.444$^{b}$ | 0.000$^{b}$ | 0.444$^{b}$ | 0.000$^{b}$ | 0.111$^{c}$ | 0.000$^{b}$ | 0.444$^{b}$ | 0.000 | 0.000$^{c}$ | 0.000 | 0.333$^{bcd}$ | 0.000 |
| November 2008 | 0.556$^{b}$ | 0.000$^{b}$ | 0.889$^{ab}$ | 0.000$^{b}$ | 0.889$^{bc}$ | 0.000$^{b}$ | 0.111$^{b}$ | 0.000 | 0.000$^{c}$ | 0.000 | 0.556$^{bc}$ | 0.000 |
| December 2008 | 0.778$^{b}$ | 0.111$^{b}$ | 0.889$^{ab}$ | 0.000$^{b}$ | 0.444$^{abc}$ | 0.000$^{b}$ | 0.667$^{ab}$ | 0.000 | 0.111$^{c}$ | 0.000 | 0.333$^{bcd}$ | 0.000 |
| LSD | 0.667 | 0.556 | 0.693 | 0.470 | 0.620 | 0.276 | 0.549 | - | 0.332 | - | 0.462 | - |
| Level of significance | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | ns | 0.01 | ns | 0.01 | ns |

**Fig. 8** Month wise resting of *Tx. splendens* (Males & Females) on different tree trunks in Gilagasa village 2009

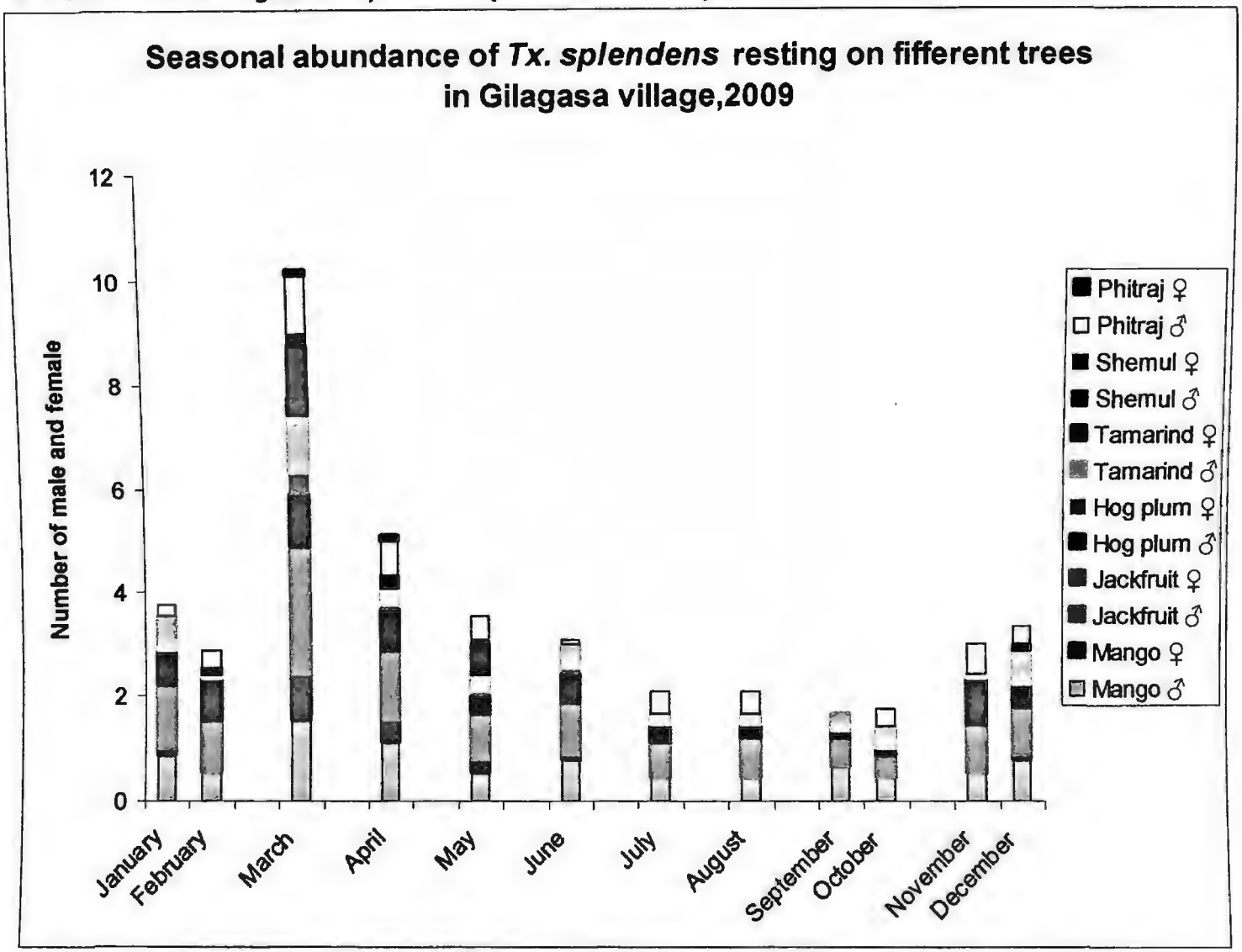


**Table 14c** Month wise resting of *Tx. splendens* (Males & Females) on different height of different tree trunks in Gilagasa village 2009

| Month | Height | Mango | | Jackfruit | | Hog plum | | Tamarind | | Shemul | | Phitraj | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| January 2009 | H1 | 0.333 | 0.000 | 1.000 | 0.000 | 0.333 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| February2009 | | 0.333 | 0.000 | 0.667 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 |
| March 2009 | | 1.000 | 0.333 | 0.667 | 0.333 | 0.667 | 0.333 | 0.667 | 0.000 | 0.667 | 0.000 | 1.000 | 0.000 |
| April 2009 | | 1.000 | 0.000 | 0.667 | 0.333 | 1.000 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 |
| May 2009 | | 0.000 | 0.333 | 0.333 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 |
| June 2009 | | 0.333 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| July 2009 | | 0.333 | 0.000 | 0.333 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 |
| August 2009 | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 |
| September2009 | | 0.333 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| October 2009 | | 0.333 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| November 2009 | | 0.333 | 0.000 | 0.667 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 |
| December 2009 | | 0.667 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| January 2009 | H2 | 0.667 | 0.333 | 1.667 | 0.000 | 1.000 | 0.000 | 1.333 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 |
| February 2009 | | 0.667 | 0.000 | 1.333 | 0.333 | 1.667 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 | 0.667 | 0.000 |
| March 2009 | | 1.667 | 2.000 | 1.667 | 1.333 | 1.333 | 0.667 | 1.333 | 0.333 | 0.333 | 0.333 | 1.333 | 0.333 |
| April 2009 | | 1.667 | 1.000 | 1.000 | 0.667 | 0.667 | 0.000 | 0.667 | 0.000 | 0.333 | 0.000 | 1.667 | 0.333 |
| May 2009 | | 1.000 | 0.333 | 1.000 | 0.333 | 1.000 | 0.333 | 0.667 | 0.000 | 0.667 | 0.333 | 1.000 | 0.000 |
| June 2009 | | 1.333 | 0.000 | 1.667 | 0.000 | 1.000 | 0.000 | 0.667 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 |
| July 2009 | | 0.333 | 0.000 | 1.000 | 0.333 | 0.333 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.667 | 0.000 |
| August 2009 | | 1.333 | 0.000 | 1.000 | 0.333 | 0.333 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.667 | 0.000 |
| September 2009 | | 1.333 | 0.000 | 1.000 | 0.000 | 0.333 | 0.000 | 0.667 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| October 2009 | | 0.667 | 0.000 | 0.667 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.667 | 0.000 |
| November 2009 | | 1.333 | 0.000 | 1.333 | 0.000 | 0.667 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| December 2009 | | 1.000 | 0.333 | 1.333 | 0.000 | 0.667 | 0.000 | 1.000 | 0.000 | 0.333 | 0.000 | 1.000 | 0.000 |
| January 2009 | H3 | 0.667 | 0.000 | 1.000 | 0.000 | 0.667 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 |
| February 2009 | | 0.667 | 0.000 | 0.667 | 0.000 | 0.333 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| March 2009 | | 2.000 | 0.333 | 2.333 | 1.000 | 1.000 | 0.333 | 1.333 | 0.667 | 1.000 | 0.333 | 1.000 | 0.000 |
| April 2009 | | 0.667 | 0.333 | 1.000 | 0.333 | 0.667 | 0.333 | 0.333 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 |
| May 2009 | | 0.667 | 0.000 | 0.667 | 0.333 | 0.000 | 0.000 | 0.333 | 0.000 | 0.667 | 0.000 | 0.333 | 0.000 |
| June 2009 | | 0.667 | 0.333 | 1.000 | 0.333 | 0.333 | 0.333 | 0.333 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| July 2009 | | 0.667 | 0.000 | 0.333 | 0.000 | 0.333 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 |
| August 2009 | | 0.000 | 0.000 | 1.000 | 0.000 | 0.333 | 0.000 | 0.667 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 |
| September 2009 | | 0.333 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| October 2009 | | 0.333 | 0.000 | 0.333 | 0.000 | 0.333 | 0.000 | 0.667 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 |
| November 2009 | | 0.000 | 0.000 | 0.667 | 0.000 | 21.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.333 | 0.000 |
| December 2009 | | 0.667 | 0.000 | 1.000 | 0.000 | 0.667 | 0.000 | 0.667 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| LSD | | - | - | - | - | - | - | - | - | - | - | - | - |
| Level of significant | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

**Table 15** ANOVA Gorgoria 2008

| Source | Degrees of freedom (d.f) | Mean Square | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mango | | Jackfruit | | Shemul | | Phitraj | |
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| Replication | 2 | 1.176 | 0.176 | 2.528 | 0.528 | 58.528 | 7.583 | 0.037 | 0.000 |
| Factor A | 2 | 5.120** | 0.176* | 7.111** | 0.333* | 28.778** | 1.194 | 0.120 | 0.000 |
| Factor B | 11 | 1.815** | 0.240** | 4.576** | 0.323** | 10.000** | 2.263** | 0.239** | 0.000 |
| AB | 22 | 0.353 | 0.045 | 0.869** | 0.051 | 3.475** | 0.578 | 0.090 | 0.000 |
| Error | 70 | 0.319 | 0.52 | 0.261 | 0.090 | 3.328 | 0.555 | 0.104 | 0.000 |

* Level of significant = 0.05, ** Level of significant = 0.01

**Table 15a** Resting of *Tx. splendens* (Males & Females) on different height of different tree trunks in Gorgoria village 2008

| No. of Replication | Height | Mango | | Jackfruit | | Shemul | | Phitraj | |
|---|---|---|---|---|---|---|---|---|---|
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 3 | H1 (00 - 84cm) | $0.250^{b}$ | $0.028^{b}$ | $0.389^{c}$ | $0.056^{b}$ | $0.833^{b}$ | 0.139 | 0.194 | 0.000 |
| | H2 (85 - 168cm) | $1.000^{a}$ | $0.167^{a}$ | $1.278^{a}$ | $0.222^{a}$ | $2.611^{a}$ | 0.500 | 0.083 | 0.000 |
| | H3 (169 - 252cm) | $0.694^{a}$ | $0.083^{ab}$ | $0.833^{b}$ | $0.056^{b}$ | $1.556^{ab}$ | 0.361 | 0.111 | 0.000 |
| LSD | | 0.352 | 0.107 | 0.319 | 0.141 | 1.139 | - | - | - |
| Level of significance | | 0.01 | 0.05 | 0.01 | 0.05 | 0.01 | ns | ns | - |

**Table 15b** Month wise resting of *Tx. splendens*(Males & Females) on different tree trunks in Gorgoria village 2008

| Month | Mango | | Jackfruit | | Shemul | | Phitraj | |
|---|---|---|---|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| January 2008 | $0.667^{bc}$ | $0.000^{b}$ | $0.778^{c}$ | $0.000^{b}$ | $1.333^{b}$ | $0.111^{b}$ | $0.000^{b}$ | 0.000 |
| February 2008 | $0.778^{bc}$ | $0.000^{b}$ | $0.667^{c}$ | $0.000^{b}$ | $2.000^{ab}$ | $0.667^{b}$ | $0.000^{b}$ | 0.000 |
| March 2008 | $1.778^{a}$ | $0.667^{a}$ | $2.778^{a}$ | $0.667^{a}$ | $4.111^{a}$ | $1.778^{a}$ | $0.444^{a}$ | 0.000 |
| April 2008 | $1.111^{ab}$ | $0.222^{b}$ | $1.667^{b}$ | $0.222^{b}$ | $2.222^{ab}$ | $0.556^{b}$ | $0.444^{a}$ | 0.000 |
| May 2008 | $0.444^{bc}$ | $0.111^{b}$ | $0.667^{c}$ | $0.111^{b}$ | $0.889^{b}$ | $0.222^{b}$ | $0.000^{b}$ | 0.000 |
| June 2008 | $0.778^{bc}$ | $0.111^{b}$ | $0.444^{c}$ | $0.111^{b}$ | $1.000^{b}$ | $0.111^{b}$ | $0.111^{b}$ | 0.000 |
| July 2008 | $0.333^{bc}$ | $0.000^{b}$ | $0.333^{c}$ | $0.000^{b}$ | $0.667^{b}$ | $0.222^{b}$ | $0.111^{b}$ | 0.000 |
| August 2008 | $0.222^{c}$ | $0.000^{b}$ | $0.333^{c}$ | $0.000^{b}$ | $0.667^{b}$ | $0.000^{b}$ | $0.222^{ab}$ | 0.000 |
| September 2008 | $0.222^{c}$ | $0.000^{b}$ | $0.778^{c}$ | $0.111^{b}$ | $3.000^{ab}$ | $0.222^{b}$ | $0.111^{b}$ | 0.000 |
| October 2008 | $0.222^{c}$ | $0.000^{b}$ | $0.444^{c}$ | $0.000^{b}$ | $0.778^{b}$ | $0.000^{b}$ | $0.000^{b}$ | 0.000 |
| November 2008 | $0.556^{bc}$ | $0.000^{b}$ | $0.333^{c}$ | $0.000^{b}$ | $1.444^{b}$ | $0.111^{b}$ | $0.000^{b}$ | 0.000 |
| December 2008 | $0.667^{bc}$ | $0.000^{b}$ | $0.778^{c}$ | $0.111^{b}$ | $1.889^{ab}$ | $0.000^{b}$ | $0.111^{b}$ | 0.000 |
| LSD | 0.7050 | 0.2846 | 0.6377 | 0.3745 | 2.277 | 0.9299 | 0.3032 | - |
| Level of significance | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.05 | - |

**Fig. 9** Month wise resting of *Tx. splendens*(Males & Females) on different tree trunks in Gorgoria village 2008

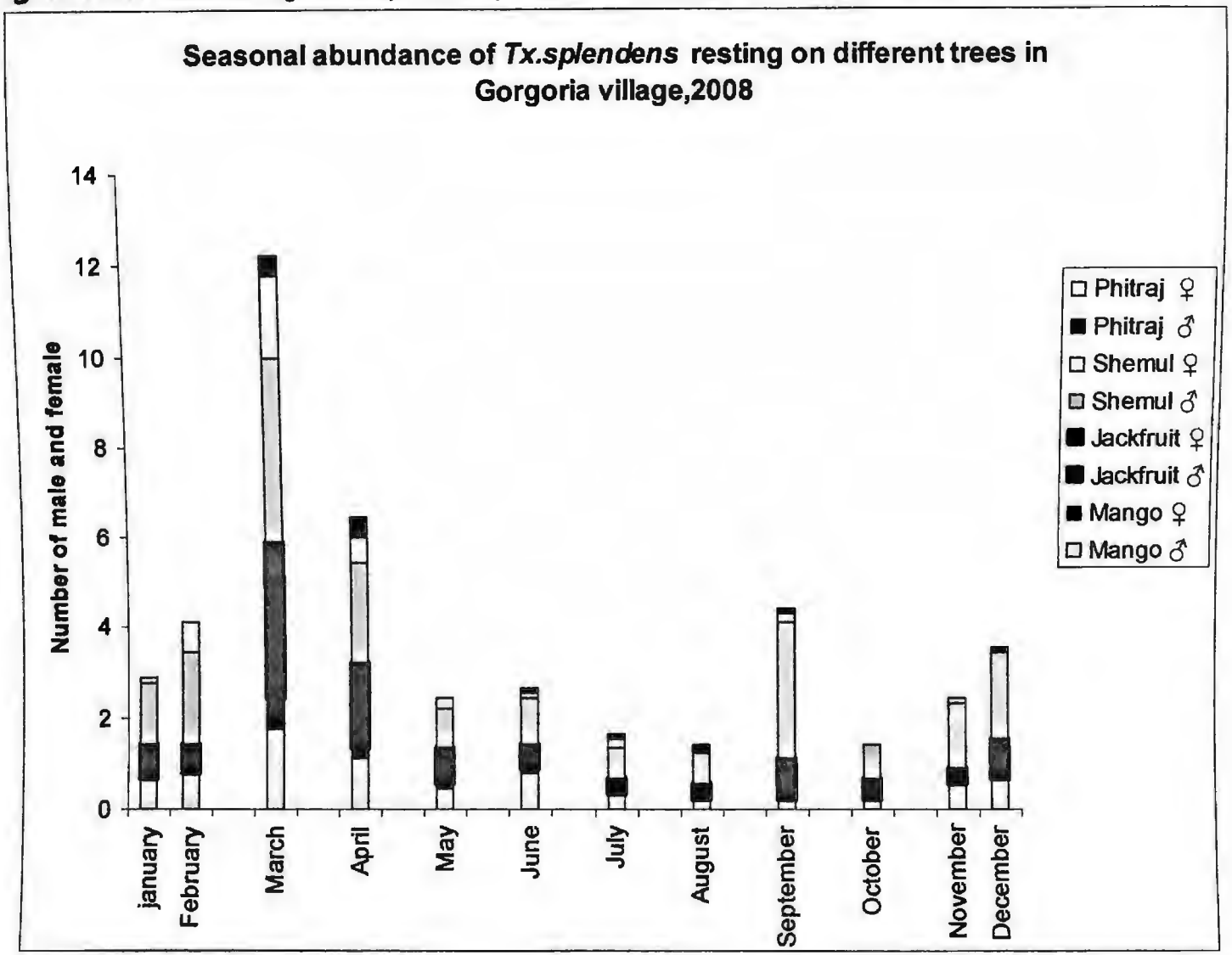


**Table 15c** Month wise resting of *Tx. splendens* (Males & Females) on different height of different tree trunks in Gorgoria village 2008

| Month | Height | Mango | | Jack fruit | | Shemul | | Phitraj | |
|---|---|---|---|---|---|---|---|---|---|
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| January 2008 | H1 | 0.333 | 0.000 | 0.333$^{d}$ | 0.000 | 0.667 | 0.000 | 0.000 | 0.000 |
| February 2008 | | 0.333 | 0.000 | 0.000$^{d}$ | 0.000 | 0.667 | 0.667 | 0.000 | 0.000 |
| March 2008 | | 1.000 | 0.333 | 1.333$^{bcd}$ | 0.333 | 1.000 | 0.333 | 0.333 | 0.000 |
| April 2008 | | 0.333 | 0.000 | 0.667$^{d}$ | 0.333 | 1.333 | 0.000 | 0.667 | 0.000 |
| May 2008 | | 0.000 | 0.000 | 0.667$^{d}$ | 0.000 | 0.333 | 0.333 | 0.000 | 0.000 |
| June 2008 | | 0.333 | 0.000 | 0.333$^{d}$ | 0.000 | 0.667 | 0.000 | 0.000 | 0.000 |
| July 2008 | | 0.000 | 0.000 | 0.333$^{d}$ | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 |
| August 2008 | | 0.000 | 0.000 | 0.000$^{d}$ | 0.000 | 0.333 | 0.000 | 0.667 | 0.000 |
| September 2008 | | 0.000 | 0.000 | 0.667$^{d}$ | 0.000 | 1.000 | 0.333 | 0.333 | 0.000 |
| October 2008 | | 0.000 | 0.000 | 0.000$^{d}$ | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 |
| November 2008 | | 0.333 | 0.000 | 0.000$^{d}$ | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 |
| December 2008 | | 0.333 | 0.000 | 0.333$^{d}$ | 0.000 | 1.667 | 0.000 | 0.333 | 0.000 |
| January 2008 | H2 | 1.000 | 0.000 | 1.333$^{bcd}$ | 0.000 | 2.667 | 0.333 | 0.000 | 0.000 |
| February 2008 | | 1.000 | 0.000 | 1.333$^{bcd}$ | 0.000 | 3.000 | 1.000 | 0.000 | 0.000 |
| March 2008 | | 3.000 | 1.000 | 4.667$^{a}$ | 1.000 | 7.667 | 3.000 | 0.333 | 0.000 |
| April 2008 | | 2.000 | 0.333 | 2.333$^{b}$ | 0.333 | 3.000 | 1.000 | 0.333 | 0.000 |
| May 2008 | | 1.000 | 0.333 | 1.000$^{cd}$ | 0.333 | 1.333 | 0.333 | 0.000 | 0.000 |
| June 2008 | | 1.000 | 0.333 | 0.667$^{d}$ | 0.333 | 1.667 | 0.333 | 0.000 | 0.000 |
| July 2008 | | 0.333 | 0.000 | 0.667$^{cd}$ | 0.000 | 0.667 | 0.000 | 0.333 | 0.000 |
| August 2008 | | 0.333 | 0.000 | 1.000$^{cd}$ | 0.000 | 0.333 | 0.000 | 0.000 | 0.000 |
| September 2008 | | 0.667 | 0.000 | 0.667$^{d}$ | 0.333 | 5.667 | 0.000 | 0.000 | 0.000 |
| October 2008 | | 0.333 | 0.000 | 0.667$^{d}$ | 0.000 | 1.333 | 0.000 | 0.000 | 0.000 |
| November 2008 | | 0.667 | 0.000 | 0.333$^{d}$ | 0.000 | 2.000 | 0.000 | 0.000 | 0.000 |
| December 2008 | | 0.667 | 0.000 | 0.667$^{d}$ | 0.333 | 2.000 | 0.000 | 0.000 | 0.000 |
| January 2008 | H3 | 0.667 | 0.000 | 0.667$^{d}$ | 0.000 | 0.667 | 0.000 | 0.000 | 0.000 |
| February 2008 | | 1.000 | 0.000 | 0.667$^{d}$ | 0.000 | 2.333 | 0.333 | 0.000 | 0.000 |
| March 2008 | | 1.333 | 0.667 | 2.333$^{b}$ | 0.667 | 3.667 | 0.000 | 0.667 | 0.000 |
| April 2008 | | 1.000 | 0.333 | 2.000$^{bc}$ | 0.000 | 2.333 | 0.667 | 0.333 | 0.000 |
| May 2008 | | 0.333 | 0.000 | 0.333$^{d}$ | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 |
| June 2008 | | 1.000 | 0.000 | 0.333$^{d}$ | 0.000 | 0.667 | 0.000 | 0.333 | 0.000 |
| July 2008 | | 0.667 | 0.000 | 0.000$^{d}$ | 0.000 | 1.000 | 0.667 | 0.000 | 0.000 |
| August 2008 | | 0.333 | 0.000 | 0.000$^{d}$ | 0.000 | 1.333 | 0.000 | 0.000 | 0.000 |
| September 2008 | | 0.000 | 0.000 | 1.000$^{cd}$ | 0.000 | 2.333 | 0.333 | 0.000 | 0.000 |
| October 2008 | | 0.333 | 0.000 | 0.667$^{d}$ | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| November 2008 | | 0.667 | 0.000 | 0.667$^{d}$ | 0.000 | 1.333 | 0.333 | 0.000 | 0.000 |
| December 2008 | | 1.000 | 0.000 | 1.333$^{bcd}$ | 0.000 | 2.000 | 0.000 | 0.000 | 0.000 |
| LSD | | - | - | 1.105 | - | 1.105 | - | - | - |
| Level of significant | | ns | ns | 0.01 | ns | 0.01 | ns | ns | - |

**Table 16** ANOVA Gorgoria 2009

| Source | Degrees of freedom (d.f) | Mean Square | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mango | | Jackfruit | | Shemul | | Phitraj | |
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| Replication | 2 | 4.287 | 0.361 | 4.481 | 1.120 | 78.037 | 5.009 | 0.009 | 0.000 |
| Factor A | 2 | 6.398** | 0.250 | 5.843** | 0.287* | 19.620** | 1.370 | 0.898** | 0.000 |
| Factor B | 11 | 1.120** | 0.545** | 1.565** | 0.615* | 9.686** | 1.807** | 0.552** | 0.000 |
| AB | 22 | 0.287* | 0.129* | 0.721** | 0.105 | 1.893 | 0.269 | 0.231** | 0.000 |
| Error | 70 | 0.144 | 0.066 | 0.243 | 0.092 | 2.656 | 0.495 | 0.104 | 0.000 |

* Level of significant = 0.05, ** Level of significant = 0.01

**Table 16a** Resting of *Tx. splendens* (Males & Females) on different height of different tree trunks in Gorgoria village 2009

| No. of Replication | Height | Mango | | Jack fruit | | Shemul | | Phitraj | |
|---|---|---|---|---|---|---|---|---|---|
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 3 | H1 (00 - 84cm) | $0.333^{c}$ | 0.028 | $0.556^{c}$ | $0.056^{b}$ | $0.917^{b}$ | 0.083 | $0.139^{b}$ | 0.000 |
| | H2 (85 - 168cm) | $1.670^{a}$ | 0.194 | $1.361^{a}$ | $0.222^{a}$ | $2.389^{a}$ | 0.472 | $0.361^{a}$ | 0.000 |
| | H3 (169-252cm) | $0.639^{b}$ | 0.111 | $0.944^{b}$ | $0.194^{ab}$ | $1.556^{ab}$ | 0.250 | $0.056^{b}$ | 0.000 |
| LSD | | 0.2368 | - | 0.3077 | 0.1426 | 1.017 | - | 0.2013 | - |
| Level of significance | | 0.01 | ns | 0.01 | 0.05 | 0.01 | ns | 0.01 | - |

**Table 16b** Month wise resting of *Tx. splendens* (Males & Females) on different tree trunks in Gorgoria village 2009

| Month | Mango | | Jackfruit | | Shemul | | Phitraj | |
|---|---|---|---|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| January 2008 | $0.778^{bc}$ | $0.000^{c}$ | $1.444^{ab}$ | $0.000^{c}$ | $1.889^{b}$ | $0.000^{b}$ | $0.000^{b}$ | 0.000 |
| February 2008 | $0.778^{bc}$ | $0.000^{c}$ | $0.880^{bc}$ | $0.111^{a}$ | $1.556^{b}$ | $0.333^{b}$ | $0.333^{b}$ | 0.000 |
| March 2008 | $1.333^{a}$ | $0.778^{a}$ | $1.667^{a}$ | $0.889^{b}$ | $4.333^{a}$ | $1.556^{a}$ | $0.889^{a}$ | 0.000 |
| April 2008 | $1.333^{a}$ | $0.444^{b}$ | $1.333^{ab}$ | $0.444^{c}$ | $2.667^{ab}$ | $0.667^{b}$ | $0.222^{b}$ | 0.000 |
| May 2008 | $0.889^{ab}$ | $0.111^{c}$ | $0.889^{bc}$ | $0.111^{c}$ | $1.556^{b}$ | $0.222^{b}$ | $0.111^{b}$ | 0.000 |
| June 2008 | $0.778^{bc}$ | $0.000^{c}$ | $0.778^{bc}$ | $0.111^{c}$ | $1.111^{b}$ | $0.000^{b}$ | $0.111^{b}$ | 0.000 |
| July 2008 | $0.444^{bcd}$ | $0.000^{c}$ | $0.444^{c}$ | $0.000^{c}$ | $0.778^{b}$ | $0.111^{b}$ | $0.000^{b}$ | 0.000 |
| August 2008 | $0.222^{d}$ | $0.000^{c}$ | $0.556^{c}$ | $0.000^{c}$ | $0.778^{b}$ | $0.000^{b}$ | $0.000^{b}$ | 0.000 |
| September 2008 | $0.333^{cd}$ | $0.000^{c}$ | $1.444^{ab}$ | $0.111^{c}$ | $0.778^{b}$ | $0.111^{b}$ | $0.000^{b}$ | 0.000 |
| October 2008 | $0.444^{bcd}$ | $0.000^{c}$ | $0.444^{c}$ | $0.000^{c}$ | $0.667^{b}$ | $0.000^{b}$ | $0.222^{b}$ | 0.000 |
| November 2008 | $0.667^{bcd}$ | $0.000^{c}$ | $0.778^{bc}$ | $0.000^{c}$ | $1.667^{b}$ | $0.111^{b}$ | $0.222^{b}$ | 0.000 |
| December 2008 | $0.556^{bcd}$ | $0.000^{c}$ | $0.778^{bc}$ | $0.111^{c}$ | $1.667^{b}$ | $0.111^{b}$ | $0.111^{b}$ | 0.000 |
| LSD | 0.473 | 0.320 | 0.615 | 0.285 | 2.043 | 0.878 | 0.402 | - |
| Level of significance | 0.01 | 0.01 | 0.01 | 0.05 | 0.01 | 0.01 | 0.01 | - |

**Fig. 10** Month wise resting of *Tx. splendens* (Males & Females) on different tree trunks in Gorgoria village 2009

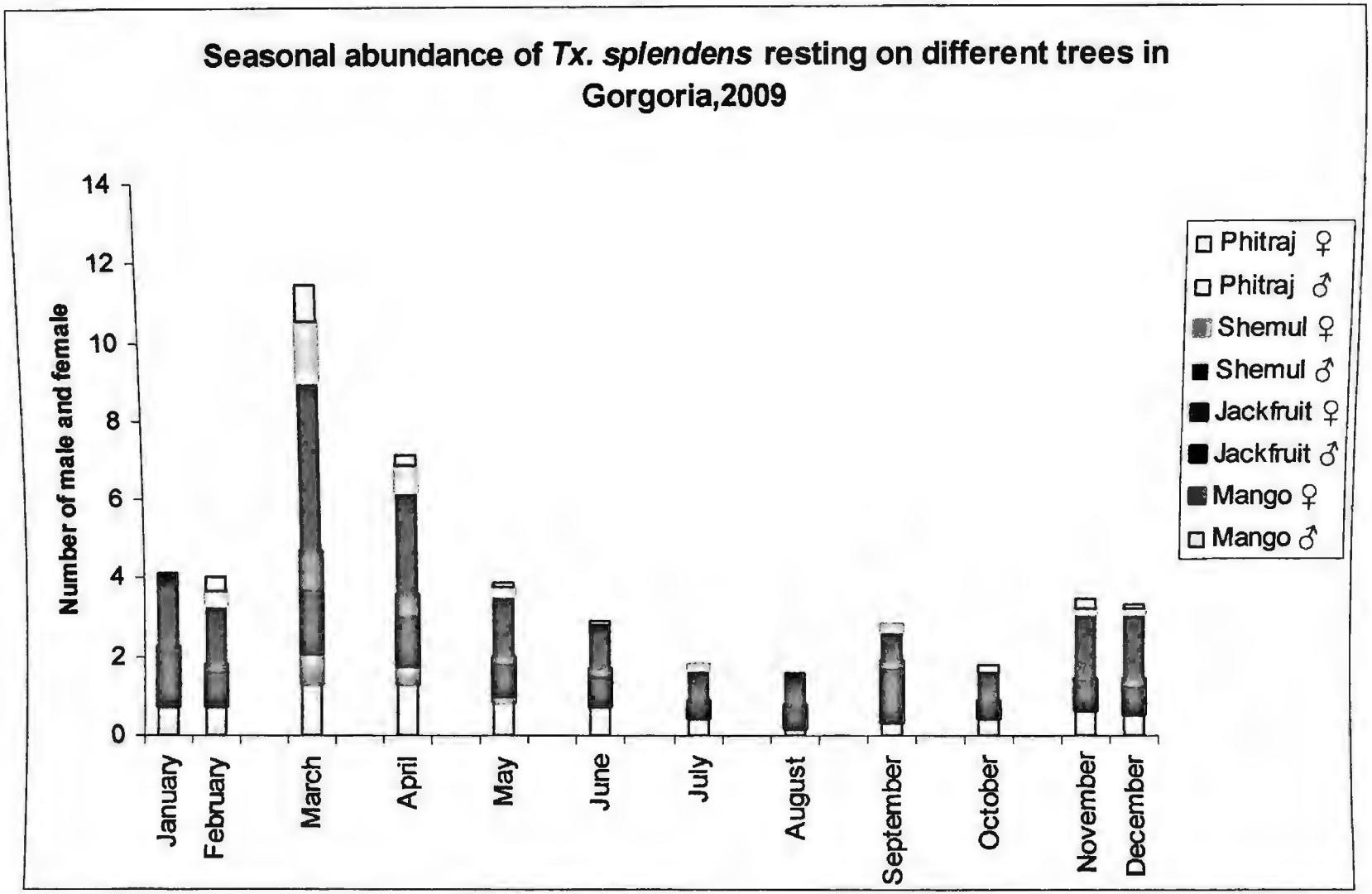


**Table 16c** Month wise resting of *Tx. splendens* (Males & Females) on different height of different tree trunks in Gorgoria village 2009

| Month | Height | Mango | | Jack fruit | | Shemul | | Phitraj | |
|---|---|---|---|---|---|---|---|---|---|
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| January 2009 | H1 | 0.333$^{de}$ | 0.000$^{d}$ | 0.667$^{def}$ | 0.000 | 1.667 | 0.000 | 0.000$^{c}$ | 0.000 |
| February 2009 | | 0.333$^{de}$ | 0.000$^{d}$ | 0.667$^{def}$ | 0.000 | 0.667 | 0.333 | 0.000$^{c}$ | 0.000 |
| March 2009 | | 1.000$^{bcd}$ | 0.333$^{cd}$ | 1.000$^{cdef}$ | 0.333 | 2.333 | 0.667 | 0.667$^{bc}$ | 0.000 |
| April 2009 | | 0.333$^{de}$ | 0.000$^{d}$ | 0.333$^{ef}$ | 0.333 | 2.000 | 0.000 | 0.333$^{bc}$ | 0.000 |
| May 2009 | | 0.333$^{de}$ | 0.000$^{d}$ | 0.667$^{def}$ | 0.000 | 0.667 | 0.000 | 0.000$^{c}$ | 0.000 |
| June 2009 | | 0.333$^{de}$ | 0.000$^{d}$ | 0.667$^{def}$ | 0.000 | 0.333 | 0.000 | 0.333$^{bc}$ | 0.000 |
| July 2009 | | 0.000$^{e}$ | 0.000$^{d}$ | 0.000$^{f}$ | 0.000 | 0.667 | 0.000 | 0.000$^{c}$ | 0.000 |
| August 2009 | | 0.000$^{e}$ | 0.000$^{d}$ | 0.667$^{def}$ | 0.000 | 0.667 | 0.000 | 0.000$^{c}$ | 0.000 |
| September 2009 | | 0.333$^{de}$ | 0.000$^{d}$ | 0.333$^{ef}$ | 0.000 | 0.333 | 0.000 | 0.000$^{c}$ | 0.000 |
| October 2009 | | 0.333$^{de}$ | 0.000$^{d}$ | 0.333$^{ef}$ | 0.000 | 0.333 | 0.000 | 0.333$^{bc}$ | 0.000 |
| November 2009 | | 0.667$^{cde}$ | 0.000$^{d}$ | 0.667$^{def}$ | 0.000 | 0.667 | 0.000 | 0.000$^{c}$ | 0.000 |
| December 2009 | | 0.000$^{e}$ | 0.000$^{d}$ | 0.667$^{def}$ | 0.000 | 0.667 | 0.000 | 0.000$^{c}$ | 0.000 |
| January 2009 | H2 | 1.000$^{bcd}$ | 0.000$^{d}$ | 2.000$^{abc}$ | 0.000 | 2.000 | 0.000 | 0.000$^{c}$ | 0.000 |
| February 2009 | | 1.333$^{bc}$ | 0.000$^{d}$ | 1.333$^{bcde}$ | 0.333 | 2.667 | 0.667 | 1.000$^{ab}$ | 0.000 |
| March 2009 | | 1.667$^{b}$ | 1.333$^{a}$ | 2.333$^{ab}$ | 1.333 | 7.667 | 2.333 | 1.667$^{a}$ | 0.000 |
| April 2009 | | 2.333$^{a}$ | 1.000$^{ab}$ | 2.333$^{ab}$ | 0.667 | 3.333 | 1.333 | 0.333$^{bc}$ | 0.000 |
| May 2009 | | 1.333$^{bc}$ | 0.000$^{d}$ | 1.000$^{cdef}$ | 0.000 | 2.333 | 0.333 | 0.000$^{c}$ | 0.000 |
| June 2009 | | 1.000$^{bcd}$ | 0.000$^{d}$ | 1.000$^{cdef}$ | 0.333 | 1.667 | 0.000 | 0.000$^{c}$ | 0.000 |
| July 2009 | | 0.667$^{cde}$ | 0.000$^{d}$ | 0.333$^{ef}$ | 0.000 | 1.000 | 0.333 | 0.000$^{c}$ | 0.000 |
| August 2009 | | 0.667$^{cde}$ | 0.000$^{d}$ | 1.000$^{cdef}$ | 0.000 | 1.333 | 0.000 | 0.000$^{c}$ | 0.000 |
| September 2009 | | 0.667$^{cde}$ | 0.000$^{d}$ | 2.667$^{a}$ | 0.000 | 1.000 | 0.000 | 0.000$^{c}$ | 0.000 |
| October 2009 | | 1.000$^{bcd}$ | 0.000$^{d}$ | 0.667$^{def}$ | 0.000 | 1.000 | 0.000 | 0.333$^{bc}$ | 0.000 |
| November 2009 | | 1.000$^{bcd}$ | 0.000$^{d}$ | 0.333$^{ef}$ | 0.000 | 2.000 | 0.333 | 0.667$^{bc}$ | 0.000 |
| December 2009 | | 1.333$^{bc}$ | 0.000$^{d}$ | 1.333$^{bcde}$ | 0.000 | 2.667 | 0.333 | 0.333$^{bc}$ | 0.000 |
| January 2009 | H3 | 1.000$^{bcd}$ | 0.000$^{d}$ | 1.667$^{abcd}$ | 0.000 | 2.000 | 0.000 | 0.000$^{c}$ | 0.000 |
| February 2009 | | 0.667$^{cde}$ | 0.000$^{d}$ | 0.667$^{def}$ | 0.000 | 1.333 | 0.000 | 0.000$^{c}$ | 0.000 |
| March 2009 | | 1.333$^{bc}$ | 0.667$^{bc}$ | 1.667$^{abcd}$ | 1.000 | 3.000 | 1.667 | 0.333$^{bc}$ | 0.000 |
| April 2009 | | 1.333$^{bc}$ | 0.333$^{cd}$ | 1.333$^{bcde}$ | 0.333 | 2.667 | 0.667 | 0.000$^{c}$ | 0.000 |
| May 2009 | | 1.000$^{bcd}$ | 0.333$^{cd}$ | 1.000$^{cdef}$ | 0.333 | 1.667 | 0.333 | 0.333$^{bc}$ | 0.000 |
| June 2009 | | 1.000$^{bcd}$ | 0.000$^{d}$ | 0.667$^{def}$ | 0.000 | 1.333 | 0.000 | 0.000$^{c}$ | 0.000 |
| July 2009 | | 0.667$^{cde}$ | 0.000$^{d}$ | 1.000$^{cdef}$ | 0.000 | 0.667 | 0.000 | 0.000$^{c}$ | 0.000 |
| August 2009 | | 0.000$^{e}$ | 0.000$^{d}$ | 0.000$^{f}$ | 0.000 | 0.333 | 0.000 | 0.000$^{c}$ | 0.000 |
| September 2009 | | 0.000$^{e}$ | 0.000$^{d}$ | 1.333$^{bcde}$ | 0.333 | 1.000 | 0.333 | 0.000$^{c}$ | 0.000 |
| October 2009 | | 0.000$^{e}$ | 0.000$^{d}$ | 0.333$^{ef}$ | 0.000 | 0.667 | 0.000 | 0.000$^{c}$ | 0.000 |
| November 2009 | | 0.333$^{de}$ | 0.000$^{d}$ | 1.333$^{bcde}$ | 0.000 | 2.333 | 0.000 | 0.000$^{c}$ | 0.000 |
| December 2009 | | 0.333$^{de}$ | 0.000$^{d}$ | 0.333$^{ef}$ | 0.333 | 1.667 | 0.000 | 0.000$^{c}$ | 0.000 |
| LSD | | 0.6180 | 0.4184 | 1.066 | - | - | - | 0.6972 | - |
| Level of significant | | 0.05 | 0.05 | 0.01 | ns | ns | ns | 0.01 | - |

Plate 1: Trap of earthen pitchers

Plate 2: Cage type-A

Plate 3: Cage type-B

Plate 4: Cage type-C

Plate 5: Ovitrap, Black plastic pot with bamboo stump

Plate 5: Ovitrap, Black plastic pot with bamboo stump

Plate 5: Ovitrap, Red plastic pot with bamboo stump

Plate 5: Ovitrap, Green plastic pot with bamboo stump

Plate 5: Ovitrap, White plastic pot with bamboo stump

Plate 5: Ovitrap, Blue plastic pot with bamboo stump

Plate 6: Bamboo-leaf infusion water

Plate 7: Love apple (*Artocarpus heterophyllus*)


Plate 8: Radish (*Raphanus sativus*)

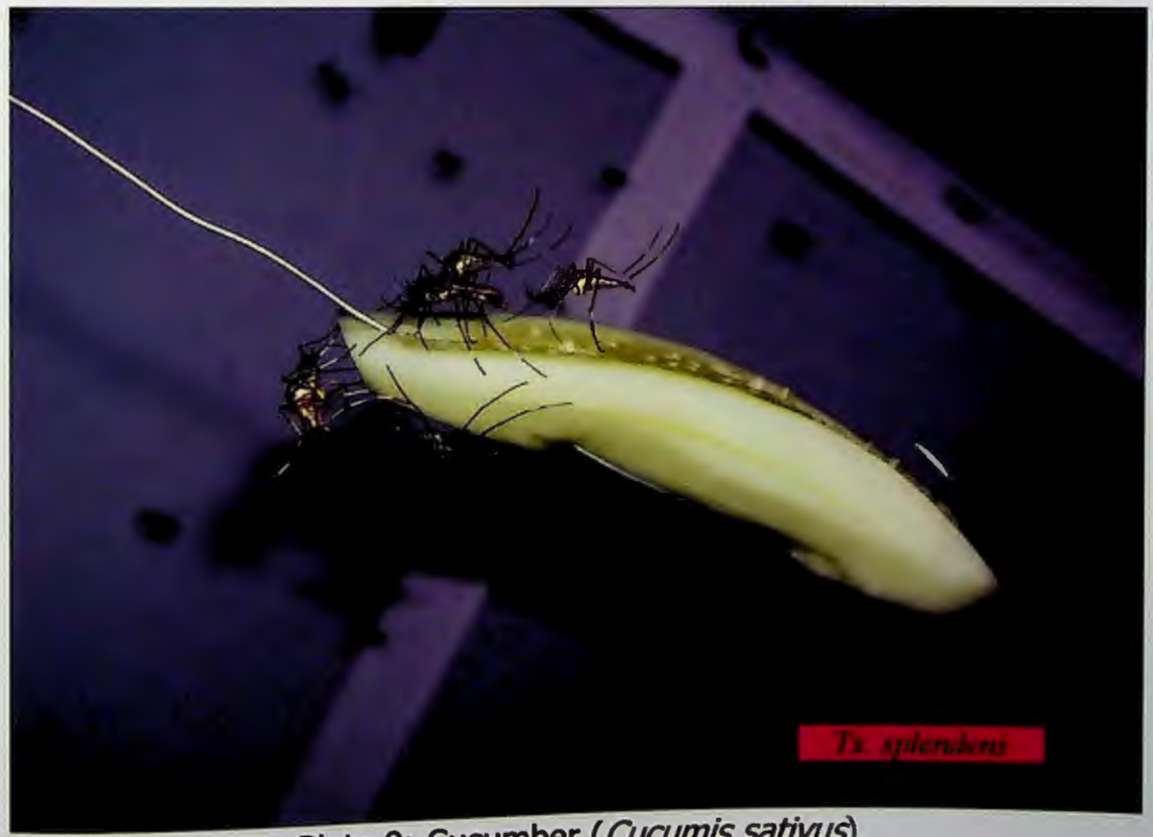



Plate 9: Cucumber (*Cucumis sativus*)

Plate 10: Carrot (*Daucas carota*)

Plate 11: Jicama (*Pachyrhizus erosus*)

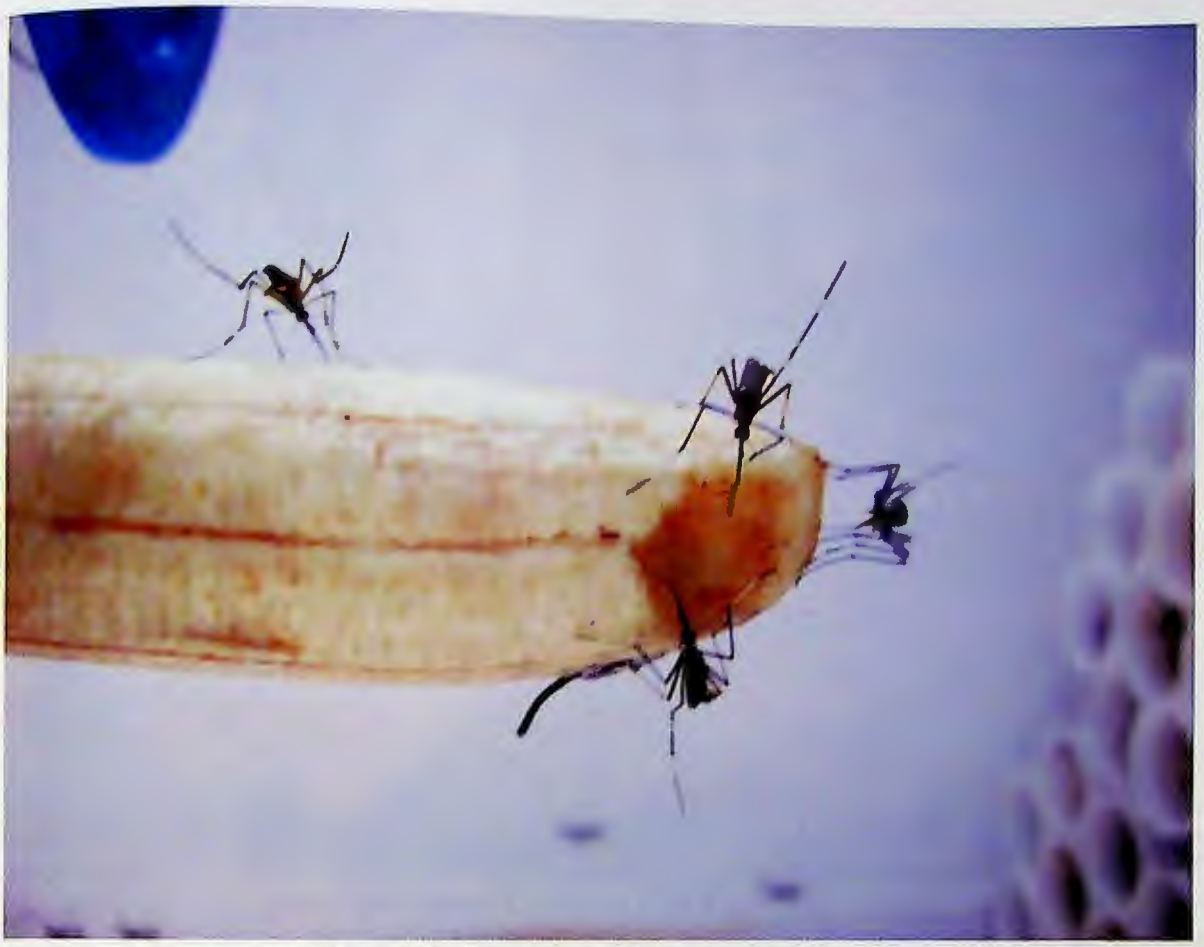


Plate 12: Banana (*Musa paradisiaca*)

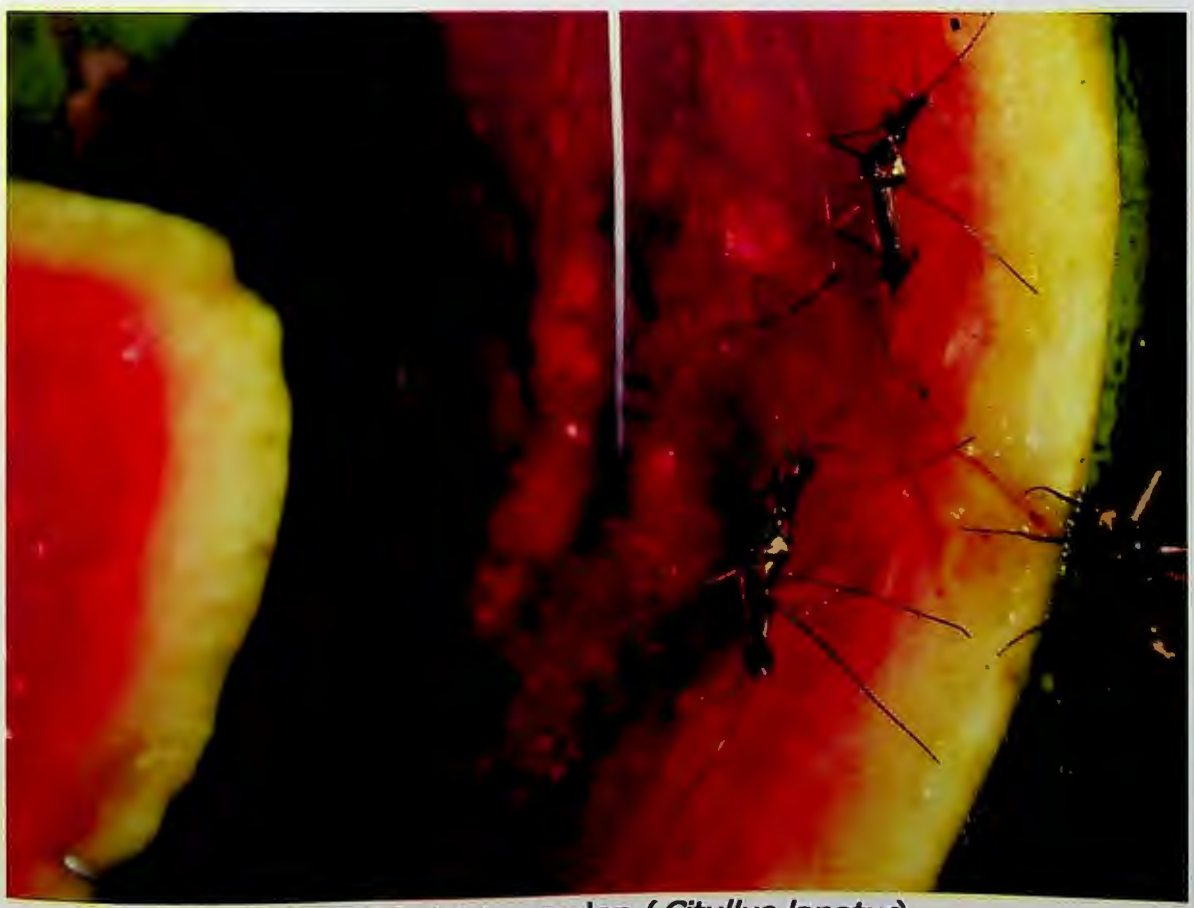


Plate 13: Water melon (*Citullus lanatus*)

Plate 14: Sweet potato (*Ipomoea batatas*)

Plate 15: Pumpkin (*Cucurbita maxima*)


Plate 16: Sugarcane (*Saccharum officinarum* )

a. Eggs of predator & prey

b. Fresh eggs

c. **Color view**

**d.** Eggs adherent one another

Plate 17: a-d, **Different view of *Tx. splendens* egg**

a. Natural view

b. Color view

Plate 18: a-b, Eggshell splitted into two halves

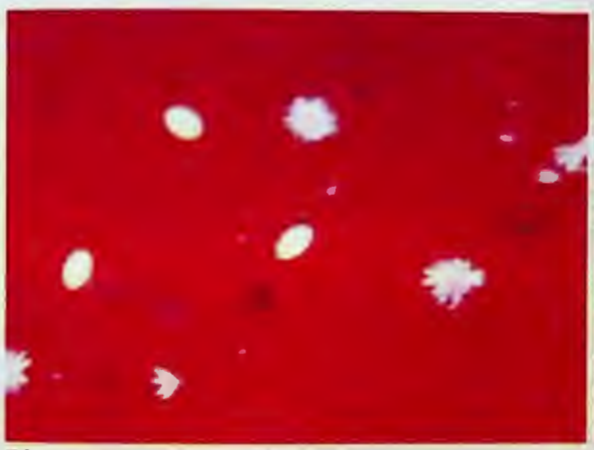


Plate 19: Unfertile egg

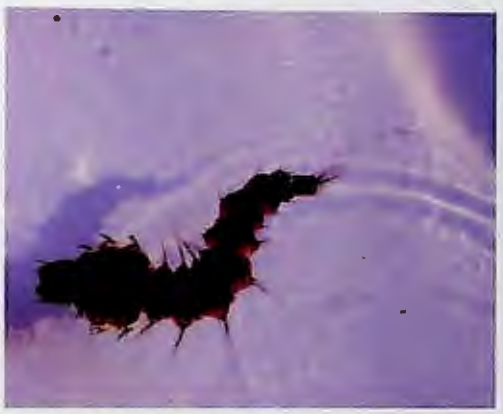

Plate 20: Individual rearing in plastic vial

Plate 21: First instarlarva of *Tx. splendens*

a. Ventral view of 4th instar

b. Dorsal view of 4th instar

c. Striking view



d. jerking view

e. Full grown of 4th instar

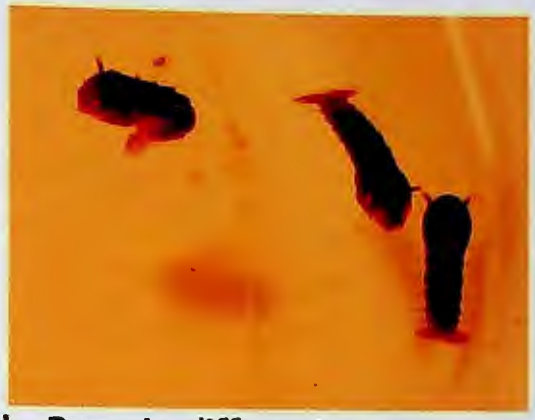
f. Association with prey larvae

g. Grasps the prey (*Cx.quinquefasciatus*)

h. . Grasps the prey ( *Ae. albopictus*)

Plate 22: Different view of 4th instar larvae ***Tx. splendens*** (a-h)

a. Pupa of *Tx. splendens*



b. Pupa in different view

c. Pupa in straight line before ecdysis

d. Pupal paddle project up & attach to rim of pot

Plate 23: a-d, different view of pupae stage

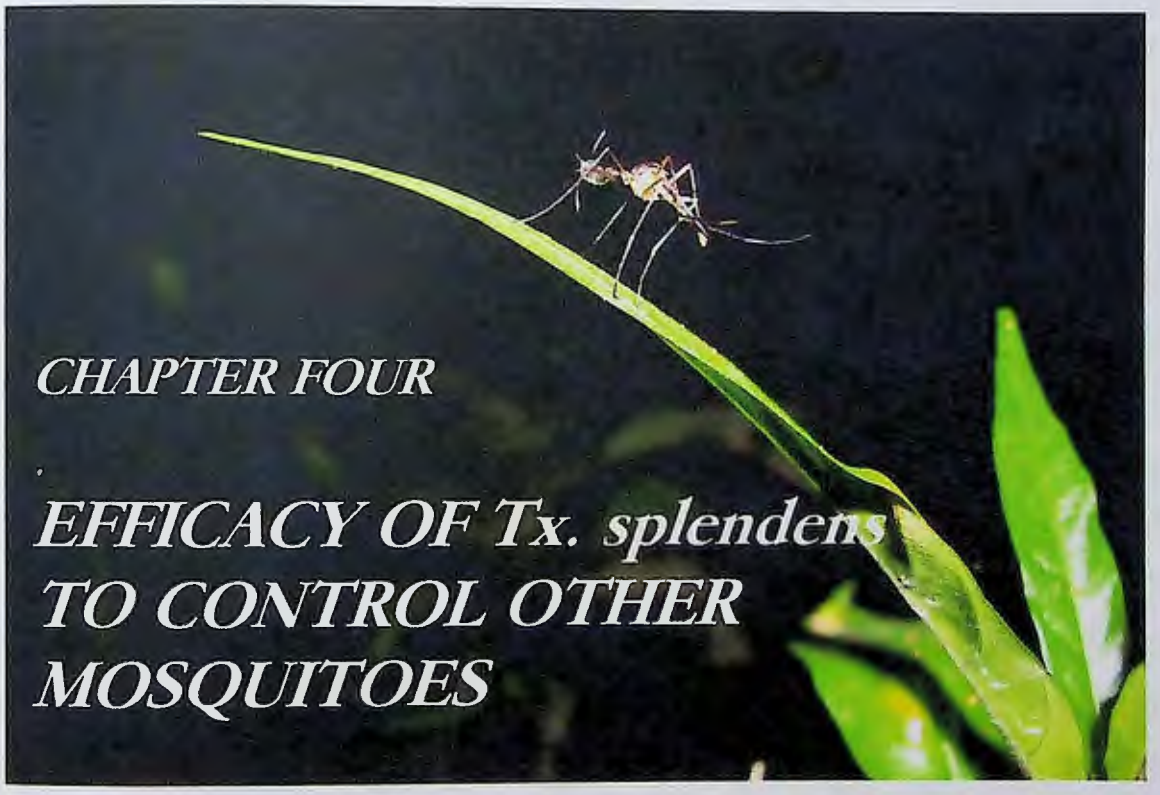
a. Larvae

b. Pupae

Plate 24: Mass rearing *Tx. splendens*, the larvae & pupae in clean bamboo-leaf infusion water


Plate 25: Adult emerged from pupa

Plate 26: Adults in cage for release in Rajshahi University Campus



## CHAPTER FOUR

# EFFICACY OF *Tx. splendens* TO CONTROL OTHER MOSQUITOES

# CHAPTER FOUR

## Efficacy of *Tx. splendens* to control other mosquitoes

### Introduction

The predator, *Tx. splendens* a container-inhabiting mosquito (Barraud, 1934; Burton and Rudnick, 1979; Chuah and Yap, 1984; Furumizo and Rudnick, 1978; GeethaBai *et al.*, 1981; Paine, 1934; Vongtangswad *et al.,* 1983).Its larvae are predacious on mosquito larvae or other small aquatic organisms in nature or man-made container (Schreiber and Jones, 1994; Steffan and Evenhuis, 1981 and Campos and Lounibos, 2000). This has wide diversity inhabit small to medium-sized containers such as tree holes, tree and bamboo stumps, leaf axils, coconut shells, pitcher plants, earthern water jars and discarded containers (Chan, 1968; Yap and Foo,1984 and Jones and Schreiber,1994; Steffan and Evenhuis,1981). Jones and Schreiber, 1994 explained that *Tx. splendens* to be better from *Tx. rutilus rutilus* contender for mosquito control in urbanized areas where discarded containers are available. Jones (1993) suggests that *Toxorhynchites* spp. larvae are well adapted for feeding on mosquito larvae and important in determining community structure and possibly species coexistence. *Toxorhynchites* species have been considered a prospective biocontrol agent for container developing vector mosquitoes such as *Aedes aegypti* and *Ae. albopictus* especially when conventional chemical control methods are undesirable (WHO, 1980). Paine,1934 was pioneer successfully used this mosquito as biocontrol agent for vector mosquitoes in the Pacific Island. *Tx. splendens* has been used successfully in India, where there were significant reductions in the number of *Ae. aegypti, Ar. subalbatus*, and *Cx.quinquefasciatus* breeding in domestic water containers (Panicker & Geetha Bai, 1983). Second-instar *Tx. splendens* larvae also suppressed *Ae. aegypti & Ae. albopictus* in Malaysia (Chuah & Yap, 1984). In Thailand, fourth-instar *Tx. splendens* reduced the *Ae. aegypti* population ( Vongtangswad *et at.,* 1983).

Bangladesh is a developing country and densely population live in substandard living condition due to poverty. In Bangladesh, vector

mosquitoes problem especially dengue disease increased day by day (Appendix Table 1) of their vector (*Ae. aegypti & Ae. albopictus*) breeding source are available both natural and arificial containers and filarial vector (*Cx. quinquefasciatus* ) also breed in container mentioned earlier. Chemical control useful other mosquitoes but imposible for prevented container-inhabiting mosquito, not only that its residual effect polluted the environment. *Tx. splendens* is a container breeding mosquito in Bangladesh (Ameen and Talukdar,1974; Nasir-Ud-Din,1952, Ahmed *et al*,1990 and Hassan,1990) and about this mosquito's efficacy is scanty. With this background,the present study was initiated to understand the efficacy i.e. control on vectors mosquito, *Ae. albopictus* and *Cx. quinquefasciatus* in the laboratory. The present effort is insufficient for the objective but it may believed to be of some help to the future researchers in this progression.

**Materials and Methods**

Prey colony of *Ae. albopictus* and *Cx. quinquefasciatus* maintain in the laboratory, eggs were reared in plastic bowl with bamboo–leaf infution water and obtain larvae were used in the experiment. The single rearing plastic vials were used (35mm Fujifilim capsule 3×5cm with a capacity of 27ml water). Known number of prey larvae of *Ae.albopictus* (II & III instar) and *Cx. quinquefasciatus* (II & III instar) were pleased in plastic vials proportionally 10:1, 15:1, 20:1, 25:1 and 30:1 with ratio prey: predator. Second and third instar of these prey larvae were applied in six replication to evaluate the experiment instarwise and another experiment vial with ratio wise prey lavae were observed 24 hours act as control. After 24 hours consumption and killing in each plastic vials (10:1, 15:1, 20:1, 25:1, 30:1) number of survived prey larva was recorded then minus from total as consumption and Killing was happen by predator with out ate prey. Moulting of each larval stsge was recorded as their duration. Consumption, killing and duration of each instar were recorded. Egg and pupal duration also noted. Data obtained from experiment were analysis statistically and was fit in "Two Factor Randomized Complete Block Design" i.e. consumption, killing and duration of each instar. Egg and pupal duration's data was put in "One Factor Randomized Complete

Block Design". ANOVA, DMRT and mean values were present in result. Experiment were conducted in room temperature and Relative Humidity mentioned earlier.

**Results and discussions**

Efficacy of *Tx. splendens* to control other mosquitoes in the laboratory, it was observed that the mosquito larvae of *Tx. splendens* were effectively antagonist to vector mosquitoes(i.e.*Ae. albopictus* and *Cx. quinquefasciatus*). The predator larvae were lavishly fed with the prey larvae of mosquito (II & III instar). Comsumption rate of the predator increased with the increaseing of food availability and this relationship was highly significant when larvae of *Ae. albopictus* and *Cx. quinquefasciatus* were offered as food. Amalraj and Das (2005), in their experiment observed that *Tx. splendens* larvae prefered *An. stephensi* as food in India. Frank *et al.*,(1984) stated that larval development time varied inversely with prey availability in *Tx. r. rutilus* and older larvae ($4^{th}$ instar) are more efficient in predation.

To determine the consumption rate of a single predator larva fed with different ratio of prey larvae, the collected data were statistically analysed by RCBD (Two Factor Randomized Complete Block Design) and the result of ANOVA (Table 17), DMRT (Table 17a) were obtained. According to data analysis the relation between consumption of a predator larva and prey larvae was found in the following ratio 10:1,15:1,20:1,25:1and 30:1. From the Table 17a, it was observed that in the p:P ratio (30:1) the comsumption of prey larvae II instar (59.875), III instar (60.792) of *Ae. albopictus* and II instar (72.125), III instar (70.042) of *Cx. quinquefasciatus* were higher than others. Statistically it was also significant at 0.01 level.There was also a statistical similarity in the following instar larvae of *Ae. albopictus* and *Cx. quinquefasciatus*, i.e. 56.792(25:1),67.375(25:1),69.125(25:1), (30:1), 59.875 (30:1), 60.792 (30:1), 70.042 (30:1) and 72.125(30:1).In this p:P ratio (10:1), the comsumption rate of prey larvae was lower but it was also statistical similar to the comsumption rate of prey larvae in the ratio of p:P (15:1).

All instars of *Tx. splendens* were predatory in nature. Steffan and Evenhuis(1981) repored that the predator larvae ambushed the prey. Russo(1986) and Linley & Darling (1993), they reported the predator larvae searched the prey, but it was observed that the wriggling behavior may induced the predator larvae to collect their food. Instars wise predation in between prey larvae and predator was presented in the Table 17b. The 4$^{th}$ instar predator larvae consumed the maximum no. of prey larvae, i.e. II & III instar of *Ae. albopictus* and *Cx. quinquefasciatus*. The rate of consumption of prey larvae at the 4$^{th}$ instar was more than the 1$^{st}$ ,2$^{nd}$ and 3$^{rd}$ instar of predator larva. At the 4$^{th}$ instar of predator larva the consumption rate of prey larvae was statistically significant at 0.01 level.

The interaction in between the consumption rate of predator(*Tx. splendens*) at different instars with the different p:P ratio was focused in the Table 17c. The higher consumption rate was obtained at the 4$^{th}$ instar of predator larva with the prey– predator ratio of 20:1,25:1, and 30:1. Similarly the lower consumption rate was found at the 1$^{st}$ and 2$^{nd}$ instars of predator larva with the prey– predator ratio of 10:1. High intensity of prey increased the consumption rate of pery larvae by the predator within the similar container.Similarly the consumption rate of prey larvae decreased with the decrease of the intensity of prey.

The killing rate of prey larvae by the larvae of *Tx. splendens* was presented in the Table 18, Table 18a. From the Table it was obtained that the killing rate of prey larvae was 2.750 for 1$^{st}$ instar and 2.708 for 2$^{nd}$ instar of *Ae. albopictus* (Prey) in the prey-predator ratio of 25:1. Simultaneously the killing rate of prey larvae was found 2.708 and 3.500 at the stage of 1$^{st}$ instar and 2$^{nd}$ instar of *Ae. albopictus* with the ratio of 30:1. In respect of killing the rate of prey larvae was 6.875 and 7.542 for 2$^{nd}$ and 3$^{rd}$ instar of *Cx. quinquefasciatus* at the prey-predator ratio of 30:1. Statistically the data were also significant at 0.01 level.Instar wise killing of prey larvae by the different instar of predator larvae showed on the Table 18b. From this

table it was found that the 1st instar of predator larva killed the highest no. (2.300) of prey larvae of *Ae. albopictus* ( II- instar). In this way of 2nd instar the 3rd instar of predator larva (*Tx. splendens*) killed the highest no.(2.733) of prey larvae of *Ae. albopictus* ( III- instar).In case of *Cx. quinquefasciatus* the predator larva(4th instar) killed the highest no.(7.967) of prey larvae(II- instar). Similarly the predator larva (4th instar) killed the the highest no.(8.267) of prey larvae(III- instar).The killing rate of *Ae. albopictus* and *Cx. quinquefasciatus* larvae by the predator larva of *Tx. splendens* at different prey-predator ratio showed in the Table 18c. From the Table it was observed that at the prey-predator ratio(30:1) the predator larva (III-instar) killed the highest no. (3.833) of prey larvae of *Ae. albopictus* (II-instar). In the same ratio (30:1) the predator larva(III-instar) killed the highest no. (4.833) of prey larvae of *Ae. albopictus* (III-instar). In case of *Cx. quinquefasciatus* the highest no. (16.17) of prey larvae(II-instar) was killed at the ratio of 20:1. In the same ratio the highest no. (15.83) of prey larvae(III-instar) was killed by the predator larva( 4th instar). Lounibos, 1979 found similar observation of *Tx. brevipalpis* in Kenya that the killing behavior was greatly reduced in lower level of prey density (12:1 p:P).

Developmental period of *Tx. splendens* fed with different prey larvae (*Ae. albopictus* and *Cx. quinquefasciatus*) was presented in Table 19 & Table 19a. From the table it was found that the duration of *Tx. splendens* larvae for development were 115.125 hrs and 114.792 hrs when the larvae fed with II & III-instar of *Ae. albopictus*. Similar observation was that the development period of *Tx. splendens* were 97.00 hrs and 101.042 hrs fed with II & III- instar of *Cx. quinquefasciatus*. In both case the prey-predator ratio was low such as 10:1. But in high prey-predator ratio i.e. 30:1 the development period of *Tx.splendens* was lower than in other prey-predator ratios. It was also observed that high density of prey larvae decreased the developmental period of *Tx. splendens*. The data were also calculated and from the calculation it was found the relation in between prey-predator ratios and the the developmental period of *Tx. splendens* was very significant at 0.01 level.

Instar wise duration of *Tx. splendens* for development fed with different prey instar was also observed. The observation placed in Table 19b. From the table it was found that developmental period of 4th instar (*Tx. splendens*) was very high among the all instars. The developmental period of 4th instar (*Tx. splendens*) were 189.600 hrs, 186.467 hrs, and 184.900 hrs, 187.500 hrs when the larvae fed with II & III-instar of *Ae. albopictus* and II & III-instar of *Cx. quinquefasciatus* respectively.Inversely the developmental periods of 1st instar (*Tx. splendens*) were low against the 4th instar of predator.

Pupal development of *Tx. splendens* depened on the quantity of food in the container which larvae fed with different prey larvae (*Ae. albopictus* and *Cx. quinquefasciatus*). From the statistically analysed by RCBD (One Factor Randomized Complete Block Design) and developmental period of pupae was presented in Table 20 & Table 20a. The result, from the calculation it was found the relation in between prey-predator ratios and the pupal developmental period of *Tx. splendens* was highly significant at 0.01 level i.e. the comsumption rate in ratio (30:1) was higher (Table 17a) and pupal developmental period was found lower.

During the experiment it was found that the quantity of food influenced the life characteristics of *Tx. splendens*, specially its immature development. The collected data showed high density of food always retarded immature development period. With decreased the immature development when high density of food the pupae of *Tx. splendens* grew rapidly and thus way a huge no. of adults were produced.By this way it may control other mosquito by ptadation and this will be treated as biological control.

Table 7 showed the life cycle of *Tx. splendens*. The average egg incubation period was 2.0 ± 0.11 days . Pupal development of *Tx. splendens* depened on the quantity of food in the container. It was observed the prey availability and high density always decreased the pupal development period. In the low density of food (15:1) the larvae of predator attained in adult within 230.80 hrs and 118.8 hrs fed with II & III- instar of *Ae. albopictus* respectively.

But in the high density of food (30:1) the pupal development period was 98.83 hs and 98.33 hrs fed with same prey larvae.With the change of food item (II & III-instar of *Cx. quinquefasciatus*) the pupal development period was 124.2 hrs and 120.5 hrs in the prey-predator ratio (10:1). But in the high density of food (30:1) the pupal development period was 98.33 hrs and 101.2 hrs. During the experiment period all collected records showed there was a negative relation between pupal development period and density of food. Amalraj and Das (2005) stated there was a significant negative correlation between immature duration and consumption rate, the *Tx. splendens* larvae in a shorter period when the prey is abundant in India. Paine mentioned that larval stage spent minimum 16 days (food abundant) and maximum 134 days ( food scarce). Furumizo and Rudnick (1978) found the average consumption of prey(*Ae.aegypti* & *Ae. albopictus*) was 150 larvae at the prey-predator ratio of 10:1 and 15:1 in *Tx. splendens*. Geetha Bai *et al.*,(1981) observed that the average number of prey killed (301) by a 4th instar of *Tx. splendens* was more than double the average number of prey consumed duting its enter larval life (144) but they did'nt mention the natural mortality of prey in the experiment. Elias *et al.*(1988) found the number of average killing and consumption (prey-*Ae.aegypti*) in *Tx. splendens* were 168.14±44.18 and 256.64±20.88 and that was high than present study.

**Table 17** ANOVA Consumption of Tx. splendens larvae at different instar within different prey ratio

| Source | Degrees of Freedom(d.f) | Mean Square | | | |
|---|---|---|---|---|---|
| | | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | *Cx.quinquefasciatus* II-instar | *Cx.quinquefasciatus* III-instar |
| Replication | 5 | 46.960 | 73.068 | 99.940 | 9.913 |
| Factor A | 4 | 2167.328** | 3018.196** | 6331.950** | 6292.938** |
| Factor B | 3 | 68398.011** | 64523.831** | 60720.567** | 69858.722** |
| AB | 12 | 268.476** | 375.240** | 1294.400** | 1570.438** |
| Error | 95 | 40.318 | 105.184 | 64.351 | 27.857 |

** Level of significant = 0.01

**Table 17a** Consumption of *Tx. splendens* larvae at different instar within different prey ratio

| Replication | Prey : Predator ratio | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | *Cx.quinquefasciatus* II-instar | *Cx.quinquefasciatus* III-instar |
|---|---|---|---|---|---|
| 6 | 10:1 | 37.625 $^{e}$ | 33.417 $^{d}$ | 33.625 $^{d}$ | 33.750 $^{d}$ |
| | 15:1 | 41.167 $^{d}$ | 41.417 $^{c}$ | 42.625 $^{c}$ | 41.667 $^{c}$ |
| | 20:1 | 49.917 $^{c}$ | 50.875 $^{b}$ | 56.000 $^{b}$ | 52.500 $^{b}$ |
| | 25:1 | 56.167 $^{b}$ | 56.792 $^{ab}$ | 67.375 $^{a}$ | 69.125 $^{a}$ |
| | 30:1 | 59.875 $^{a}$ | 60.792 $^{a}$ | 72.125 $^{a}$ | 70.042 $^{a}$ |
| Level of significance | | 0.01 | 0.01 | 0.01 | 0.01 |
| LSD | | 1.577 | 7.782 | 6.087 | 4.005 |

**Table 17b** Instar wise consumption of *Tx. splendens* at different prey instars

| Replication | Instar of predator larva | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | Cx.quinquefasciatus II-instar | Cx.quinquefasciatus III-instar |
|---|---|---|---|---|---|
| 6 | 1$^{st}$ instar | 19.133 $^{d}$ | 18.933 $^{c}$ | 27.033 $^{c}$ | 19.367 $^{d}$ |
| | 2$^{nd}$ instar | 22.933 $^{c}$ | 23.667 $^{c}$ | 25.033 $^{c}$ | 25.600 $^{c}$ |
| | 3$^{rd}$ instar | 33.767 $^{b}$ | 34.500 $^{b}$ | 44.833 $^{b}$ | 44.733 $^{b}$ |
| | 4$^{th}$ instar | 119.967 $^{a}$ | 117.533 $^{a}$ | 120.500 $^{a}$ | 123.967 $^{a}$ |
| Level of significance | | 0.01 | 0.01 | 0.01 | 0.01 |
| LSD | | 1.410 | 6.961 | 5.444 | 3.582 |

**Table 17c** Consumption of *Tx. splendens* larvae at different instar within different prey ratio

| Replication | Different prey ratio | Instar of predator larva | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | *Cx.quinquefasciatus* II-instar | *Cx.quinquefasciatus* III-instar |
|---|---|---|---|---|---|---|
| 6 | 10:1 | 1st instar | 13.83 $^{lm}$ | 13.00 $^{g}$ | 16.00 $^{ij}$ | 14.00 $^{j}$ |
| | | 2nd instar | 11.67 $^{m}$ | 12.33 $^{g}$ | 14.17 $^{j}$ | 14.83 $^{ij}$ |
| | | 3rd instar | 16.00 $^{kl}$ | 16.67 $^{fg}$ | 23.83 $^{ghij}$ | 23.33 $^{ghi}$ |
| | | 4th instar | 109.0 $^{c}$ | 91.67 $^{b}$ | 80.50 $^{d}$ | 82.83 $^{d}$ |
| | 15:1 | 1st instar | 17.67 $^{jk}$ | 17.50 $^{fg}$ | 21.83 $^{hij}$ | 18.83 $^{ij}$ |
| | | 2nd instar | 17.50 $^{jk}$ | 17.67 $^{fg}$ | 20.50 $^{hij}$ | 21.17 $^{hij}$ |
| | | 3rd instar | 23.33 $^{h}$ | 23.33 $^{efg}$ | 32.17 $^{fgh}$ | 31.67 $^{g}$ |
| | | 4th instar | 106.2 $^{c}$ | 107.20 $^{b}$ | 96.00 $^{c}$ | 95.00 $^{c}$ |
| | 20:1 | 1st instar | 21.33 $^{hi}$ | 21.67 $^{efg}$ | 36.83 $^{fg}$ | 20.83 $^{hij}$ |
| | | 2nd instar | 18.33 $^{ijk}$ | 20.00 $^{efg}$ | 31.83 $^{fgh}$ | 31.00 $^{g}$ |
| | | 3rd instar | 29.50 $^{g}$ | 29.67 $^{efg}$ | 44.83 $^{f}$ | 44.17 $^{f}$ |
| | | 4th instar | 130.5 $^{a}$ | 132.2 $^{a}$ | 110.5 $^{b}$ | 114.0 $^{b}$ |
| | 25:1 | 1st instar | 19.50 $^{ij}$ | 19.33 $^{efg}$ | 28.67 $^{ghi}$ | 21.17 $^{hij}$ |
| | | 2nd instar | 31.17 $^{g}$ | 31.83 $^{def}$ | 27.33 $^{ghij}$ | 29.17 $^{gh}$ |
| | | 3rd instar | 46.83 $^{e}$ | 47.67 $^{cd}$ | 61.50 $^{e}$ | 62.33 $^{e}$ |
| | | 4th instar | 127.2 $^{b}$ | 128.3 $^{a}$ | 152.0 $^{a}$ | 163.8 $^{a}$ |
| | 30:1 | 1st instar | 23.33 $^{h}$ | 23.17 $^{efg}$ | 31.83 $^{fgh}$ | 22.00 $^{hij}$ |
| | | 2nd instar | 36.00 $^{f}$ | 36.50 $^{de}$ | 31.33 $^{fgh}$ | 31.83 $^{g}$ |
| | | 3rd instar | 53.17 $^{d}$ | 55.17 $^{c}$ | 61.8 $^{e}$ | 62.17 $^{e}$ |
| | | 4th instar | 127.00 $^{b}$ | 128.3 $^{a}$ | 163.5 $^{a}$ | 164.2 $^{a}$ |
| **LSD** | | - | **3.154** | **15.56** | **12.17** | **8.010** |
| Level of significant | | - | 0.01 | 0.01 | 0.01 | 0.01 |

**Table 18** ANOVA Killing of different instar by *Tx. splendens* larvae within different prey ratio

| Source | Degrees of Freedom (d.f) | Mean Square | | | |
|---|---|---|---|---|---|
| | | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | *Cx.quinquefasciatus* II-instar | *Cx.quinquefasciatus* III-instar |
| Replication | 5 | 0.308 | 0.220 | 5.993 | 2.240 |
| Factor A | 4 | 12.154** | 22.096** | 99.967** | 105.250** |
| Factor B | 3 | 2.919** | 7.756** | 218.900** | 215.678** |
| AB | 12 | 2.426** | 4.485** | 89.650** | 74.456** |
| Error | 95 | 0.979 | 1.224 | 9.074 | 3.472 |

** Level of significant = 0.01

**Table 18a** Killing of different instar by *Tx. splendens* larvae within different prey ratio

| Replication | Prey : Predator ratio | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | *Cx.quinquefasciatus* II-instar | *Cx.quinquefasciatus* III-instar |
|---|---|---|---|---|---|
| 6 | 10:1 | 1.333 $^{b}$ | 1.375 $^{b}$ | 1.708 $^{c}$ | 2.125 $^{d}$ |
| | 15:1 | 1.292 $^{b}$ | 1.417 $^{b}$ | 2.792 $^{bc}$ | 3.042 $^{cd}$ |
| | 20:1 | 1.875 $^{b}$ | 1.500 $^{b}$ | 5.000 $^{ab}$ | 4.875 $^{b}$ |
| | 25:1 | 2.750 $^{a}$ | 2.708 $^{a}$ | 5.042 $^{ab}$ | 3.667 $^{bc}$ |
| | 30:1 | 2.708 $^{a}$ | 3.500 $^{a}$ | 6.875 $^{a}$ | 7.542 $^{a}$ |
| Level of significance | | 0.01 | 0.01 | 0.01 | 0.01 |
| LSD | | 0.750 | 0.839 | 2.286 | 1.414 |

**Table 18b** Instar wise killing of different prey by *Tx. splendens* instars

| Replication | Predator larval instar | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | Cx.quinquefasciatus II-instar | Cx.quinquefasciatus III-instar |
|---|---|---|---|---|---|
| 6 | 1$^{st}$ instar | 2.300 $^{a}$ | 2.167 $^{ab}$ | 4.567 $^{b}$ | 2.733 $^{b}$ |
| | 2$^{nd}$ instar | 1.833 $^{a}$ | 2.000 $^{ab}$ | 2.733 $^{bc}$ | 2.933 $^{b}$ |
| | 3$^{rd}$ instar | 2.200 $^{a}$ | 2.733 $^{a}$ | 1.867 $^{c}$ | 3.067 $^{b}$ |
| | 4$^{th}$ instar | 1.633 $^{a}$ | 1.500 $^{b}$ | 7.967 $^{a}$ | 8.267 $^{a}$ |
| Level of significance | | 0.01 | 0.01 | 0.01 | 0.01 |
| LSD | | 0.671 | 0.750 | 2.044 | 1.265 |

**Table 18c** Killing of different instar by *Tx. splendens* larvae within different prey ratio

| Replication | Different prey ratio | Instar of predator larva | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | *Cx.quinquefasciatus* II-instar | *Cx.quinquefasciatus* III-instar |
|---|---|---|---|---|---|---|
| 6 | 10:1 | 1st instar | 1.167 $^{c}$ | 1.167 $^{d}$ | 1.000 $^{c}$ | 1.000 $^{e}$ |
| | | 2nd instar | 1.333 $^{c}$ | 1.333 $^{d}$ | 1.000 $^{c}$ | 1.000 $^{e}$ |
| | | 3rd instar | 1.000 $^{c}$ | 1.167 $^{d}$ | 1.167 $^{c}$ | 1.167 $^{e}$ |
| | | 4th instar | 1.833 $^{bc}$ | 1.833 $^{cd}$ | 3.667 $^{bc}$ | 5.333 $^{cd}$ |
| | 15:1 | 1st instar | 1.667 $^{bc}$ | 1.333 $^{d}$ | 1.000 $^{c}$ | 1.333 $^{e}$ |
| | | 2nd instar | 1.167 $^{c}$ | 1.500 $^{d}$ | 1.167 $^{c}$ | 1.167 $^{e}$ |
| | | 3rd instar | 1.167 $^{c}$ | 1.500 $^{d}$ | 1.333 $^{c}$ | 1.333 $^{e}$ |
| | | 4th instar | 1.167 $^{c}$ | 1.333 $^{d}$ | 7.667 $^{b}$ | 8.333 $^{b}$ |
| | 20:1 | 1st instar | 2.00 $^{bc}$ | 1.833 $^{cd}$ | 1.667 $^{c}$ | 1.000 $^{e}$ |
| | | 2nd instar | 1.667 $^{bc}$ | 1.667 $^{cd}$ | 1.167 $^{c}$ | 1.500 $^{e}$ |
| | | 3rd instar | 1.667 $^{bc}$ | 1.333 $^{d}$ | 1.000 $^{c}$ | 1.167 $^{e}$ |
| | | 4th instar | 2.167 $^{abc}$ | 1.167 $^{d}$ | 16.17 $^{a}$ | 15.83 $^{a}$ |
| | 25:1 | 1st instar | 3.333 $^{ab}$ | 3.000 $^{bcd}$ | 5.500 $^{bc}$ | 1.167 $^{e}$ |
| | | 2nd instar | 2.667 $^{abc}$ | 1.667 $^{cd}$ | 4.500 $^{bc}$ | 2.500 $^{de}$ |
| | | 3rd instar | 3.333 $^{ab}$ | 4.833 $^{a}$ | 1.833 $^{c}$ | 3.000 $^{de}$ |
| | | 4th instar | 1.667 $^{bc}$ | 1.333 $^{d}$ | 8.333 $^{b}$ | 8.000 $^{bc}$ |
| | 30:1 | 1st instar | 3.333 $^{ab}$ | 3.500 $^{abc}$ | 13.67 $^{a}$ | 9.167 $^{b}$ |
| | | 2nd instar | 2.333 $^{abc}$ | 3.833 $^{ab}$ | 5.833 $^{bc}$ | 8.500 $^{b}$ |
| | | 3rd instar | 3.833 $^{a}$ | 4.833 $^{a}$ | 4.000 $^{bc}$ | 8.667 $^{b}$ |
| | | 4th instar | 1.333 $^{c}$ | 1.833 $^{cd}$ | 4.000 $^{bc}$ | 3.833 $^{de}$ |
| Level of significant | | - | 0.01 | 0.01 | 0.01 | 0.01 |
| LSD | | - | 1.502 | 1.679 | 4.572 | 2.828 |

**Table 19 A**NOVA Developmental period (hrs) of *Tx. splendens* fed with prey larvae

| Source | Degrees of Freedom (d.f) | Mean Square | | | |
|---|---|---|---|---|---|
| | | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | *Cx.quinquefasciatus* II-instar | *Cx.quinquefasciatus* III-instar |
| Replication | 5 | 26.268 | 83.795 | 139.853 | 75.553 |
| Factor A | 4 | 4980.125** | 5176.737** | 1514.488** | 2021.596** |
| Factor B | 3 | 135406.208** | 129827.497** | 126295.089** | 132939.756** |
| AB | 12 | 4171.458** | 4183.088** | 1217.360** | 1707.874** |
| Error | 95 | 26.640 | 85.704 | 171.169 | 55.259 |

** Level of significant = 0.01

**Table 19a** Developmental period (hrs) of *Tx. splendens* fed with prey larvae within different ratio

| Replication | Prey : Predator ratio | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | *Cx.quinquefasciatus* II-instar | *Cx.quinquefasciatus* III-instar |
|---|---|---|---|---|---|
| 6 | 10:1 | 115.125 [a] | 114.792 [a] | 97.000 [a] | 101.042 [a] |
| | 15:1 | 88.417 [b] | 85.292 [b] | 96.458 [a] | 93.208 [b] |
| | 20:1 | 85.917 [bc] | 85.042 [b] | 89.875 [ab] | 90.083 [b] |
| | 25:1 | 84.000 [c] | 83.500 [bc] | 83.333 [bc] | 83.083 [c] |
| | 30:1 | 78.000 [d] | 77.250 [c] | 79.000 [c] | 77.250 [d] |
| Level of significance | | 0.01 | 0.01 | 0.01 | 0.01 |
| LSD | | 3.916 | 7.025 | 9.928 | 5.641 |

**Table 19b** Instar wise developmental period (hrs) of *Tx. splendens* fed with prey larvae (*Ae. albopictus* & *Cx.quinquefasciatus*)

| Replication | Instar of predator larva | Fed with *Ae. albopictus* II-instar(hrs) | Fed with *Ae. albopictus* III-instar(hrs) | Fed with *Cx.quinquefasciatus* II-instar(hrs) | Fed with *Cx.quinquefasciatus* III-instar(hrs) |
|---|---|---|---|---|---|
| 6 | 1st instar | 48.200 [c] | 48.000 [c] | 47.533 [c] | 47.333 [c] |
| | 2nd instar | 50.067 [c] | 50.000 [c] | 50.633 [c] | 49.833 [c] |
| | 3rd instar | 73.300 [b] | 72.233 [b] | 73.467 [b] | 71.067 [b] |
| | 4th instar | 189.600 [a] | 186.467 [a] | 184.900 [a] | 187.500 [a] |
| Level of significance | | 0.01 | 0.01 | 0.01 | 0.01 |
| LSD | | 3.503 | 6.283 | 8.879 | 5.045 |

**Table 19c** Instar wise developmental period (hrs) of *Tx. splendens* fed with prey larvae (*Ae. albopictus* & *Cx.quinquefasciatus*) within different prey ratio

| Replication | Different prey ratio | Instar of predator larva | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | Cx.*quinquefasciatus* II-instar | Cx.*quinquefasciatus* III-instar |
|---|---|---|---|---|---|---|
| 6 | 10:1 | 1st instar | 49.83 f | 49.83 e | 48.17 f | 48.50 h |
| | | 2nd instar | 52.67 f | 52.67 e | 50.00 f | 51.67 gh |
| | | 3rd instar | 74.50 e | 75.00 d | 74.67 e | 74.00 f |
| | | 4th instar | 283.5 a | 281.7 a | 215.2 a | 230.0 a |
| | 15:1 | 1st instar | 48.83 f | 48.67 e | 48.33 f | 48.17 h |
| | | 2nd instar | 51.83 f | 50.50 e | 53.83 ef | 53.33 gh |
| | | 3rd instar | 74.17 e | 73.17 f | 74.50 e | 63.00 fg |
| | | 4th instar | 178.8 b | 168.8 b | 209.20 a | 208.3 b |
| | 20:1 | 1st instar | 49.50 f | 49.17 e | 48.00 f | 47.67 h |
| | | 2nd instar | 52.33 f | 51.67 e | 50.50 f | 50.17 h |
| | | 3rd instar | 74.67 e | 73.83 d | 72.50 e | 72.83 f |
| | | 4th instar | 167.2 c | 165.5 b | 188.5 b | 189.7 c |
| | 25:1 | 1st instar | 46.50 f | 46.50 e | 46.17 f | 45.83 h |
| | | 2nd instar | 46.67 f | 49.67 e | 48.50 f | 47.67 h |
| | | 3rd instar | 70.83 e | 67.00 d | 72.50 e | 72.83 f |
| | | 4th instar | 172.0 bc | 170.8 b | 166.2 c | 166.0 d |
| | 30:1 | 1st instar | 46.33 f | 45.83 e | 47.00 f | 46.50 h |
| | | 2nd instar | 46.83 f | 45.50 e | 50.33 f | 46.33 h |
| | | 3rd instar | 72.33 e | 72.17 d | 73.17 e | 72.67 f |
| | | 4th instar | 146.5 d | 145.5 c | 145.5 d | 143.5 e |
| Level of significant | | - | 0.01 | 0.01 | 0.01 | 0.01 |
| LSD | | - | 7.833 | 14.05 | 19.86 | 11.28 |

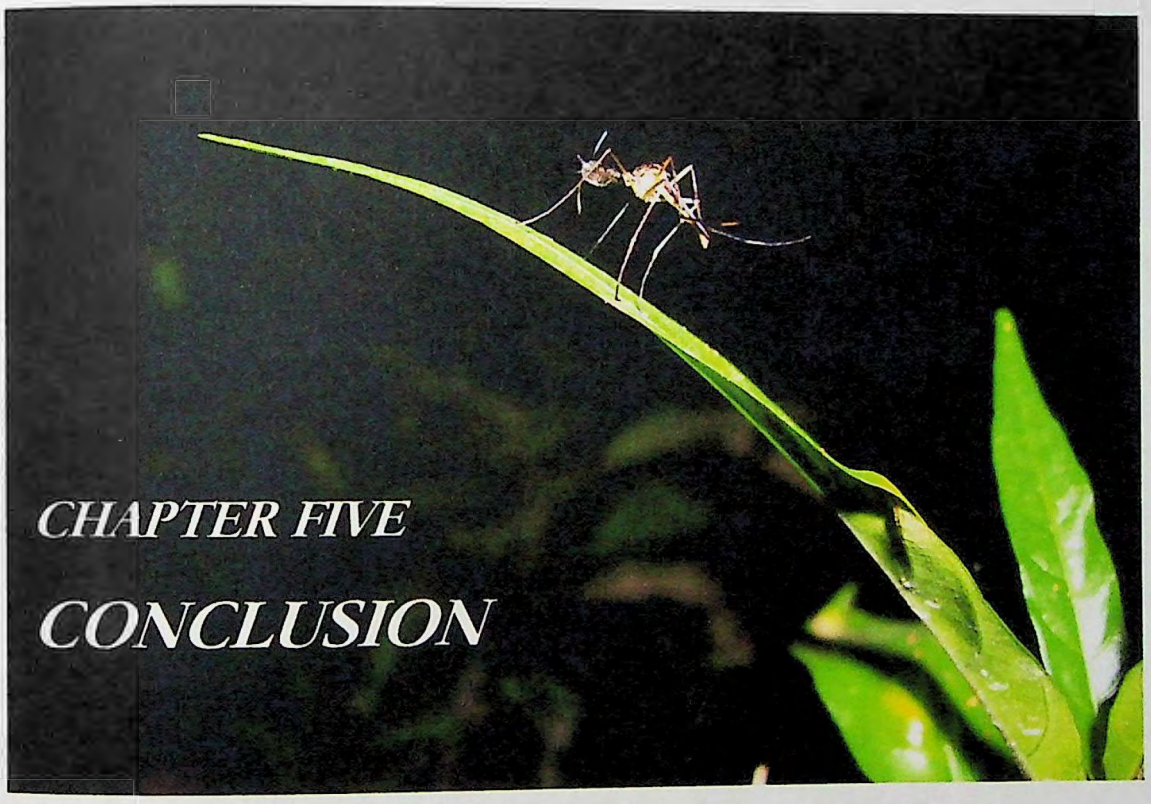
**Table 20** ANOVA Developmental duration(hrs) of Pupal of Tx. splendens at different different prey ratio

| Source | Degrees of Freedom (d.f) | Mean Square | | | |
|---|---|---|---|---|---|
| | | Pupal duration | | | |
| | | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | *Cx.quinquefasciatus* II-instar | *Cx.quinquefasciatus* III-instar |
| Replication | 5 | 35.393 | 31.493 | 116.940 | 22.293 |
| Factor A | 4 | 834.200** | 635.283** | 533.450** | 629.450** |
| Error | 20 | 14.560 | 11.443 | 30.590 | 8.310 |

** Level of significant = 0.01

**Table 20a** Developmental duration(hrs) of Pupal of Tx. splendens at different different prey ratio

| Replication | Prey : Predator ratio | Pupal duration | | | |
|---|---|---|---|---|---|
| | | *Ae. albopictus* II-instar | *Ae. albopictus* III-instar | *Cx.quinquefasciatus* II-instar | *Cx.quinquefasciatus* III-instar |
| 6 | 10:1 | $126.5^{a}$ | $121.3^{a}$ | $124.2^{a}$ | $120.5^{a}$ |
| | 15:1 | $230.8^{a}$ | $118.8^{a}$ | $119.5^{ab}$ | $119.2^{a}$ |
| | 20:1 | $114.5^{b}$ | $109.8^{b}$ | $112.7^{bc}$ | $110.7^{b}$ |
| | 25:1 | $102.5^{c}$ | $101.0^{c}$ | $106.0^{cd}$ | $101.0^{c}$ |
| | 30:1 | $98.83^{c}$ | $98.33^{c}$ | $101.2^{d}$ | $98.00^{c}$ |
| Level of significance | | 0.01 | 0.01 | 0.01 | 0.01 |
| LSD | | 6.268 | 5.557 | 9.086 | 4.736 |



# CHAPTER FIVE

# CONCLUSION

# CHAPTER FIVE
## Conclusion

The biology of *Tx. splendens* has been observed in the laboratory. Defferent types of seasonal fruits and vegetables slices were used as food for adults with addition of artificial food.

Mating were usually occurred in day light i.e diurnal mating but in extended photoperiod i.e nocturnal mating was happened. Mating periodicity, pre-ovipoition period, ovipoition and fecundity were also observed. For mating the male-female ratio was 1:1, 2:1, 3:1, 6:1 and 9:1. Factorial analysis of data indicated that mating was occurred more at predusk (4pm to 6pm) than at morning. Pre-ovipoition period was highly significant in between ratio of males: female (1:1, 2:1, 3:1, 6:1 and 9:1) at 0.01 level.Pre-ovipoition period decreased with the increasing of males. Female also produced higher number of eggs(166.66) in ratio of male- female, 9:1 it was also highly significant at 0.01 level. But ovipoition preiod and male- female ratio was found non-significant.

A speciall type of ovitrap was used for egg laying in the laboratory. The ovitrap was unique and composed of artificial and natural container. Natural container was a bamboo stump which was placed on the middle of plastic pot (artificial container) that stopped the air drafts created by females during oviposition flight, which also stopped egg from blowing out. The bamboo stump also acted as resting substratum for female during oviposition flight. Bamboo–leaf infusion water was used for rearing the mosquitoes (Predator and Prey) in the laboratory it was high-quality for rearing the mosquitoes.

*Tx. splendens* preferred to lay eggs on second quarter of the day (12.00 pm to 18.00pm).They did not lay any eggs on fourth quarter of the day (0.00am to 6am). But at extended photoperiod by artificial light *Tx. splendens* was found to oviposit at night between 0.00am to 6am (third quarter of the day).

As a ovitrap black plastic pot with bamboo stump was preferred by *Tx. splendens* for egg laying. Statiscally it was also highly significant at 0.01 level.

On the basis of egg width four categories of egg i.e W7,W8,W9 and W10 were found during the experimental period. The highest number of egg 474 out of 702 were found in group of W8.

There were four larval stages of *Tx. splendens* after egg encubation. The average egg encubation was 2.0±0.11 days.The developmental period of larvae was, 1st instar=1.8±0.14, 2nd instar=2.34±0.17, 3rd instar=3.15±0.13 and 4th instar=7.7±0.33 days.The pupa attained in adult with the average of 4.31±0.17 days. *Tx. splendens* completed it's life cycle within the average of 21.47±0.52 days. Two types of prey larvae i.e nature based prey and colony based prey were used for mass rearing of *Tx. splendens* in laboratoty.

Resting adult of *Tx. splendens* were collected from two villages (Gilagasa and Gorgogia) of district Sherpur. Adults were resting on the following trees, i.e Bamboo, Black plum, Coconut ,Hog-plum, Guava, Jackfruit, Jigar, Lichi, Mango, Pithraj, Pomelo, Rain tree, Tamarind, and Shimul during collection. The adults were found from 0 to 2.52 meter height of the trees. Total height (0 to 2.52m) was fragmented into three parts called H1, H2 & H3. The seasonal abundance of the adult population of *Tx. splendens* in different heightwise was aplenty found at the height 85-168cm (H2) of all trees and monthwise seasonal prevalence of males and females were highest in the month of March during study period.

To determine the efficacy of *Tx. splendens* to control other mosquitoes in the laboratory, it was observed that the mosquito larvae of *Tx. splendens* were effectively antagonist to vector mosquitoes (*Ae. albopictus* and *Cx. quinquefasciatus*). Comsumption rate of the predator increased with the increaseing of food availability and this relationship was highly significant. The predator larva consumed prey larvae at the following ratios i.e 10:1, 15:1, 20:1, 25:1 and 30:1. The predator larva comsumed highest no. of

II and III-instar of *Ae. albopictus* and *Cx. quinquefasciatus* at the ratio of 30:1. That was the highest rate than others comsumption. Statistically it was also significant at 0.01 level. In the p:P ratio (10:1) the comsumption rate of prey larvae was lower.During experiment it was observed the predator larva killed prey larvae but not comsumed them. In the prey-predator ratio, 30:1 the predator larva killed highest number of prey larvae.Developmental period of predator larvae was low in prey-predator ratio 30:1 and it was high in ratio 10:1. Pupal development of *Tx. splendens* depened on the quantity of food in the container. It was observed the prey availability and high density of prey always decreased the pupal development period.

Many attempts have been made to shed light on different aspects of biology of *Toxorhynchites* spp. esespecially on *Tx. splendens* with a desire to use these species for the contral of other mosquitoe. Unfortunatily no standard protocol has not been developed which could be followed for the control of other mosquitoes.

# CHAPTER SIX

# REFERENCES
# APPENDIX

# CHAPTER SIX

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# Appendix

**Appendix Table 1** Dengue cases and Deaths in Bangladesh from 2000 to 2008

| Sl no. | Year | Bangladesh | | Dhaka city | |
|---|---|---|---|---|---|
| | | Cases | Death | Cases | Death |
| 1 | 2000 | 5551 | 93 | 3964 | 51 |
| 2 | 2001 | 2430 | 88 | 2344 | 44 |
| 3 | 2002 | 6102 | 58 | 5851 | 49 |
| 4 | 2003 | 486 | 10 | 450 | 9 |
| 5 | 2004 | 3934 | 13 | 3874 | 13 |
| 6 | 2005 | 1048 | 4 | 1033 | 4 |
| 7 | 2006 | 2200 | 11 | 2144 | 11 |
| 8 | 2007 | 466 | 0 | 465 | 0 |
| 9 | 2008 | 1153 | 0 | 1151 | 0 |
| Total | | 23370 (2596.66±711.67) | 277 (30.77±12.71) | 21276 (2364±618.69) | 181 (20.11±7.15) |

Source : Directorate General of Health Services,Dhaka

**Appendix Table 2** Ad ult longevity of Male nd Female

| Replication | Male | Female |
|---|---|---|
| | Age | Age |
| 1 | 3 | 5 |
| 2 | 7 | 7 |
| 3 | 27 | 7 |
| 4 | 33 | 11 |
| 5 | 38 | 13 |
| 6 | 44 | 13 |
| 7 | 62 | 14 |
| 8 | 68 | 16 |
| 9 | 73 | 29 |
| 10 | 78 | 30 |
| 11 | 78 | 45 |
| 12 | 81 | 46 |
| 13 | 84 | 51 |
| 14 | 85 | 61 |
| 15 | 87 | 64 |
| 16 | 87 | 68 |
| 17 | 87 | 72 |
| 18 | 88 | 75 |
| 19 | 88 | 75 |
| 20 | 88 | 79 |
| 21 | 90 | 80 |
| 22 | 91 | 85 |
| 23 | 92 | 88 |
| 24 | 92 | 91 |
| 25 | 92 | 92 |
| 26 | 93 | 93 |
| 27 | 93 | 94 |
| 28 | 96 | 94 |
| 29 | 97 | 96 |
| 30 | 100 | 99 |
| 31 | 103 | 103 |
| 32 | 105 | 109 |
| 33 | 105 | 109 |
| 34 | 107 | 115 |
| 35 | 107 | 117 |
| 36 | 107 | 117 |
| 37 | 107 | 118 |
| 38 | 111 | 121 |
| 39 | 112 | 123 |
| 40 | 113 | 123 |
| 41 | 113 | 124 |
| 42 | 114 | 125 |
| 43 | 114 | 128 |
| 44 | 117 | 129 |
| 45 | 117 | 129 |
| 46 | 118 | 131 |
| 47 | 121 | 133 |
| 48 | 123 | 133 |
| 49 | 125 | 135 |
| 50 | 131 | 138 |
| 51 | 145 | 142 |
| 52 | 149 | 149 |
| 53 | 149 | 149 |
| 54 | 150 | 151 |
| Mean±SE (range) | 94.16±4.32 (3-150) | 87.85±6.00 (7-151) |