BIO-ECOLOGY OF ANGOUMOIS GRAIN MOTH AND ITS EGG PARASITOID *TRICHOGRAMMA EVANESCENS* AND PARASITIZATION PERFORMANCE OF THE PARASITOID

MST. MARIAM KHATUN

DEPARTMENT OF ENTOMOLOGY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

DHAKA 1207

DECEMBER, 2010

BIO-ECOLOGY OF ANGOUMOIS GRAIN MOTH AND ITS EGG PARASITOID *TRICHOGRAMMA EVANESCENS* AND PARASITIZATION PERFORMANCE OF THE PARASITOID

BY

MST. MARIAM KHATUN

REGISTRATION NUNBER: 08-03262

A thesis
Submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
IN
ENTOMOLOGY

SEMESTER: JULY-DECEMBER, 2010

Approved by:

(Dr. Md. Razzab Ali)	**(Dr. Md. Mizanur Rahman)**
Supervisor	**Co-Supervisor**
&	**&**
Professor	**Professor**
Department of Entomology	**Department of Entomology**
SAU, Dhaka	**SAU, Dhaka**

(Professor Dr. Md. Razzab Ali)

Chairman
Examination Committee

ACKNOWLEDGEMENTS

The author first wants to articulate her enormous wisdom of kindness to the Almighty Allah for His never ending blessing, protection, regulation, perception and assent to successfully complete the Master of Science (MS).

The author like to express her deepest sense of gratitude and sincere appreciation to her honorable supervisor Dr. Md. Razzab Ali, Professor, Department of Entomology, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh, for his continued supervision, support, encouragement, practical criticism and invaluable suggestions throughout the study period and gratuitous labor in conducting and successfully completing the research work and in the preparation of the manuscript preparation.

The author also expresses her gratefulness and best regards to respected Co-Supervisor, Dr. Md. Mizanur Rahman, Professor, Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka for his pedagogic guidance, helpful comments and constant inspiration throughout the research work and preparation of the thesis.

The author also expresses heartfelt thanks to the Chairman and all the teachers of the Department of Entomology, SAU, for their valuable suggestions, instructions, cordial help and encouragement during the period of the study.

The author expresses her sincere appreciation to her brother, sisters, relatives, well wishers and friends for their inspiration, help and encouragement throughout the study period.

Dhaka Bangladesh
December, 2010 The Author

BIO-ECOLOGY OF ANGOUMOIS GRAIN MOTH AND ITS EGG PARASITOID *TRICHOGRAMMA EVANESCENS* AND PARASITIZATION PERFORMANCE OF THE PARASITOID

BY

MST. MARIAM KHATUN

ABSTRACT

The study was conducted in the laboratory of the Department of Entomology at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from July, 2010 to January, 2011 to study on the growth and development of the host Angouimos grain moth, *Sitotroga cerealella* and its egg parasitoid *Trichogramma evanescens* as well as to find out the performance of *T. evanescens* egg parasitoid in parasitizing the eggs of *S. cerealella* and the effect of weather factors on the biology of both host and parasitoid as well as on parasitization. The experiment was laid out in Complete Randomized Design (CRD) with five replications. The incubation, larval, pupal period, egg to adult period, adult longevity and total life span of Angouimos grain moth, *S. cerealella* were ranged from 6.67 to 7.33 days, 15.67 to 22.67 days, 4.33 to 6.00 days, 26.67 to 36.00 days, 6.0 to 7.33 days and 32.67 to 44.0 days, respectively during the study period from August 2010 to January 2011. Climatic factors such temperature and relative humidity significantly influenced on the growth and development of *S. cerealella* and all these parameters regarding growth and development were negatively correlated with both temperature and relative humidity, where the rapid growth and development was observed in August 2010 maintaining 30.12^{o}C temperature and 86.52% relative humidity and slow growth was observed in January 2011 maintaining 23.21^{o}C temperature and 67.20% relative humidity. The freshly laid eggs of *S. cerealella* performed highest hatching rate (87.60%) that was better than long day stored host eggs for using in rearing of *Trichogramma evanescens* egg parasitoid.

The egg to larval period, pupal period, egg to adult period, adult longevity and total life span of *T. evanescens* egg parasitoid were ranged from 2.20 to 3.80 days, 2.0 to 3.60 days, 4.20 to 7.40 days, 3.0 days and 7.20 to 10.40 days, respectively during the study period from September 2010 to January 2011. Climatic factors such temperature and relative humidity significantly influenced on the growth and development of *T. evanescens* and all these parameters were positively correlated with both temperature and relative humidity, where the rapid growth and development was observed in January 2011 maintaining 23.21^{o}C temperature and 67.20% relative humidity and slow growth was observed in January 2011 maintaining 30.12^{o}C temperature and 86.52% relative humidity. The rate of parasitization increased with the increase of the number of Trichogramma release and the maximum parasitization (91.40%) was achieved by the release of 40 Trichogramma released at pupal stage for 100 host eggs. Among different months of the experimental period, the highest parasitization rate (84.00%) was recorded in January 2011 and lowest (65.40%) was recorded in September 2010. Comparatively lower temperature (23.21^{o}C) and relative humidity (67.20%) recorded in January 2011 favored higher parasitization rate than higher temperature (29.05^{o}C) and relative humidity (84.23%) recorded in September 2010.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

DEPARTMENT OF ENTOMOLOGY

Sher-e-Bangla Agricultural University

Sher-e-Bangla Nagar, Dhaka-1207

CERTIFICATE

This is to certify that thesis entitled, **"Bio-ecology of Angoumois grain moth and its egg parasitoid *Trichogramma evanescens* and parasitization performance of the parasitoid "** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in ENTOMOLOGY,** embodies the result of a piece of bonafide research work carried out by Mst. Mariam Khatun, Registration No. 08-03262 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: December, 2010
Place: Dhaka, Bangladesh

Dr. Md. Razzab Ali
Supervisor
&
Professor
Department of Entomology
SAU, Dhaka

CHAPTER I

INTRODUCTION

Pest problem is one of the major constraints for achieving higher production in agriculture crops. About 30% of crop loses is occurred due to pests and diseases each year. The damage due to those pests is estimated to be Tk. 60,000 cores annually (Rahman, 2006). The use of pesticides in crop protection has certainly contributed for minimizing yield losses but the pesticides, which are needed to be applied carefully; only when the threshold limits of the pest population is exceeded.

Various control strategies have been adopted against insect pest of agricultural crops, one common method being the use of synthetic insecticides, which can be environmentally disruptive and can result in the accumulation of residues in the harvested produce and creating health hazards (Awasthi,1998). Till today insecticides were the major means of insect control in all crops including vegetables in Bangladesh. Such use of pesticides in vegetables and other field crops caused several problems including the development of resistance, destruction of natural enemies, insect pest resurgence, secondary pest outbreak and harmful residues in edible fruits and farm produces (Rahman, 2006; Chinniah *et al.*, 1998).

In view of the several disadvantages associated with the unscientific use of pesticides in agriculture, there is an urgent need for minimizing the use of chemical pesticides in the management of insect pests. Growing public concern over potential health hazards of synthetic pesticides and also steep increase in cost of cultivation but low profit making, farmers has led to the exploration of eco-friendly pest management tactics. Hence, search for the alternative method of insect pest control utilizing non-toxic, environment friendly and human health hazard free methods are being pursued now-a-days.

Natural enemies of insect pests, known as biological control agents, include predators, parasitoids, and pathogens. Utilization of natural enemy aims at suppressing the pest species

with least or no emphasis on the use of insecticides (Wagge, 1983). Among these natural enemies, parasitoids are one of the most important bio-control agents. They are the organism that, during its development, lives in or on the body of a host individual, eventually killing that individual and develop as a free living adult.

Among the parasitoids *Trichogramma* is one the most effective agent now a days used as biological control agent. *Trichogramma* spp. is extremely tiny wasps in the family Trichogrammatidae under the order Hymenoptera. *Trichogramma* belongs to the category of egg parasitoid of biological agents. It attacks the pest at the egg stage itself and hence damage done by larvae is avoided. *Trichogramma* spp., the most widely used bio-control agent in the world and is effective against bollworms of cotton, stem borers of sugarcane, fruit borers of fruits and vegetables. It offers a lower cost but more effective plant protection option in comparison to insecticides.(Narayanasamy, 1999)

Trichogramma species are facultatively gregarious (Rabinovich, 1971), polyphagous egg parasitoids that are often used in inundative biological control programs (Smith, 1996) against a wide range of Lepidopterous eggs (Corrigan and Laing, 1994; Doyon and Boivin, 2005) that are totally eco-friendly and non-toxic to the consumers. Many parts of the world use *Trichogramma* sp. successfully for crop production (Hasan, 1992). The egg parasitoid *Trichogramma* can achieve a level of control that is near 100% in some years or areas (Kim and Heinrichs, 1985; Kim *et al.,* 1986).

Among different species *Trichogramm evanescens* egg parasitoid is the new introduction in Bangladesh for the management of insect pest of agricultural crops. The inundative releases of egg parasitoid *Trichogramma evanescens* Westwood adults into commodity could play an important role in suppression of Lepidopterous moth populations (Brower, 1983) especially *S. cerealella* (Alam *et al.,* 2008a; El-Wakeil,2007; Hasan and WenQing, 2001), *Corcyra cephalonica* (Alam *et al.* 2008a; Alam *et al.* 2008b); *Helicoverpa armigera* (El-Wakeil, 2007);

Mediterranean flour moth, *Ephestia kuehniella* (Ayvaz *et al.,* 2008). Alam *et al.* (2008a) reported that significantly highest egg parasitism was done by *T. evanescens* (88.6%) than other species viz. *T. chilonis* (77.6%) and *T. japonica* (43.8%) when reared on host eggs *S. cerealella.*

Trichogramma are dark colored tiny wasps and the female wasp lays 20-40 eggs into the host's eggs. The entire cycle is completed within 8-12 days. The tiny adult wasps search for the host (pest) eggs in the field and lay their eggs into the eggs of the pests. The parasitized host's eggs turn uniformly black in 3-4 days. The *Trichogramma* eggs on hatching, feed the embryonic contents of host's egg, completes its development and adult comes out of the host egg by chewing a circular hole. A single *Trichogramma*, while multiplying itself, can thus destroy over 100 eggs of the pest.

To ensure the continuous supply, easy handling mass rearing technique, bio-ecology and parasitizing efficacy of parasitoid must be known first. So, the attempt is undertaken to study the bio-ecology of host insect *Sitotroga cerealella* and egg parasitoid *T. evanescens* and the performance of parasitoid to parasitize the eggs of *S. cerealella* reared on wheat grains with the following objectives-

a. To study the growth and development of the Angoumois grain moth *S.cerealella,*

b. To study the growth and development of the *T. evanescens* on the eggs of *S. cerealella*;

c. To study the effect of weather factors (temperature, relative humidity etc.) on the growth and development of *S. cerealella* and *T. evanescens* in storage;

d. To study the performance of *T. evanescens* for parasitizing the eggs of *S. cerealella*.

CHAPTER II

REVIEW OF LITERATURE

Trichogramma species are polyphagous egg parasitoids that are often used in inundative biological control programs against a wide range of Lepidopterous eggs that are totally eco-friendly and non-toxic to the consumers. Several studies in relation to different aspects of this parasitic wasp have been reported from many countries of the world. But *Trichogramm evanescens* egg parasitoid is the new introduction in Bangladesh for the management of insect pest of agricultural crops. For better understanding of the host anguimos grain moth, *S. cerealella*; mass rearing, bio-ecology and parasitization performance of *T. evanescens* egg parasitoid on its host's eggs have been made to review the available literature related to this study.

2.1 Trichogramma

Trichogramma is a potential biological control agent against Lepidopterous insect pest. It is an egg parasitoid that kills the pest before it can cause any damage to the plant. Trichogramma are among the smallest insects, having a wingspread of about 1/50th of an inch. Despite its size, this parasitic wasp is an efficient destroyer of the eggs of more than 200 species of moths and butterflies which are leaf eaters in the larval stage. Trichogramma wasps seek out eggs, but do not feed on or harm vegetation. It is a particularly effective control agent because it kills its host before a plant can be damaged.

2.2 Taxonomic Position

Phylum: Arthropoda

Class: Insecta

Order: Hymenoptera

Family: Trichogrammatidae

Genus: *Trichogramma*

Species: *Trichogramma evanescens*

2.3. Species identification

Within the genus *Trichogramma*, there are 145 described species worldwide; 30 species have been identified from North America and an estimated 20 to 30 species remain to be described. The species most commonly collected from crops and orchards are *evanescence, atopovirilia, brevicapillum, deion, exiguum, fuentesi, minutum, nubilale, platneri, pretiosum,* and *thalense* (Olkowski and Zhang 1990). *Trichogramma* are difficult to identify because they are so small and have generally uniform morphological characters. Also, certain physical characteristics such as body color and the number and length of body hairs can vary with body size, season, rearing temperature and the host on which the adult was reared. Because of these difficulties and the lack of type specimens, species names in the literature in North America prior to 1968 were used incorrectly and inconsistently and are therefore unreliable.

2.4 Biology and Life cycle

Adults are approximately 1/25 inch (0.1-1.2 mm). They often have wing hairs (setae) arranged in rows. Their body is relatively compact and the antennae are short. *Trichogramma* species are difficult to identify due to their minute size and generally uniform morphological features. *Trichogramma* spp. undergo complete metamorphosis. The adult wasp lays an egg within a

recently laid host egg, and as the wasp larva develops, it eats the host embryo, causing the egg to turn black.

Because their life cycle from egg to adult is about 7 to 10 days, these parasites have many more generations than their hosts and their populations can increase rapidly. *Trichogramma* turns the eggs of some caterpillar species black. This is the best way to detect parasitization by *Trichogramma*. *Trichogramma* wasps primarily parasitize eggs of moths and butterflies (Lepidoptera). However, certain species of *Trichogramma* also parasitize eggs of beetles (Coleoptera), flies (Diptera), true bugs (Heteroptera), other wasps (Hymenoptera), and lacewings and their relatives (Neuroptera). The adult female wasp uses chemical and visual clues to locate host egg. The chemical clues, called kairomones, are on the moth scales left near the egg by the female moth during oviposition. Some of these same chemicals are also sex pheromones of the host. Egg shape and color also may be visual clues to the wasp. Once a female finds host egg, she drills a hole through the chorion (egg shell) and inserts two to three eggs into the host egg. The internal pressure of the host egg forces a small drop of yolk out of the oviposition hole. Females feed on this yolk, which increases their longevity. Under laboratory conditions a female parasitizes from one to ten host eggs per day during her life. Large females parasitize more eggs than smaller females. Females provided honey and young host eggs to feed on live an average of 11 days, while females receiving only honey live 3 days. Host eggs in the early stages of development are more suitable for parasite development. Older eggs, especially those in which the head capsule of the larva is visible, are not usually parasitized and if they are, parasite survival is much lower. The yolk and embryo of the parasitized host egg are digested before the *Trichogramma* egg hatches. A venom injected by the female at the time of oviposition is believed to cause this predigestion of the egg's contents. Eggs hatch in about 24 hours and the parasite larvae develop very quickly. Two *Trichogramma* larvae can consume the digested contents of a young budworm egg within 10 hours of hatching (Strand 1986). Larvae develop through three instars. During the 3rd instar (3 to 4 days after the host egg was parasitized) dark

melanin granules are deposited on the inner surface of the egg chorion, causing the bollworm egg to turn black. Larvae then transform to the inactive pupal stage. After about 4-5 days, the adult wasps emerge from the pupae and escape the bollworm egg by chewing a circular hole in the egg shell. The black layer inside the chorion and the exit hole are evidence of parasitism by *Trichogramma.*

Plate 1. Female *Trichogramma* ovipositing on a moth's egg

(www.Ibamspray.com)

The life cycle from egg to adult requires about 9 days, but varies from 8 days when mid-summer temperatures are high (90°F) to as many as 17 days at 60° F. Adults are most active at 75 to 85° F. An average of two *Trichogramma* adults will emerge from a single egg of host. A single host egg can yield wasps of the same or opposite sex. *Trichogramma* adults emerge from host eggs in the early morning. Males emerge first and remain at the host egg to mate with emerging females if they are present. Mated females produce male and female offspring. Unmated females produce only males. Females begin egg laying within a few hours of emergence. *Trichogramma* overwinter as immature forms in host eggs. Some species enter a state of diapuase which allows them to tolerate long periods of subfreezing temperatures. Other species of *Trichogramma*, slow their rate of development and may be active as adults during warm days as early as January and February.

2.5 Trichogramma as biological control agent

2.5.1 Introduction of new species

At least four species of *Trichogramma* have been imported to the U.S. and released for the control of crop pests. In 1968, *T. evanescens* was introduced from Europe into southern California and Missouri for control of imported cabbage worm and cabbage looper on cabbage. A species from Russia, *T. euproctidis*, was imported and released in cotton in Georgia in 1975. In 1993, *Trichogrammatoidea bactrae* was introduced from Australia into California and Arizona for control of the pink bollworm in cotton. The establishment of these three introduced species has not been documented. During 1993-96, *T. ostriniae* was imported from China and released in New York for control of European corn borer in sweet corn.

2.5.2 Augmentation

Augmentation is the periodic release of a natural enemy that does not occur naturally in sufficient numbers to keep a pest below damaging levels. Agumentation can be carried out by inundative releases or inoculative releases. The inundative approach is achieved by flooding the crop with multiple releases of insectary-reared natural enemies. The released insects control pests present at the time, but there is little expectation that later generations will persist at sufficient levels to provide control. This approach requires a large number of the natural enemies at the precise time when pest eggs are present and crop and weather conditions are conducive to the release. Correct timing requires good coordination between the rearing facility and field staff. Inoculative releases involve one or several releases to establish populations of the natural enemy before pest densities have begun to increase. The natural enemy reproduces on the target pest or an alternate host and its population increases to levels sufficient to control the target pest later in the season. In China, inoculative releases of *Trichogramma* in gardens in the spring produce populations of wasps which later in the season move into adjacent fields to control cotton pests (Li 1994).

2.5.3 Conservation

Conservation as a biological control method includes crop management practices that protect and encourage natural enemies and increase their impact on pests. Examples include using only selective insecticides and planting strip crops in and around fields to provide food and habitat for natural enemies. Insecticides such as Bt (formulations of *Bacillus thuringiensi*s) and some insect growth regulators have very little or no impact on *Trichogramma* and can be used in IPM programs with *Trichogramma*. Interplanting rye grass in seed corn production fields lowered soil temperatures which otherwise would be lethal to released *Trichogramma* distributed in cardboard capsules deposited on the soil (Orr *et. al*. 1997). *Trichogramma* species commonly parasitize bollworm, in corn and sorghum, and these crops may serve as an important source of adults which disperse into cotton .

2.6 Biological control status and Parasitization performance of *Trichogramma* on different crop pests

Early cotton entomologists noted that *Trichogramma* parasites commonly attacked bollworm eggs in cotton. Parasitism rates reported in 1903 and 1945 ranged from 5 to 35 percent in Texas cotton, presumably in the absence of insecticides (Quaintance and Brues 1905). In Arkansas, parasitism of bollworm and budworm eggs in untreated cotton is typically 20 percent (Kring and Smith 1995). In Louisiana, early season parasitism reached 60 to 80 percent but sharply declined once insecticide treatments began (Johnson 1985). In the Gulf Coastal region of Texas, natural parasitism of bollworm and budworm eggs in cotton increased from about 20 percent in early June to 65 percent by late July (Segers, *et. al*. 1984). In the U.S., *T. pretiosum* and *T. exiguum* are the most common

Two releases each at a rate of 185,000 pupae per acre are made beginning at the first moth flight as determined by black light traps in European corn borer. European corn borer eggs hatch after about 5 to 6 days and the egg-laying period continues for 4 to 7 weeks. In-field reproduction of released parasites is believed to be important in providing residual control of eggs deposited

after the second release. Field evaluations in Germany have shown releases result in a 70 to 93 percent reduction in corn borer larvae relative to untreated fields (Bigler 1994).

Augmentation of *Trichogramma* has also been promoted for pest control in cotton, corn, apple, spruce and avocado production. However, a recent survey found that very few state Cooperative Extension Services currently provide recommendations for controlling any insect pest with *Trichogramma.*

In California, parasitism of tomato fruit worms (bollworms and budworms) by native *T. pretiosum* in tomatoes is considered in the treatment thresholds for treating these pests with insecticides. Augmentation of *T. pretiosum* is an effective control tactic in Mexico and is part of the IPM program for fresh market tomatoes.

In California, two avocado pests, the omnivorous looper *Sabulodes aegrotata* and the moth *Amorbia cuneana*, can be managed by releasing *T. platneri* in every fourth avocado tree. Large field studies in Canada have shown that two releases each of 30 million *Trichogramma minutum* per acre resulted in 60 to 80 percent egg parasitism of spruce budworm, *Choristoneura fumiferana*, in white spruce stands. However, *Trichogramma* releases have not been adopted as a control tactic for spruce budworm in Canada.

2.7 Ecological constraints to releasing *Trichogramma*

1. Parasitism represents replaceable mortality because of competition with predators for eggs.
2. Wasp mortality caused by egg predators.
3. Potential density dependent predation of young larvae.
4. Difficulty in maintaining field populations of Trichogramma

2.8 Developing a biological control program using *Trichogramma*

2.8.1 Selecting the "Best" *Trichogramma* species

Different species and strains of *Trichogramma* typically prefer different host eggs and crop habitats and have different searching abilities and tolerance to weather conditions. Efficacy is improved by selecting the most effective and adapted species or strain for the specific crop/pest situation. Local species and strains collected from the target area and host often are the first choice for evaluation. However, exotic species and strains may be more effective and should be evaluated. If a species of interest is available commercially, it should be evaluated along with native species. However, unless the supplier maintains high quality control standards both genetic and product quality can deteriorate, leading to poor field performance. Cultures of *Trichogramma* are begun from single isolated females to ensure species purity. Host eggs are collected from the field, placed individually in gelatin capsules, and held until adults emerge. A minute streak of pure, diluted honey should be put in each capsule just before adults emerge; capsules should be examined daily for adults. A colony is initiated from a single host egg that yields both male and female adults. The species is identified once each colony contains a number of adults. Single colonies of the desired species are then combined to broaden genetic diversity. A minimum of 500 to 1,500 individuals may be required to initiate a culture without loss of genetic diversity (Greenberg *et al*., 1996). Standard laboratory trials are conducted to determine the ability of the candidate species to parasitize and develop in the target host egg, the species' preference for the target host egg, and total egg mortality caused by parasitism and adult feeding. Other important attributes include fecundity, development rate, sex ratio, and longevity. These traits indicate probable field performance and are important in mass-rearing programs. Species that show a high degree of host preference are then evaluated for searching ability by releasing adults into cages containing host eggs on the crop plant. Cage experiments are conducted under different temperatures and humidities and different ratios of parasites to host eggs. The species or strain(s) parasitizing or killing the greatest number of eggs are considered to have a high potential for success in field releases.

2.8.2 Selection of factitious hosts for mass rearing of parasitoids

Even though several factitious hosts exist for rearing *Trichogramma*, many researchers still prefer to use *S. cerealella*, because it is easy to rear, despite being less adequate for the multiplication of the parasitoid in relation to other species, such as *A. kuehniella* and *Corcyra cephalonica* (Stainton). In order to compensate for this lower nutritional quality, *T. pretiosum* Riley reared on *S. cerealella* should be released in greater numbers (in relation to those reared on *C. cephalonica* and *A. kuehniella*) to control *H. virescens* in cotton. Therefore, it is essential to associate the nutritional quality of the host, to produce parasitoids that are competitive with those in nature, with a rearing technique that enables mass production of *Trichogramma*. Since the Chinese have extensive silkworm rearing at their disposal, they also use *Philosamia ricini* (Drury) and *Antheraea pernyi* (Guérin-Méneville) eggs or ovules in *Trichogramma* rearing. In general, *A. kuehniella* has proved to be the most suitable factitious host for the Brazilian species although, *C. cephalonica* is the best rearing host for *T. galloi* Zucchi, a predominant parasitoid of *D. saccharalis* eggs in most of Brazil. The influence of biotic (mating, oviposition, adult feeding) and abiotic factors (temperature, relative humidity, and photoperiod) was exhaustively studied in a number of papers and book chapters published on the subject.

Plate 2. A healthy giant swallowtail butterfly egg

Figure 3. An egg with Trichogramma wasps inside egg

Figure 4. Trichogramma wasp emerging from the side of the egg

.

Plate 5. Turning the egg under the microscope dented the top of the egg: one wasp is emerging from a hole in the egg

Plate 6. Trichogramma wasps maturing inside an egg (cut open with a razor blade)

Plate 7. Trichogramma wasp laying eggs

(Source: www.butterflyfunfacts.com)

2.9 Mass rearing of *Trichogramma* for commercial release

Rearing *Trichogramma* requires first rearing an insect, typically a species of moth, to produce eggs in which the wasps will develop. The Angoumois grain moth, *Sitotroga cerealella*, and the Mediterranean flour moth, *Ephestia kuehniella*, are easily and inexpensively reared on wheat or other grains and are commonly used to rear *Trichogramma* (Morrison *et. al*. 1987). Studies to date indicate that there is no difference in field performance between *Trichogramma* reared on *Sitotroga* and those reared on *Ephestia*. To lower production costs, research is underway to develop an artificial egg. China has led in this area and commercially produces one species of *Trichogramma* on an artificial diet composed of insect blood. Further research should lead to major reductions in production costs. Poor quality of mass reared *Trichogramma* can result in control failures. The artificial conditions of mass rearing can select for genetic changes that reduce the effectiveness of the *Trichogramma* in the field. Such rearing conditions include rearing multiple generations on unnatural host eggs, the absence of plants, crowding and interference, rapid generation time, and failure to rejuvenate genetic stock. Except for obvious problems such as lack of adult emergence or wing deformities, growers and pest advisors cannot detect poor quality *Trichogramma* prior to release. Commercial suppliers are responsible for maintaining desirable characteristics necessary for good performance in the field. Production colonies should be periodically (i.e., every six generations) replaced with individuals from a stock culture maintained on the natural or target host. Suppliers also should assess the percent

host egg parasitization, adult emergence, and the sex ratio of emerged adults to be sure they are within acceptable standards. Standards for established cultures on *Sitotroga* are 80 ± 5 percent egg parasitization, 90± 5 percent adult emergence, and a sex ratio of 1.2 to 1.5 females per male. The Association of Natural Biocontrol Producers and the International Organization of Biological Control Subcommittee on Quality Control are developing quality control standards for *Trichogramma* and other natural enemies.

2.10 Methods for releasing *Trichogramma*

Trichogramma are typically shipped and released as pupae inside the host egg. Parasitized pupae are distributed in the field just prior to emergence of the adult wasps, although in some Latin American countries wasps are released after emergence. Parasitized host eggs can be mixed with a carrier and broadcast, or glued to cards or the inside of paper capsules which are then dropped onto or attached to the crop. Broadcasting host eggs is relatively simple but host eggs that fall on the ground may be subjected to lethal temperatures or drown in flooded areas. Also, adults emerging from loose eggs or egg capsules deposited on the ground must walk or fly to the plant to locate host eggs. It is desirable for adults to emerge quickly so they escape predation and avoid high temperatures. Adult emergence can be synchronized to occur shortly after release by refrigerating host eggs at 16.7°C in total darkness beginning on the eighth day of development. After at least 6 days and up to 10 days of refrigeration, adults will emerge within 4 hours once pupae inside host eggs are exposed to light and at least 27^0C. This reduced temperature regime results in about 73 percent of the adults emerging within 4 hours of field release. However, chilling and warming can interfere with development (Stinner *et. al*. 1974). Both manual and mechanized release methods using ground and aerial applicators have been developed. Aerial release methods using refrigeration units were developed in the U.S.A liquid spray system (Biosprayer®) is available for ground application.

2.11 Evaluating releases of *Trichogramma*

Field releases of *Trichogramma* are evaluated by measuring egg parasitism, larval densities, crop damage and economic return relative to similar fields treated with insecticides or not treated. The release method and release rate and frequency should be the same as used in commercial applications. Egg parasitism measures pest mortality directly and is determined by collecting eggs from the field and holding them for evidence of parasitism (eggs turning black or the emergence of a wasp). When eggs of the target species are uncommon or difficult to locate, egg parasitism can be estimated by placing sentinel eggs, either of the target species or other acceptable species, in the field. Sentinel eggs can be killed by UV light to prevent hatch yet still remain suitable for parasitism by *Trichogramma.* The density and distribution of sentinel eggs on the plant and in the field should be similar to that of wild eggs. Unnatural clumping of sentinel eggs increases parasitization. Also, sentinel eggs should not be washed because moth scales left by the female on and around the eggs contain chemical cues (karimones) that arrest *Trichogramma. Trichogramma* wasps also kill host eggs by feeding on them. The host egg is stung and the adult feeds on the drop of liquid appearing at the site of the sting, but no egg is laid. The host egg dies, leaving no evidence of parasitism. In some species of *Trichogramma*, host feeding contributes significantly to pest control. For this reason, egg hatch should be recorded in addition to egg parasitism. Typically, field collected or sentinel eggs are held for hatch and then scored as hatched or dead due to parasitization, predation, infertility (undeveloped) or undetermined causes. Parasitism also can be estimated by determining the ratio of parasitized (black) and unparasitized eggs in a field sample and comparing it to the actual parasitism rate as determined by holding the eggs. The length of time host eggs are in the field exposed to parasites determines the likelihood of their being parasitized. Thus, egg development at the time of collection should be recorded so that exposure time can be determined. Eggs of bollworms and budworms are pearly white when first deposited. After 15 to 18 hours, a reddish or tan ring becomes visible around the top of the egg. About 10 hours before hatch, the black head capsule of the larva is visible. Once an egg is parasitized, the band, if present, disappears

and the egg becomes tan in color for 3 to 4 days before turning black. If only white eggs are collected, parasitism would be underestimated since eggs were not exposed a full 2 days. Since unparasitized eggs are in the field for 3 days and parasitized eggs are there for about 8 or 9 days, the chances of finding a parasitized egg are three times greater based on relative numbers alone. Thus, eggs that are black at the time of sampling should be excluded from the sample to avoid overestimating parasitism. Excluding white and black eggs and using only tan eggs provides the most accurate estimate of actual parasitism (Lopez and Morrison 1985). A parasitism rate of 80 percent is desirable according to theoretical studies and population models. Field parasitism of 75 percent or more is the acceptable level for European corn borer. The densities of larvae must also be assessed because increased egg parasitism and mortality may not reduce densities of damaging larvae. For some pests an increase in egg parasitism by *Trichogramma* may represent compensatory or replaceable mortality rather than additive mortality. Comparisons of crop damage, yield and quality are important in assessing the economic return on augmenting *Trichogramma*. Other methods of evaluating releases may include trapping adult wasps to study movement and dispersion. Sticky traps, yellow pan traps and low malaise traps have been used to capture *Trichogramma* adults. When evaluating *Trichogramma* releases it is important to remember the indirect benefits.

Unlike many insecticides, *Trichogramma* have very little impact on other natural enemies which may be valuable in holding the target pest and secondary pests in check. Also, augmentation programs do not pose risks to field workers or leave toxic residues on produce (Trumble and Alvarado 1991).

2.12 Life tables of *Trichogramma evanescens*

The age-specific fecundity of *Trichogramma evanescens* Westwood and *T. cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) with *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae: Phycitinae) as host and the life span of the adults with and without hosts was determined by

Matthias and Sherif (2001) at 20, 26, 30 and 35^{0}C and 75 ± 5% relative humidity in the laboratory. At 35^{0}C, no progeny emerged. The availability of hosts prolonged the longevity in both species. Longevity, percentage of parasitism, fecundity, and the intrinsic rate of natural increase were higher in *T. evanescens* than in *T. cacoeciae. Trichogramma evanescens* is expected to be more effective than *T. cacoeciae* for use in biological control of *Ephestia* spp., especially at low and high temperatures.

Nabil *et al*. (2008) evaluate the efficacy of *Trichogramma evanescens* (Westwood) in controlling the European grape berry moth *Lobesia botrana* (Den. & Schiff.) in two vineyards, in El-Beheira and El-Gharbia Governorates, northern Egypt during the 2004 and 2005 seasons. *T. evanescens* was mass produced on *Sitotroga cerealella* (Olivier) eggs in National Research Center in Egypt. The horizontal and vertical searching activity of *T. evanescens* was studied to determine the proper way of distributing the *Trichogramma* cards in vineyards in 2004. Field experiments were also conducted to evaluate inundative releases of *T. evanescens* to control *L. botrana* on large scale in 2005. Parasitism by *T. evanescens* on *L. botrana* eggs was greatly affected with the horizontal or vertical distance from the release points. Parasitism reached over 97% and reduction percents of infestation were caused by the pest reached 96.8% in treated plots. A significant increase in the crop was achieved in treated plots. The results showed that the release cards should be distributed in every three grape rows and on height 130–170 cm to obtain good parasitism rates. *T. evanescens* could be a potential candidate for biological control of *L. botrana* in vineyards.

In Egypt, *Trichogramma evanescens* Westwood (TE) is extensively used in inundative releases against a number of lepidopterous pests of several crops reported by Essam (2010). However, the wasp had not been collected from olive groves. Field trials on the use of commercially available TE against the olive moth, *Prays oleae* (Bern.) (OM) were carried out for three successive years (2002–2004). The objective of this study was to evaluate the efficacy of

inundative releases of this wasp on damage reduction. The obtained results were encouraging since OM attacks were reduced by 42.9,71 and 69.9% and TE-treated trees yielded significantly bigger olive fruits by 10.5 and 12.5% than untreated trees in 2002 and 2004 olive seasons, respectively. However, parasitization levels indicated that the wasp is not well adapted to local environmental conditions of olive groves. The suggested measure to improve the quality of released wasps is to mass release of local wasps isolated during the present study, i.e., *T. cordubensis* Vargas and Cabello and *T. euproctidis* Girault.

The efficiency of the parasitoid *Trichogramma evanescens* (Westwood) reared on eggs of three different factitious hosts; *Sitotroga cerealella, Ephestia kuehniella* and *Galleria mellonella* was studied by Nabil (2007) for controlling bollworm *Helicoverpa armigera*. Efficiency of *Trichogramma* was studied by measuring parasitism rates, emergence rates, longevity and sex ratio. Wasps reared from each source were tested on the source host and on the target host, *H. armigera* under laboratory conditions. Rates of parasitism on *H. armigera* eggs, emergence rates of parasitoids and their longevity were the highest for wasps reared on *H. armigera*. Wasps reared on *S. cerealella* gave comparable rates. However, wasps from *E. kuehniella* gave the lower rates and *G. mellonella* gave the lowest ones. Parasitized eggs of *H. armigera* and *S. cerealella* produced more parasitoid females than eggs of *E. kuehniella* and *G. mellonella*. Results are discussed for magnifying efficiency of the parasitoid in controlling *H. armigera* in the field.

Laboratory studies were conducted by Saour (2004) to assess several life-history characteristics of three *Trichogramma* species *T. cacoeciae, T. evanescens,* and *T. principium* reared on potato tuber moth eggs. The effects of host age, parasitoid age, and different temperatures on the mean number of parasitized eggs and the percentage of emerged progeny were determined. The age of both *Trichogramma* and host eggs significantly affected the number of eggs parasitized by the wasps, but did not affect the percentage of parasitoids emerging from parasitized eggs. No

intraspecific differences for potato tuber moth eggs were found among the tested *Trichogramma* species. However, *T. principium* proved to be more effective than *T. cacoeciae* and *T. evanescens* in parasitizing host eggs at high temperatures (>33^{0}C). *Trichogramma* significantly decreased the number of potato tuber moth F_1 emerged progeny when they were released with moths in small cages either over potatoes or potato seedlings.

2.13 Environmental factors that affect field performance

The ability of released *Trichogramma* to locate host eggs depends in part on the distribution pattern of the *Trichogramma* in the field, the release technique, the size and structure of the crop, and the location of the host eggs. The distribution pattern should bring *Trichogramma* as close as possible to host eggs to reduce searching time by the wasp. Weather conditions such as low temperatures and rain can reduce searching and parasitism. Wasps are easily transported by wind and may be blown out of release fields. In corn, 40 to 60 percent of released *Trichogramma* daily move out of the field this way. In contrast, most *T. minutum* and *pretiosum* wasps in cotton remained in the release field. Host plant characteristics can influence the parasitism rate. The density and pattern of trichomes (minute, stout hairs on leaves) can slow the searching rate of *Trichogramma* and reduce parasitism. Dense canopies allow *Trichogramma* to disperse by walking and by short jumps between plants. Nectar feeding increases adult longevity, so the absence of nectaries in cotton reduces parasitism (Treacy *et al*. 1987). Parasitism can decline as leaf surface area increases, creating a greater area for wasps to search. However, studies in cotton relating leaf surface area to parasitism rates have been contradictory (Ables *et al*. 1980).

Parasitism activity of *Trichogramma sibericum* Sorkina tested in laboratory bioassays by Prasad (2002) at different ambient temperatures was not dependent on the rearing temperature of the wasps. *Trichogramma sibericum* parasitized more *Ephestia kuehniella* Zeller eggs as ambient temperature increased from 16 to 26^{0}C. Rearing temperature had a significant effect on potential fecundity (measured as egg load) and on parasitism rate. Insects reared at 21^{0}C laid significantly

more eggs than wasps reared at 16, 26^{0}C or fluctuating temperatures between 16 and 26^{0}C. Computer generated simulations with laboratory derived data suggest that rearing *T. sibericum* at 16^{0}C will improve biological control efficacy when ambient temperatures are expected to be cool (<20^{0}C). Marginal analysis also indicates that rearing at 16^{0}C is more cost effective, again when ambient temperatures are expected to be cool.

Trichogramma sibericum Sorkina was reared by Prasad (1999) in the laboratory at three temperatures: 16, 21, and 26^{0}C. Individuals from each of these treatments were then tested for propensity to initiate flight at one of four ambient temperatures: 16, 19, 21, or 26^{0}C. Both rearing and ambient temperatures had significant effects on flight initiation. Insects reared at 16^{0}C had the highest mean proportion of flyers; insects reared at 26^{0}C had the lowest. The proportion of insects initiating flight increased with increasing ambient temperature. Also, the interaction of these two temperature experiences was significant. Insects reared at 16^{0}C were more likely to initiate flight at 16^{0}C than insects reared at 21 or 26^{0}C. These results indicate that performance (as assessed by flight initiation) at ambient temperature is dependent on the temperature previously experienced during rearing.

At emergence females of Trichogramma had a lot of mature eggs in their ovaries, but some delayed parasitization or refused to parasitize a laboratory host reported by Sergey (2009). The effect of constant and alternating temperatures on the percentage of *Trichogramma buesi* females parasitizing *Sitotroga cerealella* eggs and the duration of the pre-parasitization period were investigated. The temperature dependencies of the rate of preimaginal development, pre-emergence survival, number of eggs laid daily, and total lifetime fecundity were also determined. As the temperature was increased from 12 to 35^{0}C, the median pre-oviposition period decreased from 5 days to 3 h, with maximum values of 24 and 1.5 days, respectively. The rate of induction of parasitization (reciprocal of duration of the pre-parasitization period of the females that parasitized) increased with temperature like the rate of preimaginal development

and average number of eggs laid daily by a parasitizing female. Total cumulative percentage of parasitizing females reached a maximum (60%) at temperatures of 25-30^{0}C, while at 12 and 35^{0}C, respectively, 25 and 50% of females parasitized the *S. cerealella* eggs. Average lifetime fecundity and pre-emergence survival showed a similar dependence on temperature. The influence of the thermorhythm (25^{0}C for 4 h and 15^{0}C for 20 h) was strongly dependent on its position within the photoperiod. When thermophase coincided with photophase, the percentage of females that parasitized was close to that recorded at a constant temperature of 25^{0}C. But when the high temperature pulse coincided with the dark period, the percentage of parasitizing females was the same as at 15^{0}C. Thus, the temperature dependence of ethogenesis (supposedly, an increase in motivation to parasitize or search for a host) in Trichogramma females was similar to that of morphogenesis, although the reaction to alternating temperatures may have been complicated by interaction with the light : dark regime.

The influence of temperature on lifetime attributes of *Trichogramma pretiosum* Riley and *Trichogrammatoidea annulata* De Santis (Hymenoptera: Trichogrammatidae) was evaluated by Arlei *et al*. (2003) at four constant temperatures (15, 20, 25, and 30^{o}C), RH 70 ± 10%, photophase 14 h. *Anagasta kuehniella* (Lepidoptera: Pyralidae) eggs were used as hosts. Developmental times of both parasitoid species were similar when exposed to 20, 25, or 30^{o}C. *T. annulata*, however, developed slightly faster than *T. pretiosum* at 15^{o}C. Emergence rates of both species were above 89%. The temperature threshold for *T. pretiosum* and *T. annulata* was 11^{0}C and the number of degree-days required for their development was 126.9 and 122.3, respectively. Parasitization was maximal at 25^{o}C. *T. annulata*, however, parasitized significantly more hosts than *T. pretiosum* in the entire temperature range. Temperature had no effect in brood size. *T. annulata* progeny consisted predominantly of males, except at 15^{0}C, whereas in *T. pretiosumitconsisted* predominantly of females, except at 30^{0}C. Parental females lived longer than males.

The egg parasitoid *Trichogramma turkestanica* Meyer is being evaluated by Hansen and Jensen (2002) as a biological control agent against the Mediterranean flour moth, *Ephestia kuehniella* Zeller, in flour mills. The longevity, parasitism and host-feeding of the parasitoid at four constant temperatures (15-30^{0}C) have been determined in the laboratory. The highest fecundity occurred at intermediate temperatures. The number of host eggs killed by host-feeding per female was highest at the two lower temperatures. A very conservative estimate of host-feeding showed that it accounts for approximately half of the mortality of host eggs at 20 and 25 degrees C and thus could constitute a major mortality factor for the flour moth population.

At emergence females of *Trichogramma* had a lot of mature eggs in their ovaries, but some delayed parasitization or refused to parasitize a laboratory host. The effect of constant and alternating temperatures on the percentage of *T. buesi* females parasitizing *Sitotroga cerealella* eggs and the duration of the pre-parasitization period were investigated. The temperature dependencies of the rate of preimaginal development, pre-emergence survival, number of eggs laid daily, and total lifetime fecundity were also determined. As the temperature was increased from 12 to 35°C, the median pre-oviposition period decreased from 5 days to 3 h, with maximum values of 24 and 1.5 days, respectively. The rate of induction of parasitization (reciprocal of duration of the pre-parasitization period of the females that parasitized) increased with temperature like the rate of preimaginal development and average number of eggs laid daily by a parasitizing female. Total cumulative percentage of parasitizing females reached a maximum (ca 60%) at temperatures of 25–30°C, while at 12 and 35°C, respectively, 25 and 50% of females parasitized the *S. cerealella* eggs. Average lifetime fecundity and pre-emergence survival showed a similar dependence on temperature. The influence of the thermorhythm (25°C for 4 h and 15°C for 20 h) was strongly dependent on its position within the photoperiod. When thermophase coincided with photophase, the percentage of females that parasitized was close to that recorded at a constant temperature of 25°C. But when the high temperature pulse coincided with the dark period, the percentage of parasitizing females was the same as at 15°C. Thus, the

temperature dependence of ethogenesis (supposedly, an increase in motivation to parasitize or search for a host) in *Trichogramma* females was similar to that of morphogenesis, although the reaction to alternating temperatures may have been complicated by interaction with the light : dark regime.

Delaying emergence of Trichogramma spp. is critical for commercial production reported by Rundle *et al*. (2004). Here, diapause induction was considered for three species (*T. brassicae* Bezdenko, *T. carverae* Oatman & Pinto, and *T. furculatum* Carver), and the effect of storage temperature (4^0C, 8^0C, and 10^0C) and time (1-8 week) was investigated for *T. carverae*. For all species, percentage of emergence was lowered after an initial diapause induction period (28 day at 14^0 C and a photoperiod of 8:16 [L:D] h) and lowered further after 1-mo storage at 3 degrees C and a photoperiod of 0:24 (L:D) h. No wasps emerged after 2 mo of storage, suggesting that true diapause was not induced. The effect of 1-8-wk storage on wasp quality was investigated for *T. carverae* both in the laboratory and the field. Initial fieldwork suggested that this species could be successfully stored at 10 degrees C under continuous light (after 5-d development at 25^0C and a photoperiod of 16:8 [L:D] h) without reducing the ability of wasps to parasitize eggs in the field. In a second experiment, storage temperatures lower than 10 degrees C and storage times 3 wk or longer had a negative impact on emergence and longevity, and effects were not additive. Negative effects may partly reflect size changes, because size decreased in response to storage time, and there was an interaction between time and temperature effects on size. Storage time was the major factor influencing fecundity and field success; both fitness measures were reduced after storage of 3 wk or longer. *T. carverae* can therefore be successfully stored for up to 2 week without detrimental effects, and 10^0C is the preferred storage temperature. *T. carverae* seems to survive unfavorable temperature conditions by entering a state of quiescence.

T. ostriniae Pang and Chen (Hymenoptera: Trichogrammatidae) was reared continuously for seven generations on its native host, the Asian corn borer, *Ostrinia furnacalis* (Guenée)

(Lepidoptera: Crambidae) by Baode *et al*. (2004). It took 6.7 d at 33°C and 20 d at 17°C from oviposition to adult emergence with no differences between sexes. Several theoretic models were used to fit the temperature-dependent growth curves of *T. ostriniae*. A transformed day-degree model and the Hilbert-Logan model were the most reliable for predicting temperature development of *T. ostriniae*. The wasps reared on *O. furnacalis* at 27°C by the seventh generation had a lower level of parasitism than wasps from other generations and at other temperatures (17–33^{0}C). The number of wasps emerged from individual parasitized egg, and percentage of females produced did not differ for any generations and temperatures. The differences among different generations for the amount and proportion of time female wasp spent drumming host eggs did not show any host- and generation-related trends. The time spent drumming by *T. ostriniae* female wasps reared for three generations on *O. furnacalis* and then four and six generations on the rice moth, *C. cephalonica* (Stainton), differed slightly from the other generations. High variability among the tested wasps indicated that a large number of replicates would be needed to detect the probable differences among generations.

This work was carried out by Consoli and Parra (1995) in order to study the effects of constant and alternating temperatures on the development and thermal requirements of *Trichogramma galloi*, the most common egg parasitoid of the sugarcane borer in Brazil. *T. galloi* developmental time was shorter when reared at alternating temperatures. The emergence rate of *T. galloi* decreased at constant 18°C, and the sexual ratio was affected only at 32^{0}C. *T. galloi* degree-days and threshold temperatures were similar when estimated using constant or alternating temperatures.

Parasitism activity of *Trichogramma sibericum* Sorkina tested in laboratory bioassays by Prasad *et al.* (2003) at different ambient temperatures and reported that parasitism activity was not dependent on the rearing temperature of the wasps. *T. sibericum* parasitized more *Ephestia kuehniella* Zeller eggs as ambient temperature increased from 16 to 26^{0}C. Rearing temperature

had a significant effect on potential fecundity (measured as egg load) and on parasitism rate. Insects reared at 21^0C laid significantly more eggs than wasps reared at 16, 26^0C or fluctuating temperatures between 16 and 26^0C. Computer generated simulations with laboratory derived data suggest that rearing *T. sibericum* at 16^0C will improve biological control efficacy when ambient temperatures are expected to be cool ($<20^0$C). Marginal analysis also indicates that rearing at 16^0C is more cost effective, again when ambient temperatures are expected to be cool.

Laboratory studies were conducted by Young *et al*. (2000) to investigate the effect of selected temperatures on the development, mortality, sex ratio, and emergence rate of *T. dendrolimi* Matsumura reared from a factitious host, oak silkworm, *Antheraea pernyi* (Guérin-Méneville) eggs. The comparison tests were conducted to investigate the fecundity on *Dendrolimus spectabilis* (Butler) eggs of *T. dendrolimi* reared from natural and factitious hosts, and artificial hosts. Developmental periods from egg to adult of *T. dendrolimi* reared at 26, 28, 30, and 32°C were 10.9, 9.6, 9.0, 8.6 days, respectively. Emergence rates of *T. dendrolimi* decreased 78.0, 88.4, 60.7, and 50.1% as temperature increased. The progeny sex ratios (i.e., females per male) were 7.3, 8.4, 8.2 and 6.9 at the respective temperatures. When adult *T. dendrolimi* emerged from *A. pernyi* eggs were kept in dark conditions for storage at different temperatures for 34 days, their mortality increased as temperature increased: 0% at 0°C; 10% at 4°C; 40% at 6°C; 50% at 18°C and 100% at ≥26°C. The fecundity of *T. dendrolimi* reared from three different hosts was investigated on *D. spectabilis* eggs. During the first day, the adult *T. dendrolimi* reared on *A. pernyi* eggs laid most eggs (99.0±10.7), followed by those reared on artificial hosts (76.6±24.5) and *D. spectabilis* eggs (63.4±35.9). Over the entire lifespan, *T. dendrolimi* reared on *D. spectabilis* eggs produced the highest number of eggs (218.0±27.9), followed by *A. pernyi* eggs (104.0±44.7) and artificial host (98.2±37.1). These results suggest that the temperature and three hosts factors had an effect on rearing of the *T. dendrolimi* on the factitious host, *A. pernyi* eggs.

2.14 Future prospects for *Trichogramma*

Controlling specific caterpillar pests with augmentative releases of *Trichogramma* is technically feasible in some field and forest crops. However, adoption has been slow because of variable levels of pest control in the field, high production costs, lack of application technology, and incompatibility with insecticides applied for other pests. In some pest and crop systems, targeting the egg stage may not be appropriate because of compensatory and density dependent mortality factors. These are the major challenges to researchers in developing augmentation programs (Andow, 1997):

1. Develop mass rearing methods and quality controls that consistently yield high quality parasites. The variable and often unmeasured genetic changes that *Trichogramma* undergo in mass rearing need to be under-stood and managed to preserve the behavioral and biological characteristics necessary for high efficiency in the field.
2. Understand and quantify the relationship between numbers of parasites released and their impact on the pest population and on the level of crop protection, as well as the influence of the crop and environment on the action of the natural enemy.
3. Ensure that the most suitable species or biotype is selected for augmentative use.
4. Determine the optimal size of the release area and the dispersal of the predator or parasite.
5. Develop pest management systems that eliminate or limit insecticide interference with natural enemies.
6. Develop efficient methods of producing *Trichogramma* and define environmental parameters and specifications for storage, shipment and field release.

Biological control is a component of an integrated pest management strategy. Now a day researcher give more emphasis on biological control because of its safety, host specificity,

permanency, economy & it has no adverse effect on environment. The success of biological control largely depends upon conservation of naturally occurring bio-control agents like predator, parasite, parasitoid, pathogens etc. Parasitoids are one of the most important bio-control agent. Among the parasitoids *Trichogramma* is one the most effective agent now a days used as biological control agent. *Trichogramma spp* are tiny wasps that prey on the eggs of more than 200 pests, including borers, webworms, loopers, leafworms, fruitworms, cutworms, codling moth, bollworms, and armyworms. The wasps lay their eggs in the pest eggs & killing them. So, as a safety and environmental friendly *Trichogramma* can be used to manage different types of pest species as a egg parasitoids. The successful use of augmentative releases of *Trichogramma* in IPM programs will depend on a sound and thorough research program, favorable economics, commercial investment, and the development of an Extension program to transfer this technology to crop consultants and growers.

CHAPTER III

MATERIALS AND METHODS

The study was conducted to explore the biology of host Sitotroga cerealella Oliver (Lepidoptera: Gelechiidae) and its egg parasitoid Trichogramma evanescens (Hymenoptera: Trichogrammatidae); effects of ecological factors especially temperature and relative humidity on both host and parasitoid as well as to explore the parasitization performance of the egg parasitoid reared on the eggs of S. cerealella. The details materials and methods that were used to conducting of this experiment are presented below under the following sub-headings:

3.1. Location and duration of the study

The experiment was conducted in the laboratory of the Department of Entomology at Sher-e-Bangla Agricultural University during the period from July 2010 to January 2011.

3.2. Design of experiments

The study was conducted using Complete Randomized Design (CRD) with various replications for various parameters.

3.3. Maintenance of Sitotroga cerealella stock culture

The anguimous grain moths/rice moths (S. cerealella) were reared on unhusked wheat grains in the metallic rearing cage (specially made for rearing of rice moth) in the laboratory of the Department of Entomology at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. For this purpose the wheat grains were sterilized, where the grains were kept in boiled water at 55-60oC for 3-5 minutes to kill other insects and pathogens living in the grains as well as to avoid the secondary infection by the pathogens. About 2 kg of sterilized wheat grains then kept in the steel stray (Plate 8). One gram freshly laid eggs of S. cerealella collected from Safe Agro-BioTech Ltd, Mirpur then released on the wheat grains to infest them and kept the tray with

grains and released Sitotroga eggs in the ambient condition of the laboratory. Necessary take care was also done to inhibit the infestation from ants. When a few number of S. cerealella adult emergence were seen from the wheal grains, then the whole grains were transferred to especially made metallic rearing cage (Plate 9) to facilitate adult emergence and easy collection of numerous population of adult moths. After emerging from the grains, the collected virgin (0-24 hrs old) adults of S. cerealella from the cage were allowed in the special glass tube (Plate 10) to facilitate them to lay their eggs. The every day collected eggs were then stored in the refrigerator at 4oC temperature to ensure continuous supply for future use in the study. The eggs of S. cerealella reared on unhusked wheat grains were used to parasitize by the Hymonopteran egg parasitoid T. evanescens.

Plate 8. Hot water sterilized wheat grains and Sitotroga eggs kept in steel tray.

Plate 9. Metallic cage especially made for rearing and facilitating the adult emergence of S. cerealella.

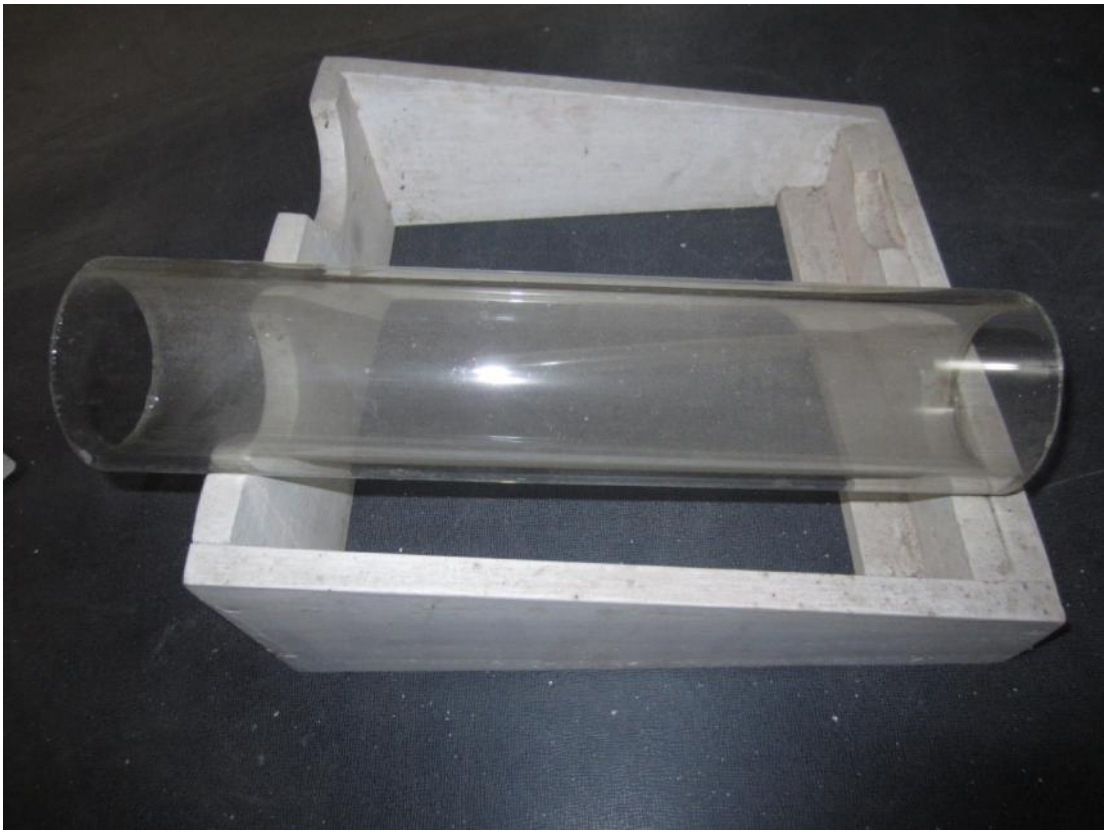

Plate 10. Glass tube used to facilitate egg laying by Sitotroga adult months

3.3.1. Biology study of Sitotroga cerealella

The eggs of S. cerealella from the stock culture were used to rear in the wheat grains from August 2010 to January 2011. Different growth and developmental stages of S. cerealella such as incubation period, larval period, pupal period and adult longevity were studied for each months of the experimental period separately. Incubation period was recorded by the identification of freshly laid eggs (Plate 11) through their transparent egg shells on the wheat grains and the hatching of eggs into larvae (Plate 12) beneath the egg shell with the help of magnifying glass as well as simple microscope. Larval and pupal (Plate 13) period were recorded by cutting infested grains with the help of blade and observation under the microscope. After emerging from the grains, the adults (Plate 14) of S. cerealella were kept in the glass vial upto death and thus the adult longevity of S. cerealella was recorded. The experiment was replicated 5 times for each parameter during each month of the study period.

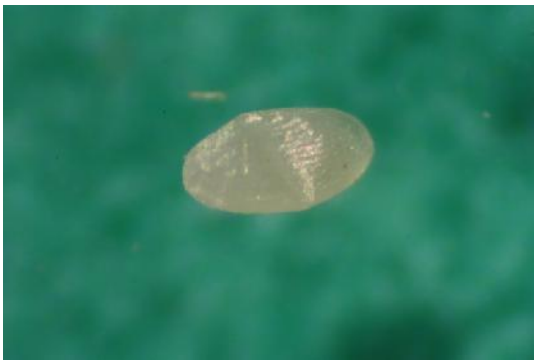

Plate 11. Freshly laid egg of S. cerealella

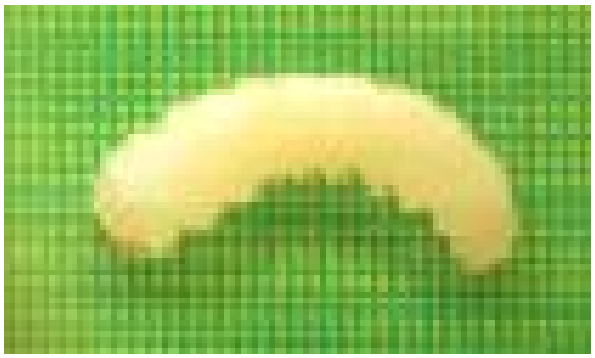

Plate 12. Larva of S. cerealella

Plate 13. Pupa of S. cerealella

Plate 14. Adults of S. cerealella

3.3.2. Viability test of the Sitotroga eggs

Viability test of the eggs laid by S. cerealella were tested considering the variable ages of eggs after laid. To conduct this part of the study, freshly laid eggs (0-1 day old), and different age period such as 10 days old eggs, 20 days old eggs, 30 days old eggs and 40 days old eggs stored at 4oC in the refrigerator were evaluated to find out the hatching percentage of the different age groups of the Sitotroga eggs.

3.4. Maintenance of Trichogramma evanescens stock culture

The inoculum of egg parasitoid Trichogramma evanescens were collected from the Safe Agro-Bio Tech Ltd, Mirpur, Dhaka, Bangladesh. The collected T. evanescens were reared on the eggs

of S. cerealella and the necessary study were done as per needed in the laboratory of the Department of Entomology at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

3.4.1. Biology study of Trichogramma evanescens

The eggs of S. cerealella from the stock culture were kept in glass vial in the laboratory at ambient room conditions. At the pupal stage of Trichogramma, the parasitized host eggs are seen dark black in color. These dark black colored Trichogramma parasitized Sitotroga eggs were released in the glass vials (Plate 15) to parasitize the eggs of Sitotroga. After releasing the parasitized eggs, the mouths of respective glass vials were covered with fine meshed Siphon cloth and tied with rubber guarder to facilitate normal aeration inside the vials. The Trichogramma parasitized eggs of host were identified with the help of magnifying glass and microscope. Usually the freshly laid eggs of Sitotroga are white in color and it turns into red color when it becomes mature and reaches to hatch. But the Trichogramma parasitized host egg become black due to presence of the parasitoid's pupae inside the host egg. After identifying the parasitized host eggs, data were recorded on egg to larval period, pupal, egg to adult period. After emerging from the parasitized Sitotroga eggs, the adults of T. evanescens were kept in the glass vial upto death and thus the adult longevity of T. evanescens was recorded.

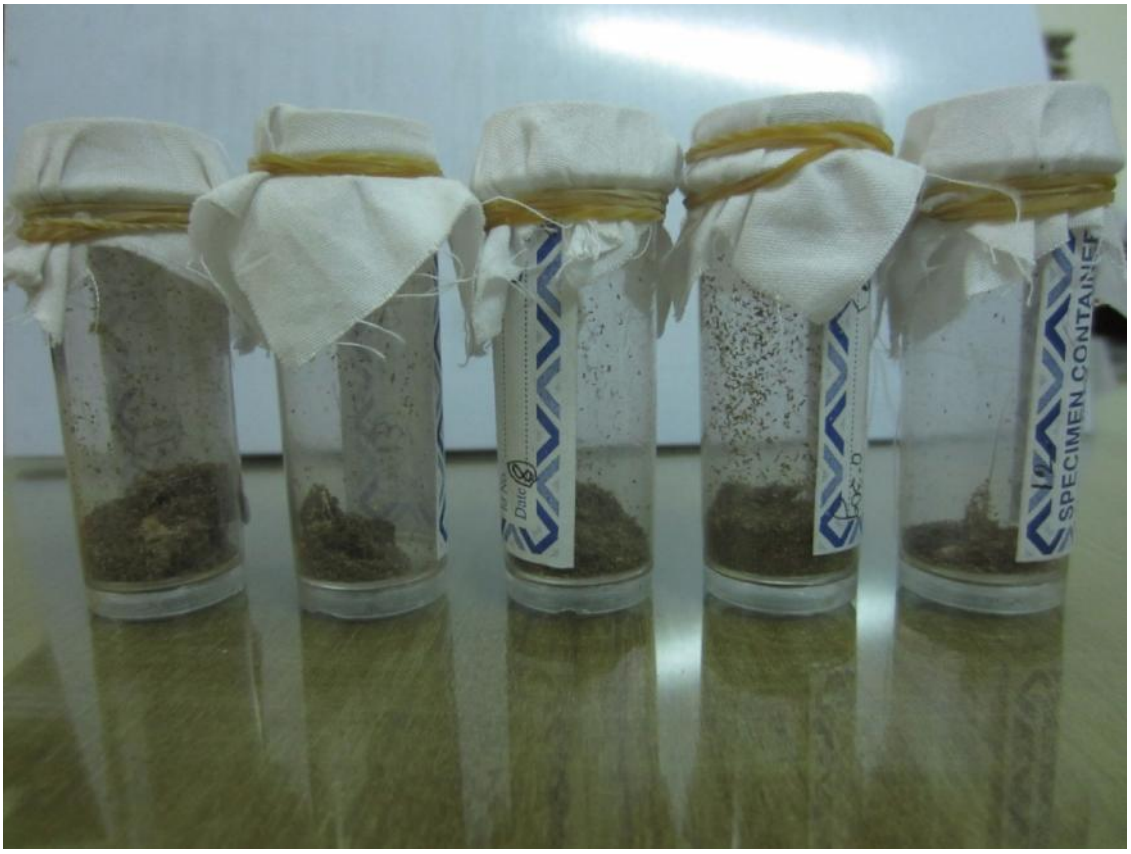

Plate 15. Vials used for parasitization practice of Sitotroga eggs by Trichogramma egg parasitoid

3.4.2 Parasitizing performance of T. evanescens

The pre-designed number such as 40, 35, 30, 25 and 20 of Trichogramma parasitized host eggs were released for each 100 Sitotroga eggs separately kept in the glass vials. After releasing the parasitized eggs, the mouths of respective glass vials were covered with Shiphon cloth and tied with rubber guarder to facilitate normal aeration inside the vials. The eggs of Sitotroga were parasitized by the released Trichogramma and then the data were recorded on the parasitized and unparasitized Sitotroga eggs for each batch to get the rate of parasitization and optimum number of Trichogramma required for effective parasitization.

3.4.3. Variations of parasitization performance during study period

The definite number (20) of Trichogramma parasitized host eggs were released for each 100 Sitotroga eggs separately kept in the glass vials. After releasing the parasitized eggs, the mouths of respective glass vials were covered with Shiphon cloth and tied with rubber guarder to

facilitate normal aeration inside the vials. The eggs of Sitotroga were parasitized by the released Trichogramma and then the data were recorded on the parasitized and unparasitized Sitotroga eggs for each batch to get the rate of parasitization. This study was continued upto January 2011 starting from September 2010.

3.5. Data recorded on weather factors

During the experimental period from August 2010 to January 2011, the weather data such as temperature and relative humidity of the ambient room condition of the laboratory were recorded for each day of the months. Then the mean values of temperature and relative humidity for each month were calculated and used to make correlation with growth and developmental period of S. cerealella and T. evanescens, as well as with the parasitization performance of T. evanescens during the different months of the study period.

3.6. Statistical analysis

The data obtained for different parameters were statistically analyzed to find out the significant difference. The mean values of all the characters were calculated and analysis of variance was performed by the ‘F’ test. The significance of the difference among the treatment means was estimated by the Least Difference Test (LSD) at 1% and 5% level of probability (Gomez and Gomez, 1984). Correlation and regression analysis were also done to make the relationship among different parameters especially for weather factors.

CHAPTER IV

RESULTS AND DISCUSSION

The results of the present study regarding the parasitization performance of *Trichogramma evanescens* egg parasitoid on the eggs of rice moth, *Sitotroga cerealella* Oliver as well as the effect of weather factors on the performance of parasitization conducted in the laboratory of the Department of Entomology at Sher-e-Bangla Agricultural University, Dhaka during April 2010 to January 2011 have been discussed and presented with interpretations under the following sub-headings:

4.1. Growth and development of *Sitotroga cerealella*

Variations amongst the growth and developmental periods of *Sitotroga cerealella* were observed among different experimental months of study.

4.1.1. Incubation period

The incubation period was ranged between 6.67 to 7.33 days from the month of August, 2010 to January, 2011. The minimum incubation period was required in August, which was statistically similar with all other months of the experimental period, whereas the maximum period was required in January.

4.1.2. Larval period

The significant variations ($P < 0.01$) among the larval period were observed in different months of the study. The larval period of rice moth, *Sitotroga cerealella* was ranged between 15.67 to 22.67 days from the month of August, 2010 to January, 2011 (Table 1). The maximum larval period was recorded in January, which was statistically similar with the month of December (21.33 days) followed by November (18.67 days). On the other hand, the minimum larval period was recorded in August that was statistically identical with September and similar with October (16.67 days).

From these findings, it was revealed that the larval period of rice moth was lowest in August and it was increased gradually and highest in January. As a result the increasing order of the larval period among different experimental months was August, September > October > November > December > January.

4.1.3. Pupal period

The significant variations (P <0.01) among pupal period were also observed in different months of the study. The pupal period of rice moth, *Sitotroga cerealella* was ranged between 4.33 to 6.00 days from the month of August, 2010 to January, 2011 (Table 1). The maximum pupal period was recorded in January, which was statistically similar with the month of December (5.67 days) and November (5.00 days) but different from all other months of the study period. On the other hand, the minimum pupal period was recorded in August that was statistically identical with September and similar with October (4.67 days).

From these findings, it was revealed that the pupal period of rice moth was lowest in August and it was increased gradually and highest in January. As a result the increasing order of the pupal period among different experimental months was August, September > October > November > December > January.

Table 1. Variations of growth and developmental period of *Sitotroga cerealella* during the month of August 2010 to January 2011

Month	Incubation period (±sd) (Day)	Larval period (±sd) (day)	Pupal period (±sd) (day)	Egg to adult (±sd) (day)	Adult longevity(±sd) (day)	Total life span (±sd) (day)
August 2010	6.67±0.58a	15.67±0.58c	4.33±0.58c	26.67±1.15c	6.00±0.00c	32.67±1.15c
September 2010	6.67±0.58a	15.67±0.58c	4.33±0.58c	26.67±0.58c	6.33±0.58bc	33.00±1.00c
October 2010	6.67±0.58a	16.67±0.58c	4.67±0.58bc	28.00±1.00bc	6.33±0.58bc	34.33±0.58bc
November 2010	6.67±0.58a	18.67±0.58b	5.00±0.00abc	30.33±1.15b	6.67±0.58bc	37.00±1.73b
December 2010	7.00±0.00a	21.33±0.58a	5.67±0.58ab	34.00±1.00a	7.33±0.58ab	41.33±1.15a
January 2011	7.33±0.58a	22.67±0.58a	6.00±0.00a	36.00±1.00a	8.00±0.00a	44.00±1.00a
$LSD_{(0.01)}$	1.315	1.439	1.175	2.494	1.175	2.879
CV (%)	7.71	3.13	9.43	3.30	6.96	3.12

In column, numeric data represent the mean value of 5 replications and means having similar letter(s) are statistically similar at 0.01 level of probability

4.1.4. Egg to adult period

The egg to adult period, i.e., the period between eggs laying to adult emergence, was recorded from the cumulative results of the incubation period, larval and pupal period. From these findings, the significant variations (P <0.01) among egg to adult period were observed in different months of the study. The egg to adult period of *Sitotroga cerealella* was ranged between 26.67 to 36.00 days from the month of August, 2010 to January, 2011 (Table 1). The maximum egg to adult period was recorded in January, which was statistically different from all other months followed by December (34.00days) and November (30.33 days). On the other hand, the minimum egg to adult period was recorded in August that was statistically identical with September and similar with October (28.00 days).

From these findings, it was also revealed that the egg to adult period of rice moth was lowest in August and it was increased gradually and highest in January. As a result the increasing order of the egg to adult period among different experimental months was August, September > October > November > December > January.

4.1.5. Adult longevity

The adult longevity, i.e., the period between adult emergence and its death, slightly varied significantly (P <0.01) in different months of the study and it was ranged between 6.00 to 8.00 days from the month of August, 2010 to January, 2011 (Table 1). The maximum period of adult longevity was recorded in January, which was statistically similar with December (7.33 days) followed by November (6.67 days). On the other hand, the minimum period of adult longevity was recorded in August, which was statistically similar (6.33 days) with September and October. From these findings, it was revealed that the period of adult longevity of rice moth was lowest in August and it was increased gradually and highest in January. As a result the increasing order of the period of adult longevity among different experimental months was August > September > October > November > December > January.

4.1.6. Total life span

The total life span, i.e., the period between eggs laying to the death of the adult, was recorded from the cumulative results of the incubation, larval and pupal period, and adult longevity. From these findings, the significant variations (P <0.01) among total life span were observed in different months of the study. The total life span of *S. cerealella* was ranged between 32.67 to 44.00 days from the month of August, 2010 to January, 2011 (Figure 1). The longest life span was observed in January, which was statistically similar with December (41.33 days) but different from all other months followed by November (37.00days) and October (34.33 days). On the other hand, the shorted life span was observed in August, which was statistically similar with September (33.00 days).

From these findings, it was revealed that the total life span of *S. cerealella* was shortest in August and it was increased gradually, whereas longest in January. As a result the increasing order of the total life span of *S. cerealella* among different experimental months was August > September > October > November > December > January.

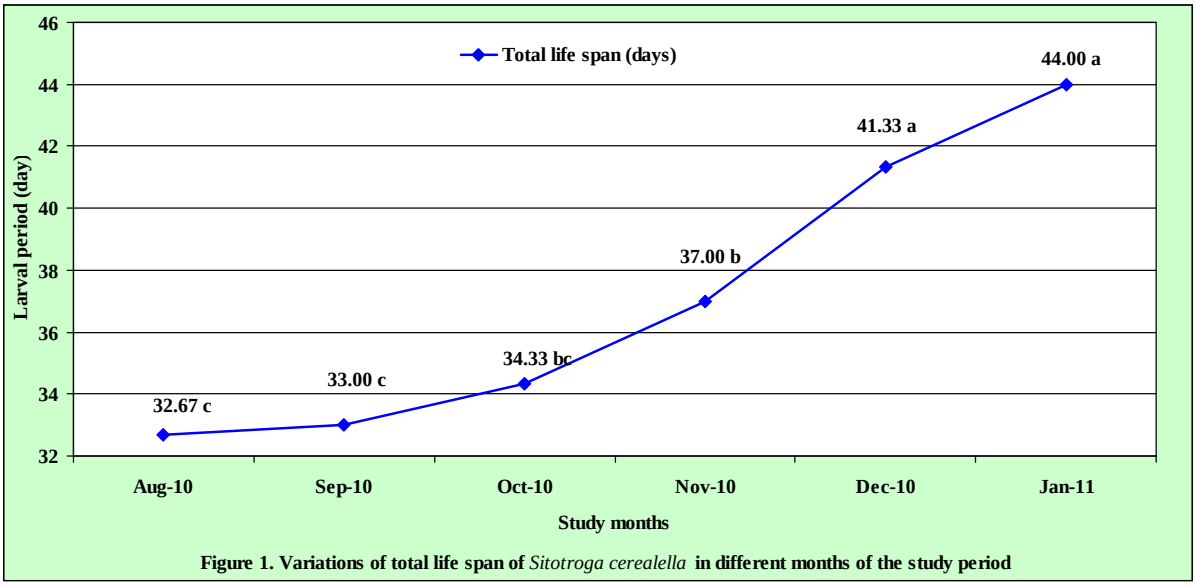

Figure 1. Variations of total life span of *Sitotroga cerealella* in different months of the study period

4.2. Factors for the variations of growth and development of *S. cerealella* in different months of the study period

Now the question arises, what factors are really responsible for such variations in the growth and developmental period of rice moth, *S. cerealella* in different months of the study periods. This question necessitates the in-depth analysis of the actual factors that varied due to the seasonal variations and their possible attributions to the variations in periods of growth and development. The in-depth analyses of all these relevant factors are presented below:

4.2.1. Climatic variations in study periods

Sharp variations in weather factors in months of the study periods were observed (Table 2). The highest mean temperature (30.12^{o}C) and relative humidity (86.52%) were recorded in the month of August 2010 followed by September ((29.05^{o}C & 84.23%, respectively), October 2010 (28.30^{o}C & 75.50%, respectively). On the other hand, the lowest mean temperature (23.21^{o}C) and relative humidity (67.20%) were recorded in the month of January 2011 followed by December 2010 (25.15^{o}C & 70.10%, respectively) and November 2010 (27.20^{o}C & 72.15%, respectively).

Table 2. Variations of weather factors during the month of August 2010 to January 2011

Month	Weather factors	
	Mean temperature (^{o}C)	Mean relative humidity (%)
August, 2010	30.12	86.52
September, 2010	29.05	84.23
October, 2010	28.30	75.50
November, 2010	27.20	72.15
December, 2010	25.15	70.10
January, 2011	23.21	67.20

4.2.2. Relationship between weather factors and developmental period of *S. cerealella*

There was a sharp and significant relationship observed between the weather factors (temperature and relative humidity) and the growth and developmental period (incubation period, larval, pupal period and adult longevity) of *S. cerealella* during the months of the experimental period (Figure 2 to Figure 9).

4.2.2.1a. Relationship between temperature and incubation period of *S. cerealella*

The correlation study was done to establish a relationship between temperature and incubation period of *S. cerealella*. The regression equation y = -0.0984x + 9.51 gave a good fit to the data and the value of the co-efficient of determination (R^2 = 0.8492) (Figure 2). From the correlation study and regression line it was revealed that a highly significant ($p < 0.01$) and strongly (r =0.8492) negative correlation was observed between temperature and incubation period, i.e., the incubation period of *S. cerealella* was decreased with the increase of temperature; conversely, the incubation period was increased with the decrease of temperature. For this reason, the incubation period of *S. cerealella* was shorter in August when temperature was higher, whereas the incubation period was longer in January, when the temperature was lower.

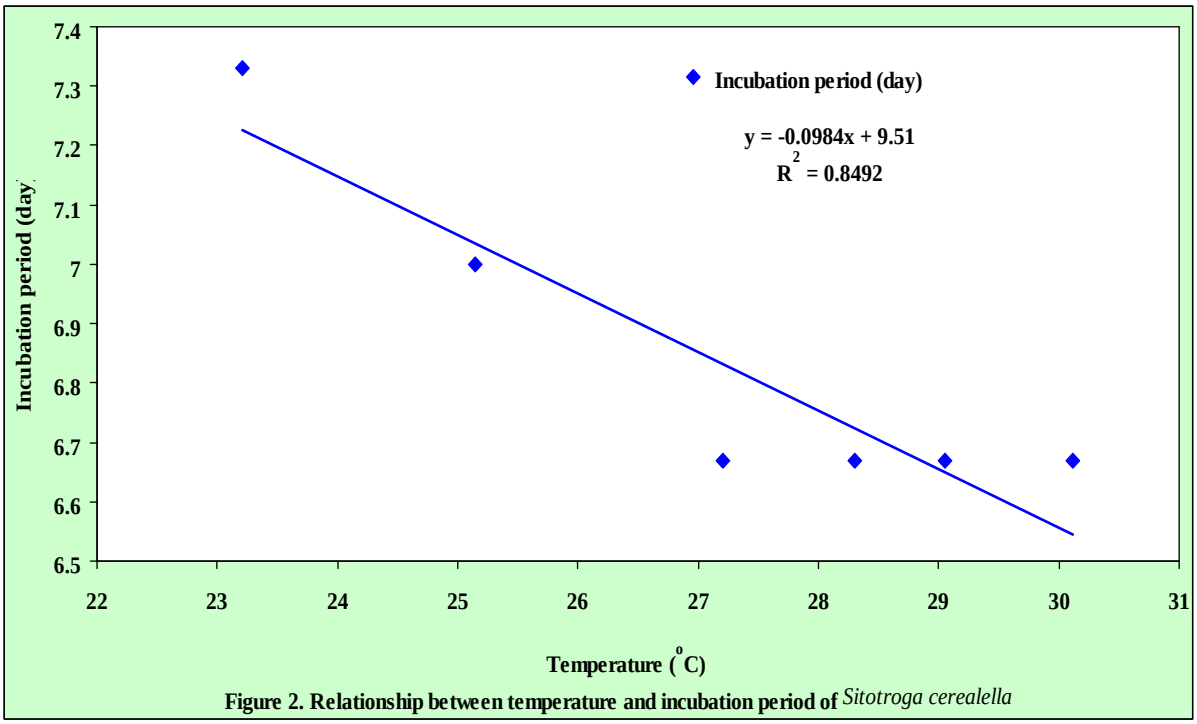

Figure 2. Relationship between temperature and incubation period of *Sitotroga cerealella*

4.2.2.1b. Relationship between relative humidity and incubation period of *S. cerealella*

The correlation study was done to establish a relationship between relative humidity and incubation period of *S. cerealella*. The regression equation y = -0.0252x + 8.75 gave a good fit to the data and the value of the co-efficient of determination (R^2 = 0.5096) (Figure 3). From the correlation study and regression line it was revealed that a highly significant ($p < 0.01$) and moderately (r =0.5096) negative correlation was observed between relative humidity and incubation period, i.e., the incubation period of *S. cerealella* was decreased with the increase of relative humidity; conversely, the incubation period was increased with the decrease of relative humidity. For this reason, the incubation period of *S. cerealella* was shorter in August when relative humidity was higher, whereas the incubation period was longer in January, when the relative humidity was lower.

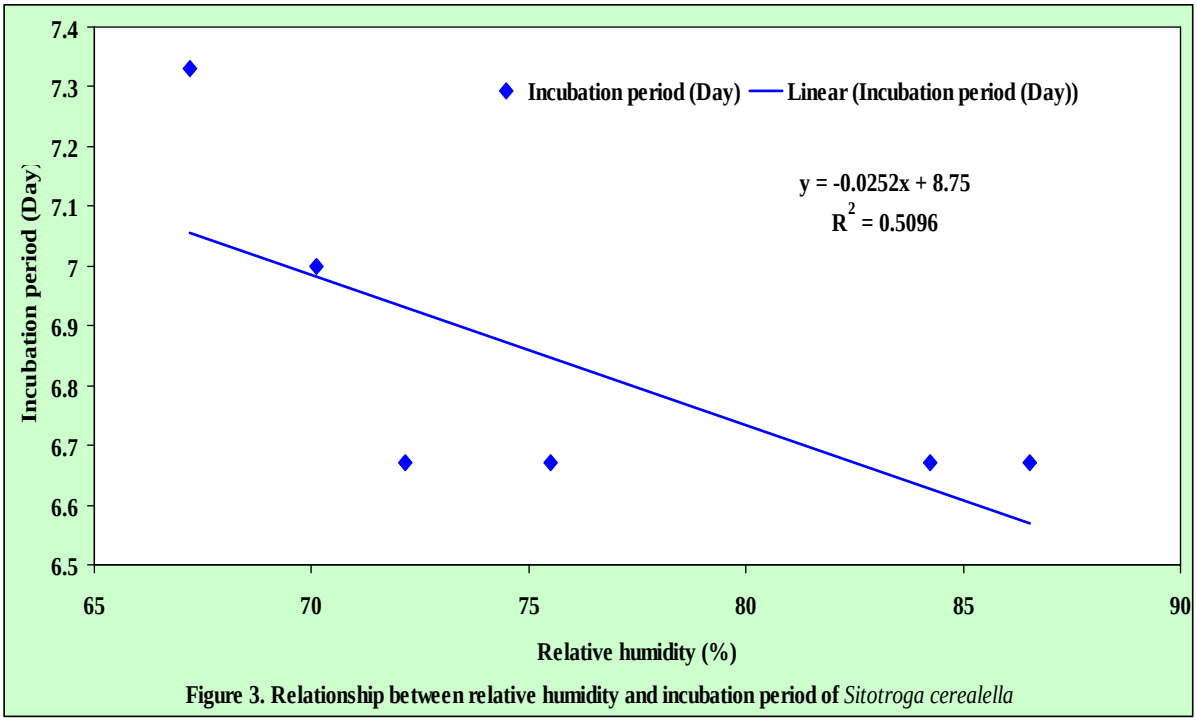

Figure 3. Relationship between relative humidity and incubation period of *Sitotroga cerealella*

4.2.2.2a. Relationship between temperature and larval period of *S. cerealella*

The correlation study was done to establish a relationship between temperature and larval period of *S. cerealella*. The regression equation y = -1.1406x + 49.44 gave a good fit to the data and the value of the co-efficient of determination (R^2 = 0.9671) (Figure 4). From the correlation study and regression line it was revealed that a highly significant ($p < 0.01$) and strongly ($r = 0.9771$) negative correlation was observed between temperature and larval period, i.e., the larval period of *S. cerealella* was decreased with the increase of temperature; conversely, the larval period was increased with the decrease of temperature. For this reason, the larval period of *S. cerealella* was shortest in August when temperature was highest maximum, whereas the larval period was longest in January, when the temperature was lowest.

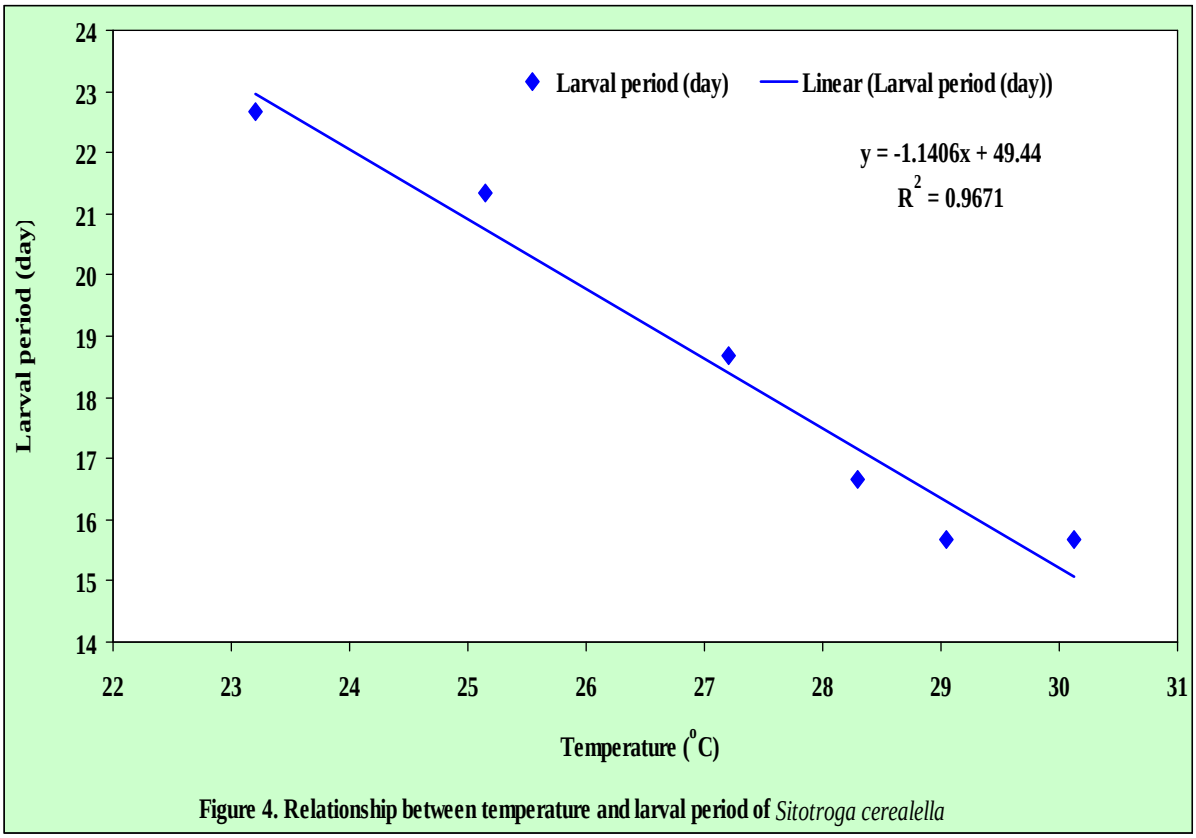

Figure 4. Relationship between temperature and larval period of *Sitotroga cerealella*

4.2.2.2b. Relationship between relative humidity and larval period of *S. cerealella*

The correlation study was done to establish a relationship between relative humidity and larval period of *S. cerealella*. The regression equation y = -0.3474x + 44.83 gave a good fit to the data and the value of the co-efficient of determination (R^2 = 0.8241) (Figure 5). From the correlation study and regression line it was revealed that a highly significant ($p < 0.01$) and strongly (r =0.8241) negative correlation was observed between relative humidity and larval period, i.e., the larval period of *S. cerealella* was decreased with the increase of relative humidity; conversely, the larval period was increased with the decrease of relative humidity. For this reason, the larval period of *S. cerealella* was shortest in August when relative humidity was highest, whereas the larval period was longest in January, when the relative humidity was lowest.

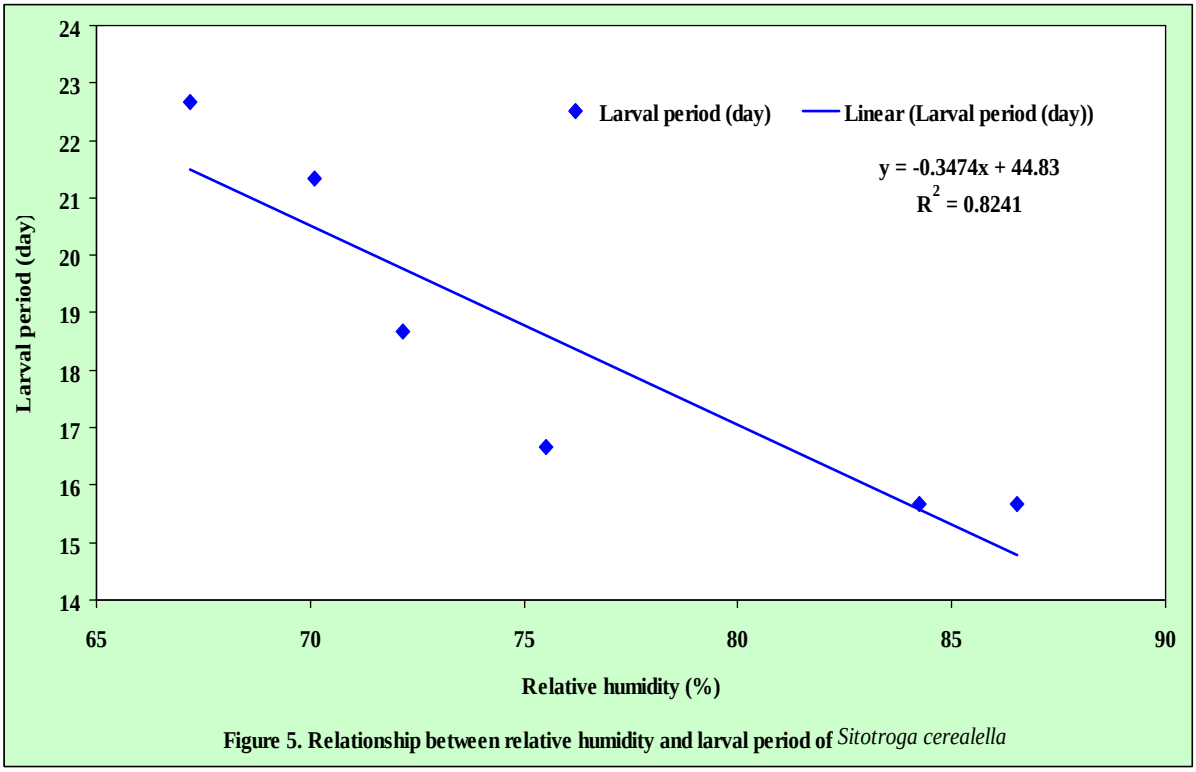

Figure 5. Relationship between relative humidity and larval period of *Sitotroga cerealella*

4.2.2.3a. Relationship between temperature and pupal period of *S. cerealella*

The correlation study was done to establish a relationship between temperature and pupal period of *S. cerealella*. The regression equation y = -0.2683x + 12.29 gave a good fit to the data and the value of the co-efficient of determination (R^2 = 0.9742) (Figure 6). From the correlation study and regression line it was revealed that a highly significant ($p < 0.01$) and strongly ($r = 0.9742$) negative correlation was observed between temperature and pupal period, i.e., the pupal period of *S. cerealella* was decreased with the increase of temperature; conversely, the pupal period was increased with the decrease of temperature. For this reason, the pupal period of *S. cerealella* was shortest in August when temperature was highest maximum, whereas the pupal period was longest in January, when the temperature was lowest.

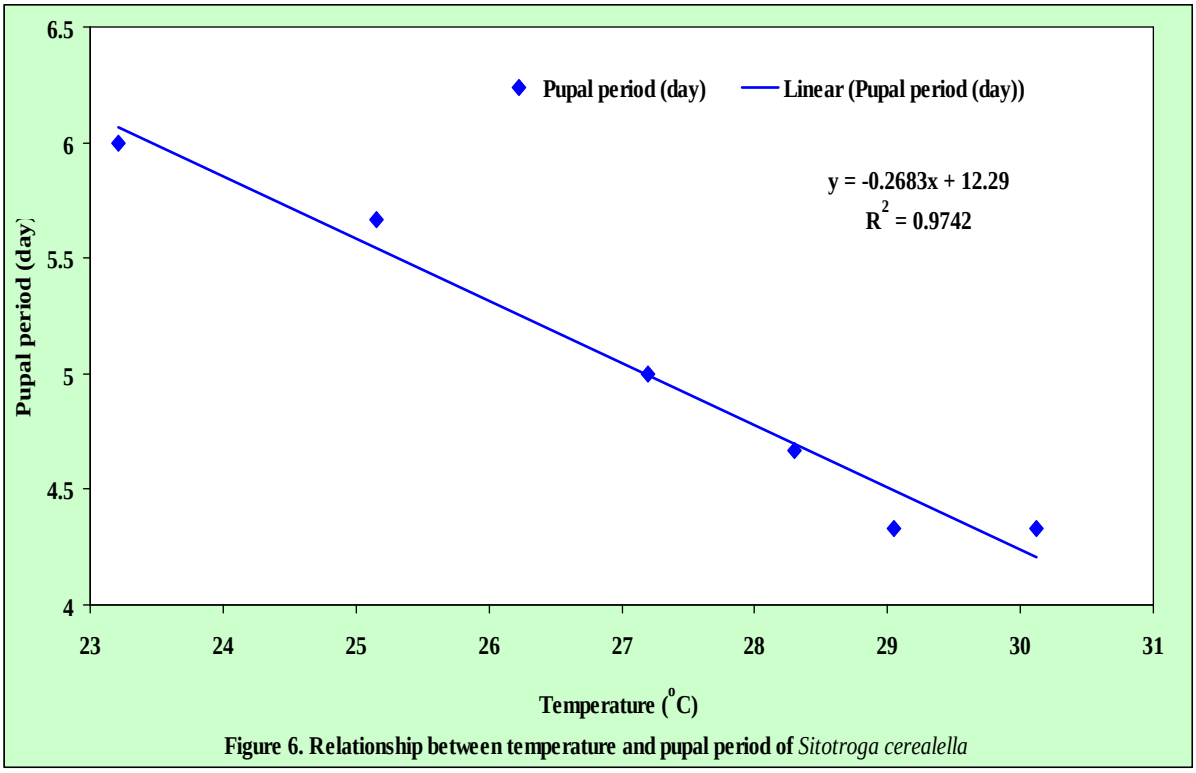

Figure 6. Relationship between temperature and pupal period of *Sitotroga cerealella*

4.2.2.3b. Relationship between relative humidity and pupal period of *S. cerealella*

The correlation study was done to establish a relationship between relative humidity and pupal period of *S. cerealella*. The regression equation y = -0.0823x + 11.25 gave a good fit to the data and the value of the co-efficient of determination (R^2 = 0.8426) (Figure 7). From the correlation study and regression line it was revealed that a highly significant ($p < 0.01$) and strongly (r =0.8426) negative correlation was observed between relative humidity and pupal period, i.e., the pupal period of *S. cerealella* was decreased with the increase of relative humidity; conversely, the pupal period was increased with the decrease of relative humidity. For this reason, the pupal period of *S. cerealella* was shortest in August when relative humidity was highest, whereas the pupal period was longest in January, when the relative humidity was lowest.

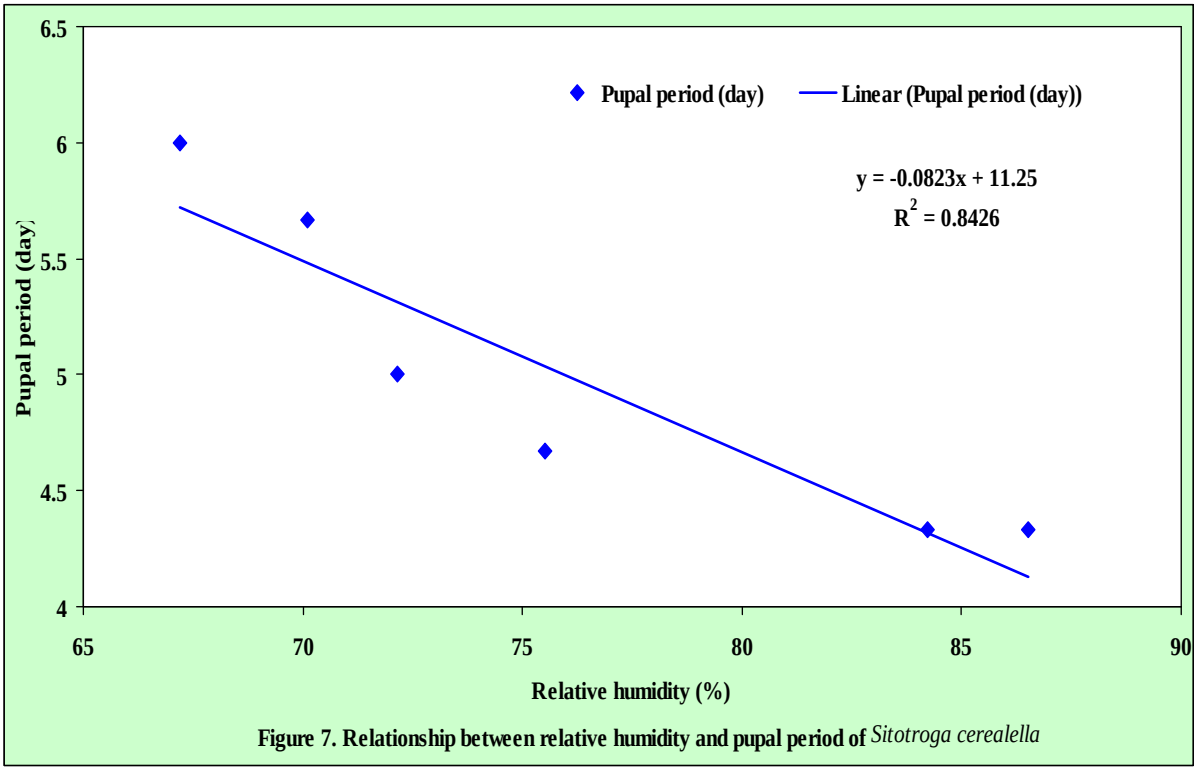

Figure 7. Relationship between relative humidity and pupal period of *Sitotroga cerealella*

4.2.2.4a. Relationship between temperature and adult longevity of *S. cerealella*

The correlation study was done to establish a relationship between temperature and adult longevity of *S. cerealella*. The regression equation y = -0.2887x + 14.62 gave a good fit to the data and the value of the co-efficient of determination (R^2 = 0.9836) (Figure 8). From the correlation study and regression line it was revealed that a highly significant ($p < 0.01$) and strongly (r =0.9836) negative correlation was observed between temperature and adult longevity, i.e., the adult longevity of *S. cerealella* was decreased with the increase of temperature; conversely, the adult longevity was increased with the decrease of temperature. For this reason, the adult longevity of *S. cerealella* was shortest in August when temperature was highest maximum, whereas the adult longevity was longest in January, when the temperature was lowest.

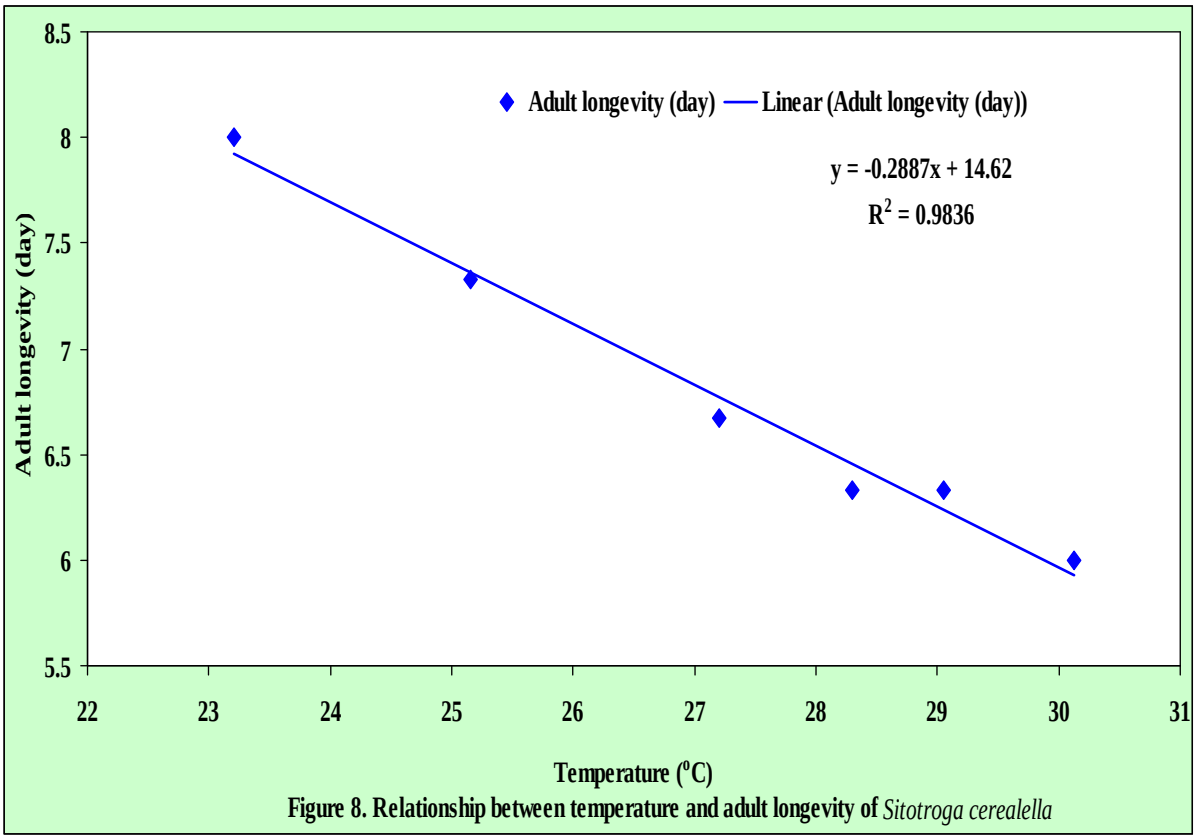

Figure 8. Relationship between temperature and adult longevity of *Sitotroga cerealella*

4.2.2.3b. Relationship between relative humidity and adult longevity of *S. cerealella*

The correlation study was done to establish a relationship between relative humidity and adult longevity of *S. cerealella*. The regression equation y = -0.0826x + 13.05 gave a good fit to the data and the value of the co-efficient of determination (R^2 = 0.7401) (Figure 9). From the correlation study and regression line it was revealed that a highly significant ($p < 0.01$) and strongly (r =0.7401) negative correlation was observed between relative humidity and adult longevity, i.e., the adult longevity of *S. cerealella* was decreased with the increase of relative humidity; conversely, the adult longevity was increased with the decrease of relative humidity. For this reason, the adult longevity of *S. cerealella* was shortest in August when relative humidity was highest, whereas the adult longevity was longest in January, when the relative humidity was lowest.

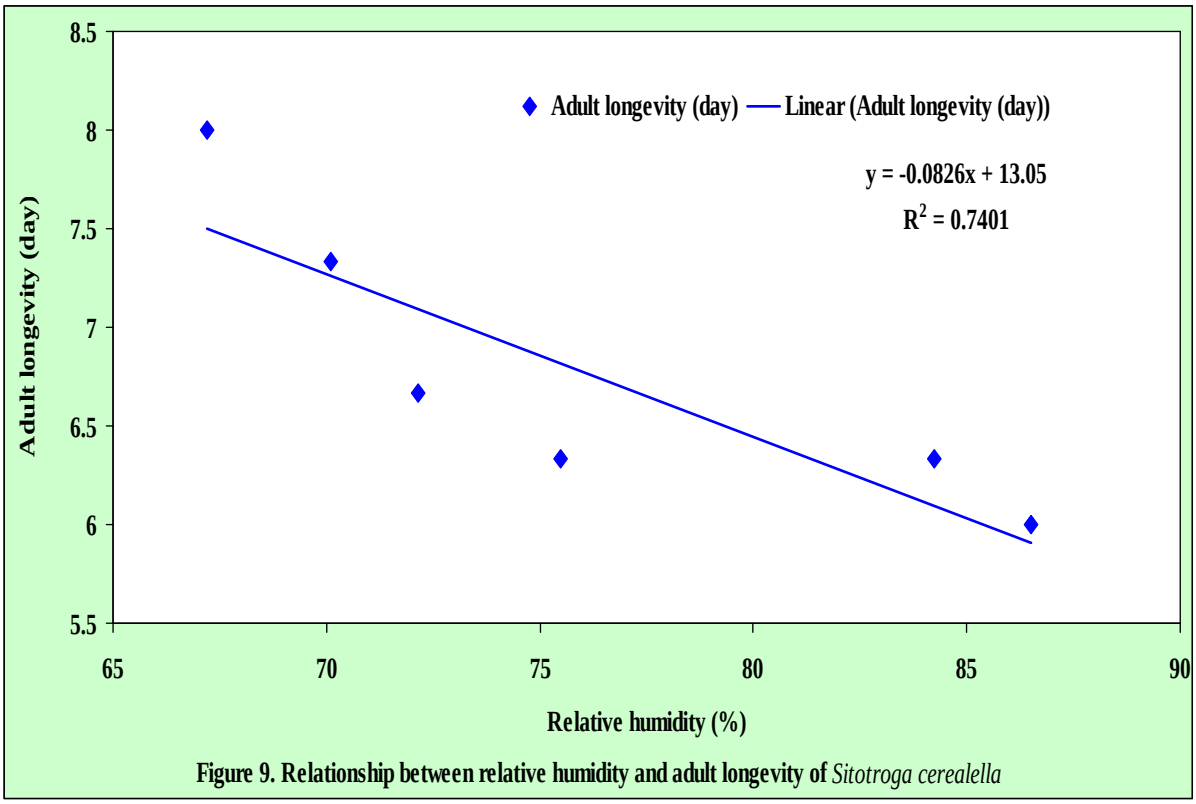

Figure 9. Relationship between relative humidity and adult longevity of *Sitotroga cerealella*

From the above findings it was revealed that the growth and developmental period viz. incubation period, larval period, pupal period and adult longevity of *S. cerealella* were negatively correlated with both the temperature and relative humidity. For this reason, these growth and developmental periods were shortest in August, when temperature and relative humidity were highest, and the growth and developmental periods were longest in January, when temperature and relative humidity were lowest, i.e., the growth and developmental periods of *S. cerealella* were increased with the decrease of the temperature and relative humidity.

4.3. Effect of age on the viability of the Sitotroga eggs

The significant variations were observed among different ages of the host's (Sitotroga) eggs on their viability in terms of hatched eggs, unhatched eggs and percent hatching illustrated in the Table 3. The maximum number (43.80) hatched eggs among 50 tested eggs of *S. cerealella* were recorded from the freshly laid eggs (0-1 day-old), which was closely followed by 10 days old eggs (40.20) and 20 days old eggs (36.00) refrigerated at 4^{o}C temperature. On the other hand, the lowest number of hatched eggs (30.60) was recorded from 40 days old eggs followed by 30

days old eggs (33.00) refrigerated at 4^{o}C temperature. The highest percentage (87.60%) of eggs were also hatched from the freshly laid eggs (0-1 day-old) of rice moth, which statistically different from all other age groups followed by 10 days old eggs (80.40%), 20 days old eggs (72.00%) refrigerated at 4^{o}C temperature. On the other hand, the lowest percentage (61.20%) of eggs was hatched from 40 days old eggs followed by 30 days old eggs (66.00%) refrigerated at 4^{o}C temperature.

Table 3. Effect of ages on the viability of host (*S. cerealella*) eggs

Age of eggs	Eggs tested (No.)	Hatched eggs (No.)	Unhatched eggs (No.)	Percent hatching
Freshly laid eggs (0-1 day old)	50	43.80 a	6.200 e	87.60 a
10 days old eggs stored at 4^{o}C	50	40.20 b	9.80 d	80.40 b
20 days old eggs stored at 4^{o}C	50	36.00 c	14.00 c	72.00 c
30 days old eggs stored at 4^{o}C	50	33.00 d	17.00 b	66.00 d
40 days old eggs stored at 4^{o}C	50	30.60 e	19.40 a	61.20 e
$LSD_{(0.05)}$	-	1.055	1.055	2.111
CV (%)	-	2.18	6.02	2.18

In column, numeric data represent the mean value of 5 replications and means having similar letter(s) are statistically similar at 0.05 level of probability

From the above findings, it was revealed that the highest percent of hatching of Sitotroga eggs recoded from the freshly laid eggs and the percent hatching was decreased with the increase of the age of Sitotroga eggs, i.e., the freshly laid eggs were better than long day refrigerated host eggs for using in rearing of *T. evanescens* egg parasitoid to ensure the maximum culture of the parasitoid.

4.4. Growth and development of *T. evanescens* egg parasitoid

Variations amongst the growth and developmental periods of *T. evanescens* egg parasitoid were observed among different experimental months of the study.

4.4.1. Egg to larval period

The significant variations (P <0.05) among the egg to larval period of *T. evanescens* egg parasitoid were observed in different months of the study. The egg to larval period was ranged between 3.80 and 2.20 days from the month of August, 2010 to January, 2011 (Table 4). The longest egg to larval period was recorded in September, which was statistically different from all other months of the study period followed by the month of October (3.20 days), November (2.80 days) and December (2.80 days). On the other hand, the shortest egg to larval period was recorded in January, which was statistically different from all other months of the study period.

From these findings, it was revealed that the egg to larval period of rice moth was longest in September and it was decreased gradually and shortest in January. As a result the descending order of the egg to larval period among different experimental months was September > October > November, December > January.

4.4.2. Pupal period

The significant variations (P <0.03) among pupal period were also observed in different months of the study. The pupal period of *T. evanescens* egg parasitoid was ranged between 3.60 and 2.00 days from the month of September, 2010 to January, 2011 (Table 4). The longest pupal period was recorded in September, which was statistically similar with the month of October (3.20 days), but different from all other months of the study period followed by November (2.60 days). On the other hand, the shortest pupal period was recorded in January, which was statistically similar with December (2.40 days) and November (2.60 days).

From these findings, it was revealed that the pupal period of *T. evanescens* egg parasitoid was longest in September and it was decreased gradually and shorted in January. As a result the

descending order of the pupal period among different experimental months was September > October > November > December > January.

4.4.3. Egg to adult period

The egg to adult period, i.e., the period between eggs laying to adult emergence, was recorded from the cumulative results of the egg to larval period and pupal period. From these findings, the significant variations ($P < 0.05$) among egg to adult period were observed in different months of the study. The egg to adult period of *T. evanescens* was ranged between 7.40 and 4.20 days from the month of September, 2010 to January, 2011 (Table 4). The longest egg to adult period was recorded in September, which was statistically different from all other months followed by October (6.40 days) and November (5.40 days). On the other hand, the shortest egg to adult period was recorded in January, which was also statistically different from all other months of the study period followed by December (5.20 days).

From these findings, it was also revealed that the egg to adult period of *T. evanescens* was longest in September and it was decreased gradually and shortest in January. As a result the descending order of the egg to adult period among different experimental months was September > October > November > December > January.

4.4.4. Adult longevity

The adult longevity, i.e., the period between adult emergence and its death, did not vary significantly ($P < 0.05$) in different months of the study and it was 3.00 day in all months (from the September, 2010 to January, 2011) of the study period (Table 4).

Table 4. Variations of growth and developmental period of *T. evanescens* egg parasitoid during the month of September 2010 to January 2011

Month	Egg to larval	Pupal period	Egg to adult	Adult longevity

	period (days)	(days)	(days)	(days)
September, 2010	3.80 a	3.60 a	7.40 a	3.0 a
October, 2010	3.20 b	3.20 ab	6.40 b	3.0 a
November, 2010	2.80 b	2.60 bc	5.40 c	3.0 a
December, 2010	2.80 b	2.40 c	5.20 c	3.0 a
January, 2011	2.20 c	2.00 c	4.20 d	3.0 a
$LSD_{(0.05)}$	0.59	0.6188	0.6727	0.00
CV (%)	15.11	16.99	8.91	0.00

In column, numeric data represent the mean value of 5 replications and means having similar letter(s) are statistically similar at 0.05 level of probability

4.4.5. Total life span of *T. evanescens* egg parasitoid

Significant variations (P <0.05) among total life span of *T. evanescens* were observed in different months of the study period. The total life span of *T. evanescens* was ranged from 10.40 to 7.20 days from the month of September, 2010 to January, 2011 (Figure 10). The longest life span was observed in September, which was statistically different from all other months of the study period followed by October (9.40 days). On the other hand, the shortest life span was observed in January, which was statistically different from all other months followed by December (8.20 days), which was statistically similar with November (8.40 days).

From these findings, it was revealed that the total life span of *T. evanescens* egg parasitoid was longest in September and it was decreased gradually, whereas shortest in January. As a result the descending order of the total life span of *T. evanescens* among different experimental months was September > October > November > December > January.

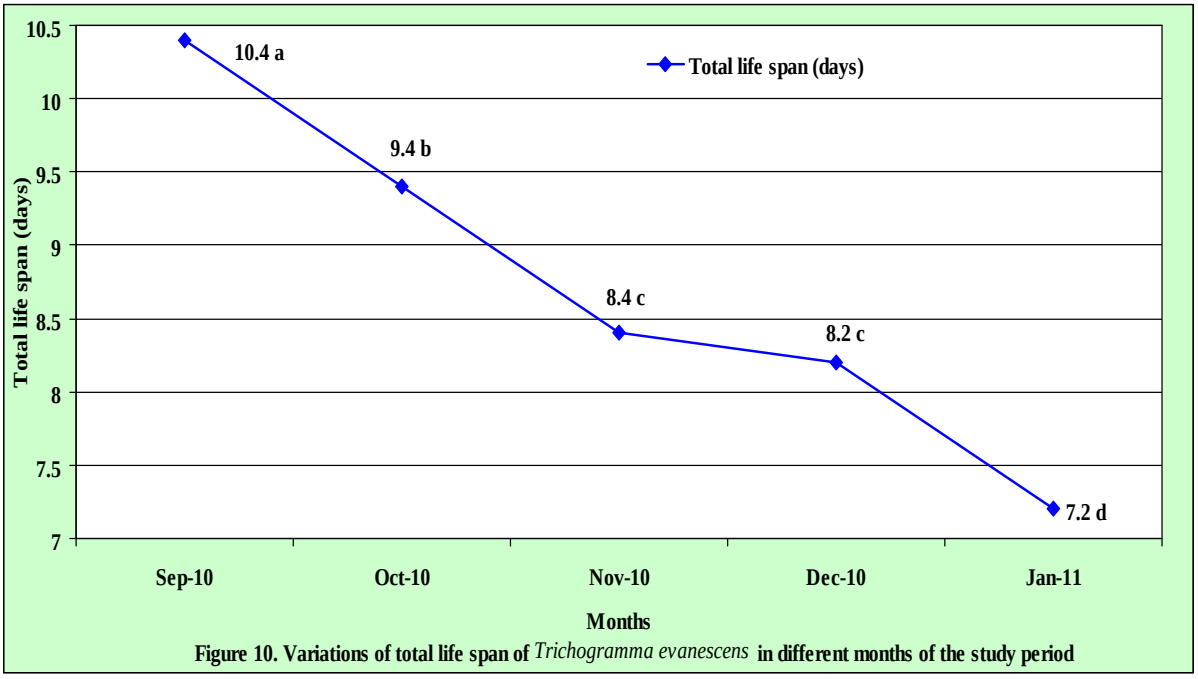

Figure 10. Variations of total life span of *Trichogramma evanescens* in different months of the study period

4.5. Factors for the variations of growth and development of *T. evanescens* in different months of the study period

Now the question arises, what factors are really responsible for such variations in the growth and developmental period of *T. evanesnens* egg parasitoid in different months of the study periods. This question necessitates the in-depth analysis of the actual factors that varied due to the seasonal variations and their possible attributions to the variations in periods of growth and development. The in-depth analyses of all these relevant factors are presented below:

4.5.1. Climatic variations in study periods

Sharp variations in weather factors in months of September, 2010 to January, 2011 were observed (Table 2). The highest mean temperature (29.05^{o}C) and relative humidity (84.23%) were recorded in the month of September. After the month of September 2010, both temperature and relative humidity were decreased gradually and the lowest mean temperature (23.21^{o}C) and relative humidity (67.20%) were recorded in the month of January 2011.

4.5.2.1a. Relationship between temperature and egg to larval period of *T. evanescens* egg parasitoid

The correlation study was done to establish a relationship between temperature and egg to larval period of *T. evanescens* egg parasitoid. The regression equation y = 0.2268x – 3.07 gave a good fit to the data and the value of the co-efficient of determination (R^2 = 0.8432) (Figure 11). From the correlation study and regression line it was revealed that a highly significant ($p < 0.01$) and strongly (r =0.8432) positive correlation was observed between temperature and egg to larval period, i.e., the egg to larval period of *T. evanesnens* was increased with the increase of temperature; conversely, the egg to larval period was decreased with the decrease of temperature. For this reason, the egg to larval period of *T. evanesnens* was longest in September when temperature was highest, whereas the egg to larval period was shortest in January, when the temperature was lowest.

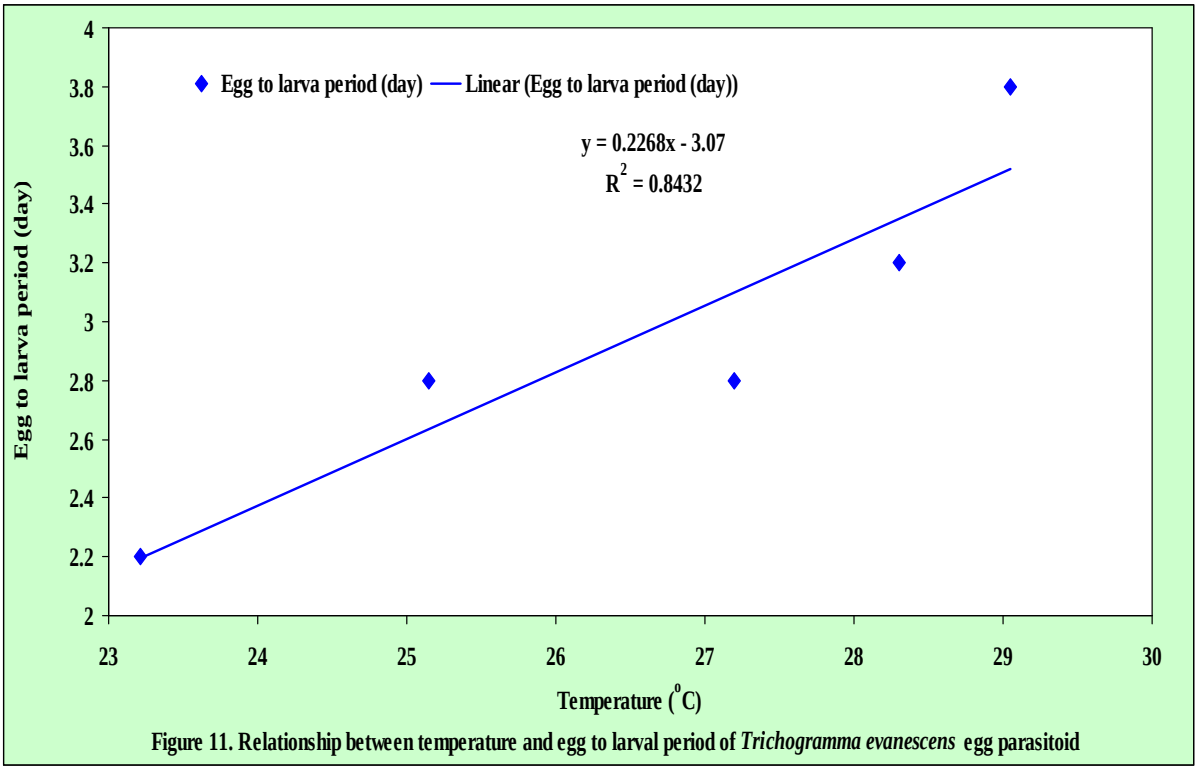

Figure 11. Relationship between temperature and egg to larval period of *Trichogramma evanescens* egg parasitoid

4.5.2.1b. Relationship between relative humidity and egg to larval period of *T. evanesnens* egg parasitoid

The correlation study was done to establish a relationship between relative humidity and egg to larval period of *T. evanescens* egg parasitoid. The regression equation y = 0.0942x – 4.0 gave a good fit to the data and the value of the co-efficient of determination (R^2 = 0.9415) (Figure 12). From the correlation study and regression line it was revealed that a highly significant ($p < 0.01$) and strongly (r =0.9415) positive correlation was observed between relative humidity and egg to larval period, i.e., the larval period of *T. evanescens* egg parasitoid was increased with the increase of relative humidity; conversely, the egg to larval period was decreased with the decrease of relative humidity. For this reason, the egg to larval period of *T. evanescens* egg parasitoid was longest in September when relative humidity was highest, whereas the egg to larval period was shortest in January, when the relative humidity was lowest.

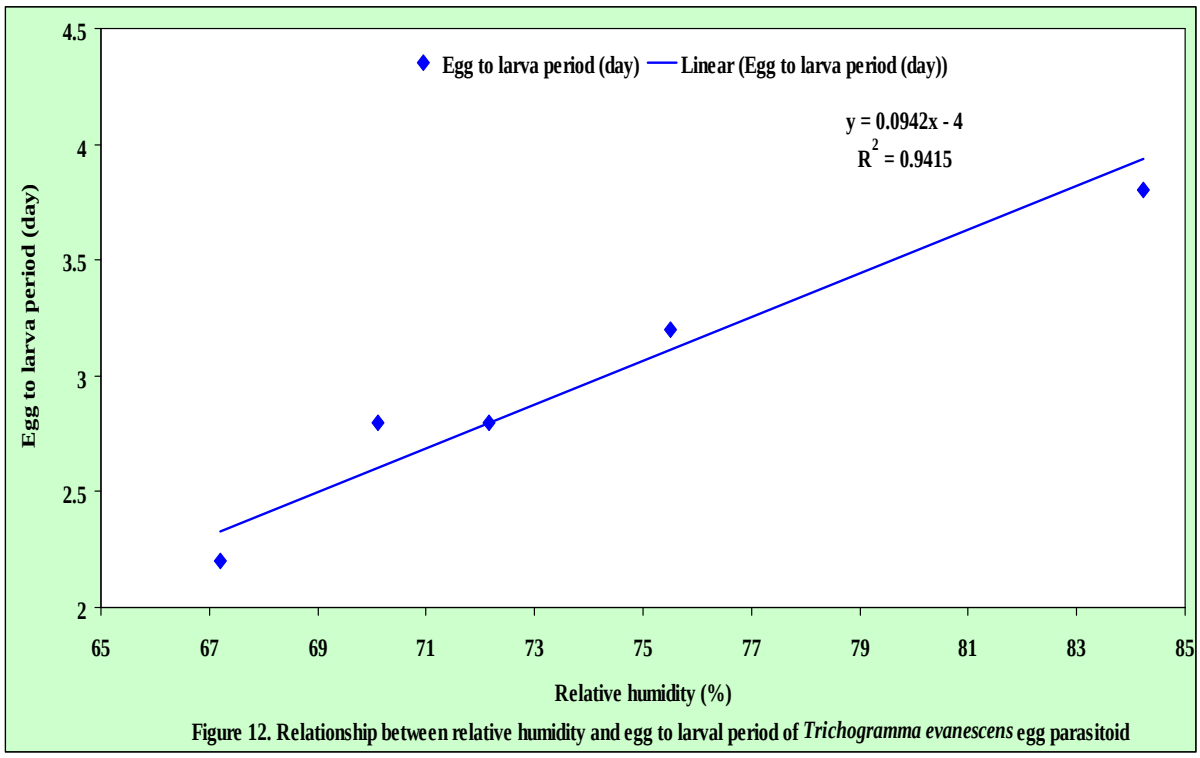

Figure 12. Relationship between relative humidity and egg to larval period of *Trichogramma evanescens* egg parasitoid

4.5.2.2a. Relationship between temperature and pupal period of *T. evanescens* egg parasitoid

The correlation study was done to establish a relationship between temperature and pupal period of *T. evanescens* egg parasitoid. The regression equation y = 0.236x – 3.51 gave a good fit to the

data and the value of the co-efficient of determination (R^2 = 0.9002) (Figure 6). From the correlation study and regression line it was revealed that a highly significant ($p < 0.01$) and strongly (r =0.9742) positive correlation was observed between temperature and pupal period, i.e., the pupal period of *T. evanescens* egg parasitoid was increased with the increase of temperature; conversely, the pupal period was decreased with the decrease of temperature. For this reason, the pupal period of *T. evanescens* egg parasitoid was longest in September when temperature was highest, whereas the pupal period was shortest in January, when the temperature was lowest.

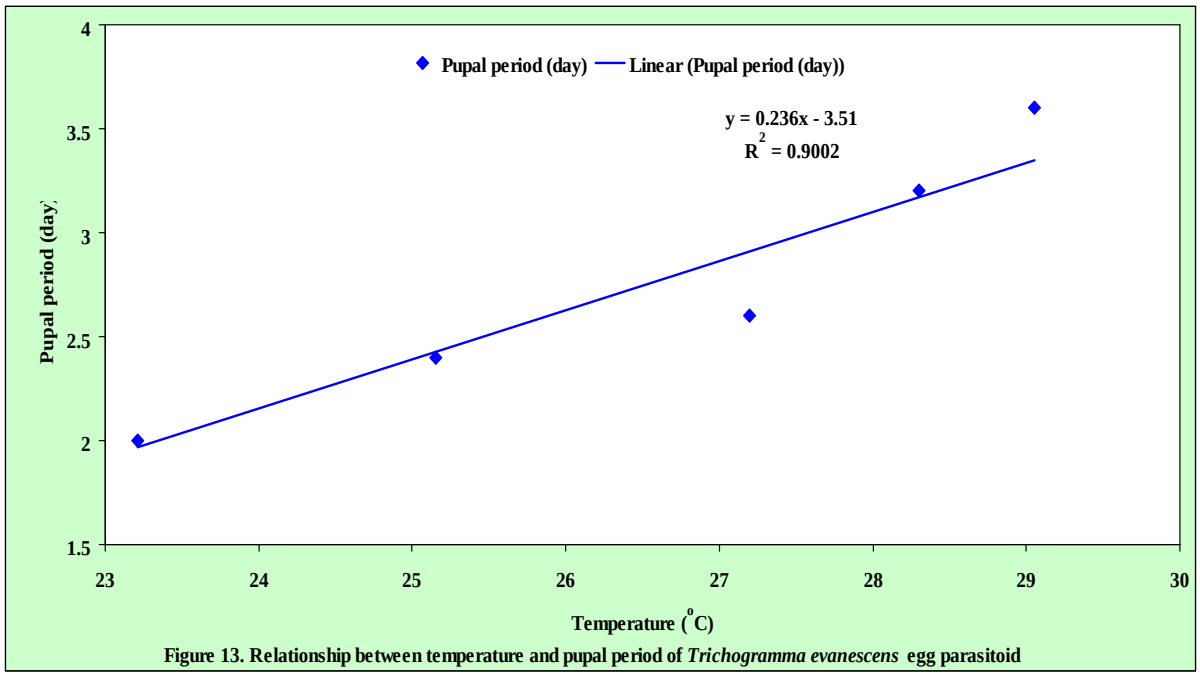

Figure 13. Relationship between temperature and pupal period of *Trichogramma evanescens* egg parasitoid

4.5.2.2b. Relationship between relative humidity and pupal period of *T. evanescens* egg parasitoid

The correlation study was done to establish a relationship between relative humidity and pupal period of *T. evanescens* egg parasitoid. The regression equation y = 0.0939x – 4.17 gave a good fit to the data and the value of the co-efficient of determination (R^2 = 0.9278) (Figure 14). From the correlation study and regression line it was revealed that a highly significant ($p < 0.01$) and strongly (r =0.9278) positive correlation was observed between relative humidity and pupal period, i.e., the pupal period of *T. evanescens* egg parasitoid was increased with the increase of

relative humidity; conversely, the pupal period was decreased with the decrease of relative humidity. For this reason, the pupal period of *T. evanescens* egg parasitoid was longest in September when relative humidity was highest, whereas the pupal period was shortest in January, when the relative humidity was lowest.

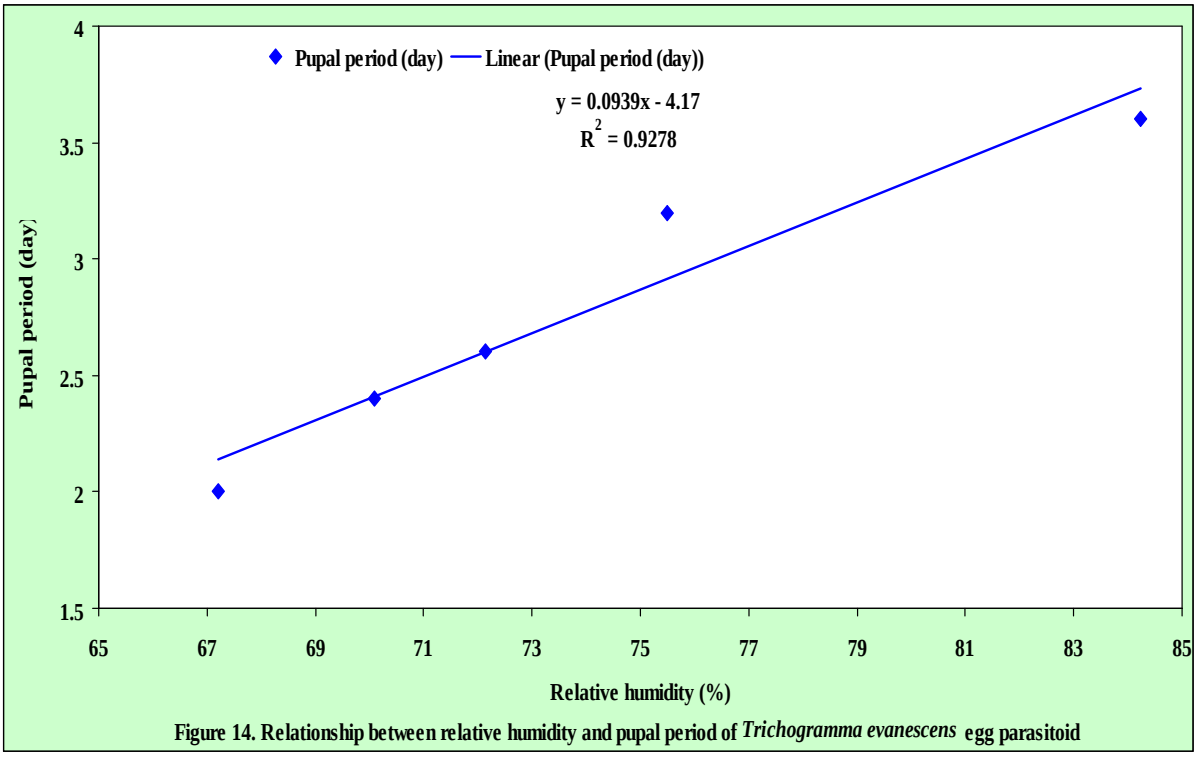

Figure 14. Relationship between relative humidity and pupal period of *Trichogramma evanescens* egg parasitoid

4.6. Performance of *Trichogramma evanescens* in parasitizing Sitotroga eggs

The statistically significant variations were observed in relation to parasitization rate of Sitotroga eggs by releasing variable number of *Trichogramma* egg parasitoid to the 100 Sitotroga eggs for each batch (Table 5) in September, 2010. Considering the number of Sitotroga eggs parasitized by Trichogramm, the highest mean number (91.40) of Sitotroga eggs were parasitized for Batch-1 comprised with 40 Trichogramma released at pupal stage. This number was statistically similar with Batch-2 (88.80) comprised with 35 Trichogramma released at pupal stage followed by Batch-3 (84.00) comprised with 30 Trichogramma released at pupal stage. The lowest mean number (68.40) of Sitotroga eggs were parasitized for Batch-5 comprised with 20 Trichogramma released at pupal stage followed by Batch-4 (76.00) comprised with 25 Trichogramma released at pupal stage. Considering the unparasitized

Sitotroga eggs, reverse trend was observed for different number of Trichogramm released, i.e., the highest number of unparasitized eggs was recorded for Batch-5 (31.40) and lowest was recorded for Batch-1. Considering the parasitization rate of Sitotroga eggs by Trichogramma, the highest percentage (91.40) of parasitization of Sitotroga eggs were observed for Batch-1, which was statistically similar with Batch-2 (88.80%) followed by Batch-3 (84.00%). The lowest parasitizaiton rate (68.40%) of Sitotroga eggs were observed for Batch-5 followed by Batch-4 (76.00%).

From the above findings, it was revealed that the parasitization rate of *Sitotroga* eggs by *Trichogramma* egg parasitoid increased with the increase of the number of *Trichogramma* released.

Table 5. Parasitization performance of *Trichogramma evanescens* egg parasitoid on Sitotroga eggs in ambient condition during September, 2010

Trichogramma released (No./batch)	Sitotroga eggs tested (No./batcth)	Mean parasitized eggs (No./batch)	Mean pnparasitized eggs (No./batch)	Parasitization rate (%)
Batch-1: 40 Trichogramma released	100	91.40 a	8.60 d	91.40 a
Batch-2: 35 Trichogramma released	100	88.80 a	11.20 d	88.80 a
Batch-3: 30 Trichogramma released	100	84.00 b	16.00 c	84.00 b
Batch-4: 25 Trichogramma released	100	76.00 c	24.00 b	76.00 c
Batch-5: 20 Trichogramma released	100	68.40 d	31.60 a	68.40 d
$LSD_{(0.05)}$	-	3.648	3.648	3.648
CV (%)	-	1.53	6.83	1.53

In column, numeric data represent the mean value of 5 replications and means having similar letter(s) are statistically similar at 0.05 level of probability

4.7. Variations of parasitization performance of *T. evanescens* during study period

The significant variations were observed in relation to the parasitization performance of the Trichogramma egg parasitoid among different months of experimental period from September, 2010 to January, 2011 when released a constant number (20.00) of Trichogramma at pupal stage

to the 100 freshly laid Sitotroga eggs for each batch (Table 6). Considering the number of parasitized eggs of Sitotroga, the highest number (84.00) of parasitized eggs was observed in January 2011, which was statistically different from all other months followed by December 2010 (77.60). On the other hand, the lowest number (65.40) of parasitized Sitotroga eggs was recorded in September 2010, which was statistically different from all other months followed by October 2010 (70.00) and November 2010 (72.00). Considering the unparasitized Sitotroga eggs, similar but reverse trend was observed, where the highest number (34.60) of unparasitized Sitotroga eggs was recorded in September 2010 and the lowest number (16.00) of unparasitized Sitotroga eggs was recorded in January 2011. Considering the parasitization rate of Sitotroga eggs, the highest parasitization rate (84.00%) was recorded in January 2011, which was statistically different from all other months followed by December 2010 (77.60%). On the other hand, the lowest parasitization rate (65.40%) was recorded in September 2010, which was statistically different from all other months followed by October 2010 (70.00%) and November 2010 (72.00%).

Table 6. Variations of parasitization performances of *Trichogramma evanescens* egg parasitoid during the month of September 2010 to January 2011

Month	Trichogramma released (No./batch)	Sitotroga eggs tested (No./batch)	Mean parasitized eggs (No.)	Mean unparasitized eggs (No.)	Rate of Parasitization (%)
September, 2010	20	100	65.40 d	34.60 a	65.40 d
October, 2010	20	100	70.00 c	30.00 b	70.00 c
November, 2010	20	100	72.00 c	28.00 b	72.00 c
December, 2010	20	100	77.60 b	22.40 c	77.60 b
January, 2011	20	100	84.00 a	16.00 d	84.00 a
$LSD_{(0.05)}$	-	-	2.010	2.010	2.010
CV (%)	-	-	2.07	5.77	2.07

In column, numeric data represent the mean value of 5 replications and. means having similar letter(s) are statistically similar at 0.05 level of probability

From the above findings, it was revealed that the significant variations for the rate of parasitization of Sitotroga eggs by the Trichogramma were observed in different months of the study period, where the highest parasitization rate was recorded in January and lowest was recorded in September.

4.8. Factors for the variations of parasitization performance of *T. evanescens* egg parasitoid in different months of the study period

Now question arises, what factors are really responsible for such variations in the parasitization performance of *T. evanescens* egg parasitoid in different months of the study periods? This question necessitates the in-depth analysis of the actual factors that varied due to the seasonal variations and their possible attributions to the variations in parasitization performance. The in-depth analyses of all these relevant factors are presented below:

4.8.1. Relationship between weather factors (temperature and relative humidity) and parasitization performance of *T. evanescens* egg parasitoid

There was a sharp and significant relationship observed between the weather factors (temperature and relative humidity) and parasitization rate of Sitotroga eggs by the Trichogramma egg parasitoid. With the months of September 2010, the lowest parasitization rate (65.4%) was observed when the highest temperature (29.05^{o}C) and highest relative humidity (84.23%) were recorded. After the month of September 2010, both the temperature and relative humidity were decreased and these were lowest in January (23.21^{o}C and 67.20%, respectively). After the month of September 2010, the parasitization rate was increased gradually and reached at highest rate (84.0%) in January 2011. From this finding it was revealed that the parasitization rate was increased with the decrease of the temperature and relatively humidity, and the highest rate (84.0%) of parasitization of Sitotroga eggs by the Trichogramma was occurred at 23.21^{o}C temperature and 67.20 % relative humidity (Figure 15).

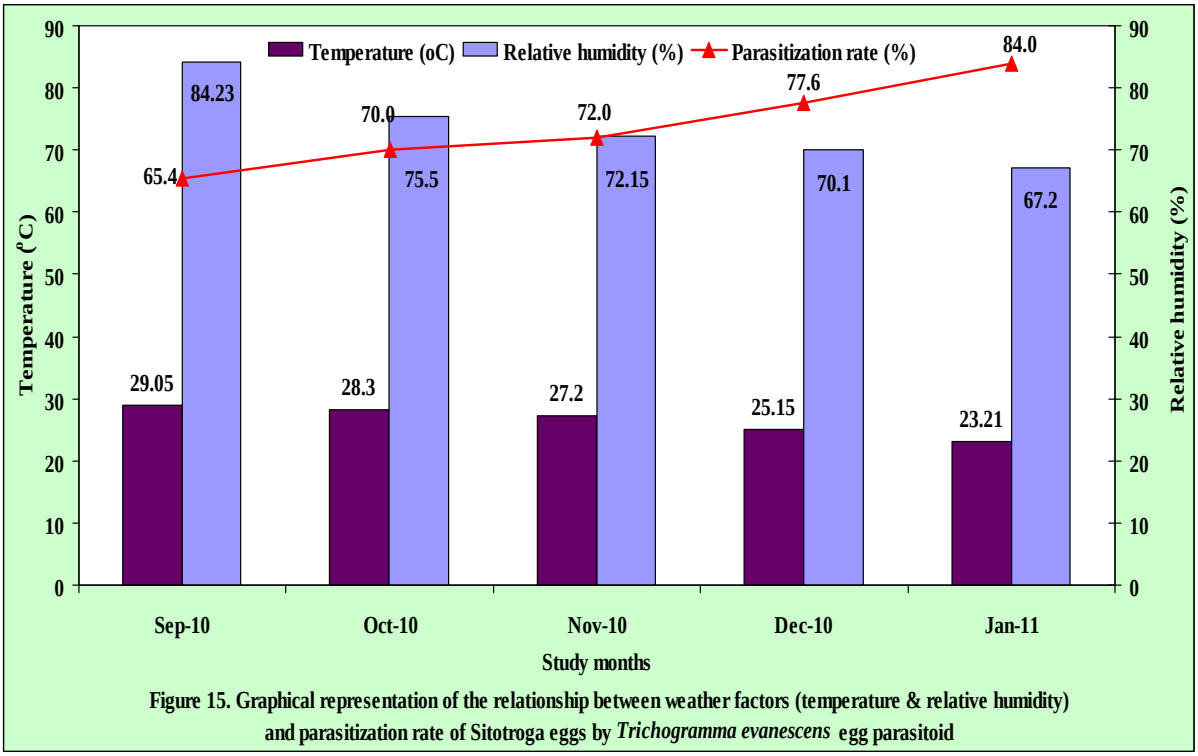

Figure 15. Graphical representation of the relationship between weather factors (temperature & relative humidity) and parasitization rate of Sitotroga eggs by *Trichogramma evanescens* egg parasitoid

CHAPTER V

SUMMARY AND CONCLUSION

The study was conducted in the laboratory of the Department of Entomology at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from July, 2010 to January, 2011 to study on the growth and development of the host Angouimos grain moth, *Sitotroga cerealella* and its egg parasitoid *Trichogramma evanescens* as well as to find out the performance of *T. evanescens* egg parasitoid in parasitizing the eggs of *S. cerealella* and the effect of weather factors on the biology of both host and parasitoid as well as on parasitization. The experiment was laid out in Complete Randomized Design (CRD) with five replications.

The incubation, larval, pupal period, egg to adult period, adult longevity and total life span of Angouimos grain moth, *S. cerealella* were ranged from 6.67 to 7.33 days, 15.67 to 22.67 days, 4.33 to 6.00 days, 26.67 to 36.00 days, 6.0 to 7.33 days and 32.67 to 44.0 days, respectively during the study period from August 2010 to January 2011. The rapid growth and development was recorded in August 2010 and slow growth was recorded in January 2011. The variations of climatic factors such as temperature and relative humidity during different months of the study period were responsible for such variations of growth and development of *S. cerealella,* where the rapid growth and development was observed in August 2010 maintaining 30.12^{o}C temperature and 86.52% relative humidity and slow growth was observed in January 2011 maintaining 23.21^{o}C temperature and 67.20% relative humidity.

All the parameters regarding growth and development were highly significant and negatively correlated with both temperature and relative humidity, i.e., the incubation period, larval period, pupal period and adult longevity were increased with the decrease of both temperature and relative humidity and vice-versa. The freshly laid eggs of *S. cerealella* performed highest

hatching rate (87.60%) that was better than long day stored host eggs for using in rearing of *T. evanescens* egg parasitoid.

The egg to larval period, pupal period, egg to adult period, adult longevity and total life span of *T. evanescens* egg parasitoid were ranged from 2.20 to 3.80 days, 2.0 to 3.60 days, 4.20 to 7.40 days, 3.0 days and 7.20 to 10.40 days, respectively during the study period from September 2010 to January 2011. The variations of climatic factors such temperature and relative humidity during different months of the study period were responsible for such variations of growth and development of *T. evanescens*, where the rapid growth and development was observed in January 2011 maintaining 23.21^{o}C temperature and 67.20% relative humidity and slow growth was observed in August 2010 maintaining 30.12^{o}C temperature and 86.52% relative humidity. All the parameters regarding growth and development of *T. evanescens* were positively correlated with both temperature and relative humidity, where the rapid growth and development was observed in January 2011 maintaining 23.21^{o}C temperature and 67.20% relative humidity and slow growth was observed in January 2011 maintaining 30.12^{o}C temperature and 86.52% relative humidity.

The rate of egg parasitization increased with the increase of the number of Trichogramma release and the maximum parasitization (91.40%) was achieved by the release of 40 Trichogramma released at pupal stage for 100 host eggs. Among different months of the experimental period, the highest parasitization rate (84.00%) was recorded in January 2011 and lowest (65.40%) was recorded in September 2010. Comparatively lower temperature (23.21^{o}C) and relative humidity (67.20%) recorded in January 2011 favored higher parasitization rate than higher temperature (29.05^{o}C) and relative humidity (84.23%) recorded in September 2010.

Considering the findings of the present experiment, further studies in the following areas may be suggested:

1. Such study is needed in control condition with highly equipped laboratory facilities;

2. Other rearing materials may be included in the future study.

CHAPTER VI

REFERENCES

Ables, J. R., Mc. Commas, Jr.D.W., Jones S.L. and Morrison.R.K. (1980). Effect of cotton plant size, host egg location, and location of parasite release on parasitism by *Trichogramma pretiosum*. *Southwestern Entomol.* **5**:261-264.

Alam, S.N.,Mondal, M.F.and Nabi, M.(2008a).Parasitism efficacy of three *Trichogramma* spp. on two hosts eggs, *Sitotroga cerealella* and *Corcyra cephalonica*. Entomology Report 2007-2008. Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. pp. 14-15.

Alam, S.N., Mondal, M.F.and Nabi, M.(2008b).Optimization of number of *Trichogramma* for effective host egg parasitism. Entomology Report 2007-2008. Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. pp. 15-17.

Alencar, J.A.,Haji, F.N.P, Oliveira, J.V. and Moreira, A.N.(2000). Biology of *Trichogramma pretiosum* Riley in eggs of *Sitotroga cerealella* (Olivier). *Pesquisa Agropecuaria Brasileira*. **35**(8): 1669-1674.

Andow, D.A.(1997). Integrating biological control in IPM systems. *In* Ecological Interactions and Biological Control, *eds*. D. A. Andow, D. W. Ragsdale and R. F. Nyvall, pp. 71-86. Westview Press.

Arlei M., Celso, L.H., Honorio, R. S. (2003) Temperature effects on *Trichogramma pretiosum* Riley and *Trichogrammatoidea annulata* De Santis. *Brazilian Archives Biol. Tech*. **46**(1): 45-49.

Awasthi, M.D.(1998). Pesticides residues in fruits and vegetables.Integrated Pest Management in Horticultural Ecosystems. pp.263-273.

Ayvaz, A., Karasu, E., Karabörklü, S. and Tunçbilek, A. S. (2008). Effects of cold storage, rearing temperature, parasitoid age and irradiation on the performance of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). *J. Stored Prod. Res*. **44**(3):232-240.

Baode, W., David, N., Ferro, J.W. and Suqin, W.(2004). Temperature-Dependent Development and Oviposition Behavior of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae), a Potential Biological Control Agent for the European Corn Borer (Lepidoptera: Crambidae). *Environ. Entomol*. 33(4):787-793.

Bigler, F. (1994). Quality control in *Trichogramma* production. *In* Biological Control with Egg Parasitoids, Wajnberg eds. E and S.A. Hassan, pp. 93-111. Oxon, U.K.: CAB International. *Biological Control*. **25**(2): 110-115.

Brower, J. H. (1983). Eggs of stored-product Lepidoptera as hosts for *Trichogramma evanescens* [Hym.: Trichogrammatidae]. *Bio Control*. 28**(4): 355-361.**

Chinniah, C., Kuttalam, S, Regupaty, A. (1998). Harvest time residue of lindane and chiorpyriphos in paddy. *Pestic. Re. J.* **10**(l):91-94.

Consoli, F. L. and Parra, J.R.P. (1995). Effects of constant and **alternating** temperatures on *Trichogramma galloi* Zucchi (Hymenoptera., Trichogrammatidae) biology. I. Development and thermal requirements. *J. Ap. Entomol*. **119**(1-5): 415–418.

Corrigan, J.E., Laing. J.E. (1994). Effects of the rearing host species and the host species attacked on performance by *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae). *Biol. Control*. **23**: 755–760.

Doyon, J. and Boivin, G. (2005). The effect of development time on the fitness of female *Trichogramma evanescens*. *J. Insect Sci*. **5**(4):1-5

El-Wakeil, N. (2007). Evaluation of efficiency of *Trichogramma evanescens* reared on different factitious hosts to control *Helicoverpa armigera. J. Pest Sci.* 80**(1):29-34.**

Essam, A. (2010). Field evaluation of the egg parasitoid, *Trichogramma evanescens* West. against the olive moth *Prays oleae* (Bern.) in Egypt. *J. Pest Sci*. **83**(1): 53-58.

Fye, R.E. and D.J. Larsen. (1969). Preliminary evaluation of *Trichogramma minutum* as a released regulator of Lepidopterous pests of cotton. *J. Econ. Entomol*. **62**:1291-1296

Greco, C.F. and Stilinovic, D. (1998). Parasitization performance of *Trichogramma* spp. (Hym., Trichogrammatidae) reared on eggs of *Sitotroga cerealella* Oliver (Lep., Gelechiidae), stored at freezing and subfreezing conditions. *J. App. Entomol.* **122**(6):311-314.

Greenberg, S.M., Nordlund, D.A. and King, E.G. (1996). Mass production of Trichogramma spp.: experiences in the former Soviet Union, China, the United States and Western Europe. *Biocontrol News Info*. **17**:51-61.

Hansen, L.S. and Jensen, K.M. (2002). Effect of temperature on parasitism and host-feeding of *Trichogramma turkestanica* (Hymenoptera: Trichogrammatidae) on Ephestia kuehniella (Lepidoptera: Pyralidae). *J. Econ. Entomol.* **95**(1): 50-56.

Hasan, S.A. (1992). Stem borer of graminacious crop in South East Asia. *Trop. Agric. Res.*, **8***:*145-153.

Hassan, S.A. and WenQing, Z. (2001). Variability in quality of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) from commercial suppliers in Germany. *Biological Control*. 22(2): 115-121.

Johnson, S.J. (1985). Low-level augmentation of *Trichogramma pretiosum* and naturally occurring *Trichogramma parasitism* of *Heliothis* spp. in cotton in Louisiana. *Environ. Entomol*. **14**:28-31.

Kim, H. S. and Heinrichs, E. A. (1985). Parasitization of yellow stem borer eggs (YST) *Scirpophaga incertu!as* eggs. *IRRI Newsl.* **10**: 14.

Kim, H. S., Heinrichs, E.A. and Mylvaganam, P. (1986). Egg parasitism of *Scirpophaga incertu!as* Walker (Lepidoptera: Pyralidae) by Hymenopterous parasitoids in IRRI rice fields. *Korean J. Plant Protec*. **25***:* 37-40.

Kring, T. J. and Smith.T.B. (1995). *Trichogramma pretiosum* efficiency in cotton under Bt-insecticide combinations. Proc. Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, Tennessee. pp. 856-857.

Lewis, W. Nordlund, J., Donald, A., Gross, H.R., Perkins, W.D., Knipling, E.F. and Voegele, J. (1976). Production and performance of *Trichogramma* reared on eggs of *Heliothis zea* and other hosts. *Environ. Entomol.* **5** (3):449-452.

Li, Li-Ying. (1994). Worldwide use of *Trichogramma* for biological control on different crops: A survey. *In* Biological Control with Egg Parasitoids, eds. E. Wajnberg and S. A. Hassan, pp. 37-53. Oxon, U.K., CAB International. *J. Asia Pacific Entomol*. **3**(2): 65-70.

Lopez, J.D.Jr. and Morrison, R.K. (1985). Parasitization of *Heliothis* spp. eggs after augmentative releases of *Trichogramma pretiosum* Riley. *Southwestern Entomol*. *Supp*. **8**:110-137.

Matthias, S. and Sherif, A.H. (2001). Comparative biology and life tables of *Trichogramma evanescens* and *T. cacoeciae* with Ephestia elutella as host at four constant temperatures. *Entomol. Exp. Applic*. **98**(1): 35-40.

Morrison, R.K., Stinner R.E. and Ridgway, R.L. (1976). Mass production of *Trichogramma pretiosum* on eggs of the Angoumois grain moth. *Southwestern Entomol*. **2**:74-80.

Nabil, E. E.W. (2007). Evaluation of efficiency of *Trichogramma evanescens* reared on different factitious hosts to control *Helicoverpa armigera*. *J. Pest Sci*. **80**(1): 29-34.

Nabil, E.E.W., Hamza, T.F. and Zakia, A.R. (2008). Attracted Article: Efficacy of inundative releases of *Trichogramma evanescens* in controlling *Lobesia botrana* in vineyards in Egypt. *J. Pest Sci*. **81**(1): 49-55.

Narayansamy, p. (1999). Traditional Pest Management for sustainable agriculture. Proceeding of Biopesticides in Insect Pest Management, Rajmundry. pp.225-231.

O'Neil, R.J., Giles, K.L., Obrycki, J. J., Mahr, D. L., Legaspi J. C. and Katovich. K.(1998). Evaluation of the quality of four commercially available natural enemies. *Biolo. Control*. 11:1-8.

Olkowski, W. and Zhang, A. (1990). *Trichogramm*aa modern day frontier in biological control. *The IPM Practitioner*. **12:**1-15.

Orr, D.B., Landis, D.A., Mutch, D. R., Manley, G.V., Stuby, S.A. and . King.R.L., (1997). Ground cover influence on microclimate and *Trichogramma* (Hymenoptera:

Trichogrammatidae) augmentation in seed corn production. *Environ. Entomol*. **26:**433-438.

Prasad, R. P., Bernard, D.R. and Deborah E.H. (2002). The effect of rearing temperature on parasitism by *Trichogramma sibericum* Sorkina at ambient temperatures.Prasad, R. P., Bernard, D. R. and Deborah, H. (1999). The Effect of Rearing Temperature on Flight Initiation of *Trichogramma sibericum* Sorkina at Ambient Temperatures. *Biolo. Control*. **16**(3): 291-298.

Prasad, R.P., Roitberg, B.D., Henderson, D. E. (2003). The effect of rearing temperature on parasitism by *Trichogramma sibericum* Sorkina at ambient temperatures. *Biological Control*, 25**(2): 110-115.**

Quaintance, A.L. and Brues. C. T. (1905). The Cotton Bollworm. USDA Bureau of Entomology Bulletin no. 50. U. S. Govt Printing Office. Washington. D.C.

Rabinovisch J. E. (1971). Population dynamics of *Telenomus fariai* (Hymenoptera: Scelionidae), a parasite of Chaga's disease vector. *Revista de Biologia Tropical.* 19:109–120.

Rahman, M.M. (2006). Vegetable IPM in Bangladesh, *In:* E.B. Radcliffe and W.D. Hutchison [eds.], Radcliffe's IPM World Textbook, URL: http://www.ipmworld.umn.edu/chapters/rahman.htm, University of Minnesota, St. Paul, MN., USA.

Rundle, B.J., Thomson, L.J., Hoffmann, A.A. (2004). Effects of cold storage on field and laboratory performance of *Trichogramma*

carverae **(Hymenoptera: Trichogrammatidae) and the response of three *Trichogramma* spp. (*T. carverae*, *T. brassicae*, and *T. funiculatum*) to cold. *J. Econ Entomol.* 97(2):213-21.**

Saour, G. (2004). Efficacy assessment of some *Trichogramma* species (Hymenoptera: Trichogrammatidae) in controlling the potato tuber moth *Phthorimaea operculella* Zell. (Lepidoptera: Gelechiidae). *J. Pest Sci*. 77(4): 229-234.

Segers, J. C., J. H. Benedict and M. F. Treacy. (1984). Natural parasitism of *Heliothis* spp. eggs on cotton and corn in the lower gulf coast of Texas. *Southwestern Entomol.* **9**:245-248.

Sergey Y.R., Natalia, D.V. and Nina, P.V. (2009). Effect of temperature on the reproduction and development of *Trichogramma buesi* (Hymenoptera: Trichogrammatidae). *European. J. Entomol.* **106** (4): 535–544.

Sergey, Y.R., Natalia, D.V., Nina P.V. (2009). Effect of temperature on the reproduction and development of *Trichogramma buesi* (Hymenoptera: Trichogrammatidae). *European. J. Entomol*. **12**(2): 23-28.

Smith S. M. (1996). Biological control with Trichogramma: Advances, successes, and potential of their use. *Ann. Rev. Entomol.* **41**:375–406.

Stinner, R.E., Ridgway, R.L., Coppedge, J.R., Morrison, R.K. and Dickerson, W.A. (1974). Parasitism of *Heliothis* eggs after field releases of *Trichogramma pretiosum* in cotton. *Environ. Entomol*. **3**:492-500.

Strand, M. R. (1986). Physiological interactions of parasitoids and hosts. *In* Insect Parasitoids, (eds.) J. Waage and D. Greathead, pp. 109-118. Academic Press, London.

Treacy, M.F., Benedict, J.H., Walmsley, M.H., Lopez, J.D. and Morrison, R.K. (1987). Parasitism of bollworm (Lepidoptera: Noctuidae) eggs on nectaried and nectariless cotton. *Environ. Entomol*. **16**: 420-423.

Waage, J. K. (1983). Agregation in field parasitoids populations of *Diodegma* (Hymenoptera: Ichneumonidae). *Ecol. Entomol*., **8**:.447-459.

Young, K.P., Hai, P.L. and Ki, S.L. (2000). Effect of Temperature on the Biology of *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) Reared on a Factitious Host, *Antheraea pernyi* (Lepidoptera: Saturniidae) Egg.